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SEROTONIN AND THE EATING DISORDERS

THE EFFECTS OF DIETING, ACUTE PLASMA TRYPTOPHAN DEPLETION AND mCPP ADMINISTRATION ON BRAIN 5-HT FUNCTION

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"One can never be too thin or too rich"

Wallis Warfield Simpson, Duchess of Windsor
ABSTRACT

The past two decades have seen an upturn in interest in the psychiatric disorders anorexia and bulimia nervosa. Although this renewed interest has been reflected in the amount of research being undertaken, little is understood about the aetiology of these often devastating disorders. In particular, only limited work has been carried out examining the possible neurobiological abnormalities that may underlie, or impart vulnerability to the development of anorexia and bulimia nervosa. While evidence now exists to suggest that serotonergic dysregulation occurs during clinical episodes of both disorders, the precise nature of the dysregulation remains unclear. Furthermore, interpretation of eating disorder research is complicated by confounding factors of the illnesses themselves, including weight loss and the physical consequences of behaviours such as bingeing and purging, and by a paucity of knowledge regarding the normal control of human feeding behaviour. The work in this thesis uses three experimental paradigms, i.e. the neuroendocrine challenge test, a test meal procedure and administration of an amino acid drink free in tryptophan, to examine the role of serotonin in the development of the eating disorders. More specifically, the neuroendocrine approach was used to examine the effects of weight loss on d-fenfluramine and meta-chlorophenylpiperazine-induced prolactin responses in healthy controls and, in combination with a test meal, to examine the effects of m-chlorophenylpiperazine on food intake and prolactin response in healthy women. In addition, the effect of a tryptophan-free amino acid drink on mood and food intake was examined in both healthy women and in women recovered from bulimia nervosa. Experimental findings reported in this thesis demonstrate no effect of tryptophan-free amino acid drink administration on mood and food intake. In contrast, evidence is presented in support of the hypothesis that moderate dieting is associated with alterations in brain serotonin function, and that such effects are confined to women, suggesting that women are more vulnerable than men to the effects of weight loss and altered food intake. The mechanism underlying dieting-induced alterations in brain serotonin function is discussed, with experimental evidence suggesting a possible pre-synaptic site of action. Studies utilising the serotonin agonist meta-chlorophenylpiperazine confirm the importance of serotonergic pathways in the control of food intake in humans and suggest involvement of 5-HT2C receptors in mediating the effects of human feeding behaviour.
Many people have given help over the three years in which the work included in this thesis was carried out and I would like to acknowledge my debt to them. I wish to thank my supervisor Dr Philip Cowen, for not only providing me with the opportunity and encouragement to carry out the work, but also for his guidance and constructive criticism in preparing this manuscript. Throughout all stages of the project he has been readily available for consultation and has provided practical assistance. I am indebted to all the staff at the Research Unit, Littlemore Hospital, Oxford, for their moral support and the technical, secretarial and nursing assistance so freely and cheerfully given. A special thanks to Claire Williams, Mike Franklin and Verlaine Bowden in this respect and to my colleague Anna Oldman with whom I worked closely on this project. Thanks must also go to Professor Gelder for allowing me to carry out this work within his department.

I would like to express my gratitude to the subjects who gave of their time to take part in these studies, especially those women patients who participated willingly in the hope of furthering our understanding of bulimia nervosa at the expense of some personal discomfort.

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Abbreviations

ACTH: adrenocorticotrophic hormone
ANOVA: two way repeated measures analysis of variance
AUC: area under the curve
AUC-B: area under the curve minus baseline
5-HT: serotonin
CSF: cerebral spinal fluid
CV: coefficient of variation
DOI: 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
DSM-III: Diagnostic and Statistical Manual of Mental Disorder (Third Ed.)
DSM-III-R: Diagnostic and Statistical Manual of Mental Disorder (Third Ed.-Revised)
EAT: Eating Attitudes Test
EDE-Q3: The Eating Disorder Examination: Questionnaire 3
GH: growth hormone
5-HIAA: 5-hydroxyindoloacetic acid
5-HT: 5-hydroxytryptamine
kJ: kilojoules
LNAA: large neutral amino acid
MAO: monoamine oxidase
mCPP: meta-chlorophenylpiperazine
8-OH-DPAT: 8-hydroxy-2-(di-n-propylamino)-tetralin
POMS: Profile of Mood State
PRL: prolactin
PVN: paraventricular nucleus
TFMPP: 1-[3-(trifluoromethyl)-phenyl] piperazine
TRH: thyroid releasing hormone
TRP: tryptophan
VA: visual analogues
Chapter 1

5-HYDROXYTRYPTAMINE AND EATING DISORDERS

1.1 OVERVIEW OF EATING DISORDERS AND DIETING BEHAVIOUR

Although Morton published the first case reports of anorexia nervosa in the literature as early as 1694, little is understood about the aetiology of this often devastating disorder. The past two decades have seen an upturn in interest in anorexia nervosa and the more recently defined eating disorder syndrome, bulimia nervosa, an interest reflected in the increased research into many aspects of these illnesses. Although such research has led to a better understanding of the eating disorders in general, particularly in the area of epidemiology, many questions with respect to aetiology remain unanswered.

1.1.1 CLASSIFICATION OF EATING DISORDERS AND ISSUES RELATING TO RESEARCH

For the purposes of this thesis, the term "eating disorders", will refer to the two main psychiatric syndromes, anorexia nervosa and bulimia nervosa as defined below.

**DSM-III-R Diagnostic criteria for Anorexia Nervosa:**

For a diagnosis of anorexia nervosa, all of the following must be met:

A. Refusal to maintain body weight over a minimal normal weight for age and height, eg., weight loss leading to maintenance of body weight 15% below that expected; or failure to make expected weight gain during period of growth leading to body weight 15% below that expected.

B. Intense fear of gaining weight or becoming fat, even though underweight.

C. Disturbance in the way in which one's body weight, size or shape is experienced, eg., the person claims to "feel fat" even when emaciated, believes that one area of the body is "too fat" even when obviously underweight.
D. In females, the absence of at least three consecutive menstrual cycles when otherwise expected to occur (primary or secondary amenorrhoea). (A woman is considered to have amenorrhoea if her periods occur only following hormone administration, e.g. the oral contraceptive pill).

**DSM-III-R Diagnostic Criteria for Bulimia Nervosa:**

For a diagnosis of bulimia nervosa, all of the following must be met:

A. Recurrent episodes of binge-eating (rapid consumption of a large amount of food in a discrete period of time).

B. A feeling of lack of control over eating behaviour during the eating binges.

C. The person regularly engages in either self-induced vomiting, use of laxatives or diuretics, strict dieting or fasting, or vigorous exercise in order to prevent weight gain.

D. A minimum average (sic) of two binge-eating episodes a week for at least three months.

E. Persistent overconcern with body shape and weight.

These clinically defined disorders are closely related but the exact nature of their relationship remains unclear. Not only do they share many demographic, clinical and psychometric features but considerable movement occurs from one disorder to the other, especially in the direction from anorexia to bulimia nervosa (Fairburn, 1983; Garner *et al.*, 1985). Indeed definitions of the two syndromes remain in flux and considerable literature exists to suggest that subgroups of the disorders (other than the two main categories defined above) can be distinguished on certain factors such as history, weight loss and psychopathological characteristics (Kaye and Weltzin, 1991b). It may also be possible that underlying psychobiological correlates further separate and/or distinguish such subgroups.

In anorexia nervosa two main eating patterns can be distinguished: a restrictor subtype where weight loss is obtained purely through food restriction and bulimic anorectics who may qualify for the diagnosis of both DSM-III-R anorexia nervosa and DSM-III-R bulimia nervosa (Kaye and Weltzin, 1991b). The third main subgroup of the eating disorders is composed of normal weight patients meeting the diagnostic criteria for bulimia nervosa.

The confounding factors of starvation along with the biological consequences of bingeing and purging have further encouraged researchers in the area of psychopharmacology to study the eating disorders in terms of the above subgroups rather than as the two clinically defined entities. Equally important is the increasing trend to look at different stages of the disorder, such as when the patient is acutely ill and upon "recovery", usually defined as abstinence from bingeing and purging behaviour in the
presence of weight restoration. This is especially significant in helping distinguish possible biological "state" (i.e. those abnormalities present only during the acute illness) from "trait" (i.e. those abnormalities present upon recovery) markers. Research looking at biological aspects of the eating disorders, which will be discussed below, can be divided into two categories. In earlier work patient definition was limited to allocating subjects to either of the broad clinical entities of anorexia or bulimia nervosa, while more recent studies have been heavily influenced by the factors outlined above. Wherever possible, research findings in this thesis will be reported and discussed within eating disorder subgroups (i.e. restrictor anorectics, bulimic anorectics and normal weight bulimics) with the stage of the illness identified.

1.1.2 ANOREXIA NERVOSA

1.1.2.1 Epidemiology

The first study looking at the incidence of anorexia nervosa within a defined population was carried out by Theander (1970) who examined ninety-four female cases in southern Sweden and reported an annual incidence rate in women of 0.24 per 100,000 inhabitants. Kendell et al (1973) studied the incidence of anorexia nervosa in three different regions: North-east Scotland (the Aberdeen case register, between 1966-9), Monroe county, New York State (1960-9), and Camberwell, London (1965-71). Using a case register approach, he found the incidence of anorexia nervosa varied between 0.37 per 100,000 per year in Monroe County to 1.6 per 100,000 per year in North-east Scotland. These earlier studies share methodological problems, such as diagnostic criteria variability, making it difficult to draw firm conclusions, but all demonstrated a trend suggesting an increase in case numbers over time. The trend was also seen in follow-up studies carried out in New York State, Zurich and Aberdeen looking at case registers up until the late 1970s (Jones and Fox, 1980; Szmukler et al, 1986; Willi et al, 1990), although an increase over time was not a universal finding (Williams and King, 1987). While improved recognition of the disorder may have played a part, these figures are thought to reflect a true increase in the disorder.

More recent studies have suggested that the incidence of anorexia nervosa may be levelling out with Willi et al (1990) and Hall and Hay (1991) reporting stable incidence and referral rates over the periods 1983-1985 and 1977-86 respectively. While Willi et al (1990) found a significant increase from 0.38 cases per 100,000 females (12-25 years) for 1956-1958 to 0.55 per 100,000 for 1963-1965 to 1.12 per 100,000 for 1973-1975, in contrast, they found no further increase between 1983-1985. Similarly, Hall and Hay (1991) reported a constant referral rate of 5 per 100,000 population (34 per 100,000 for females aged 15-34 years) for the period 1977-1986. These findings are in keeping with those of Hoek (1991) who found 6.3 cases (both hospital and general practitioner) per 100,000 population over the years 1985 and 1986.
1.1.2.2 Clinical Features

The essential features of anorexia nervosa centre around a refusal to maintain body weight, over a minimal normal weight for height, consequent upon an intense fear of gaining weight or becoming fat. This is usually accompanied by a distorted body image manifested by patients stating that they "feel fat" although underweight or even severely emaciated. In women, amenorrhoea is also present (American Psychiatric Association, 1987). A preoccupation with body shape and dissatisfaction with some aspect of their physical appearance is usual. The condition usually begins in adolescence and is seen predominantly in females (Fairburn, 1983).

Low body weight is usually accomplished by severe food restriction despite normal feelings of hunger and a preoccupation with food. Excessive exercise and laxative abuse are common as is the use of self-induced vomiting, with some studies reporting a prevalence rate of concomitant problems with bulimia amongst 40-50% of diagnosed anorectic patients (Mitchell et al, 1990b). Chronic starvation results in a number of body changes such as gastric constriction, the appearance of lanugo hair on the face, back and limbs and cardiovascular compensation reflected in bradycardia, postural hypotension and poor peripheral perfusion. A low basal metabolic rate and low circulating sex steroid hormone levels indicate the presence of endocrine abnormalities (Sharp and Freeman, 1993). Affective symptoms, especially depression and mood lability are common features and are often accompanied by social withdrawal and a lack of sexual interest (Fairburn, 1983).

1.1.2.3 Aetiological Theories

While the fundamental cause underlying anorexia nervosa is not understood, it is now usually viewed as arising from a complex interaction of biological, psychological and social factors. Fairburn (1983) has suggested that these factors may function by imparting vulnerability, or by acting as precipitants to, or perpetuators of the illness.

Early studies were heavily influenced by traditional psycho-analytical teaching, viewing the disorder as a defence against fantasies of impregnation. This idea was later replaced by that of Bruch (1962), who saw the disorder as arising from a disturbance of body image, seen alongside the anorectic's inability to interpret her own bodily sensations and paralysed by an overwhelming sense of ineffectiveness and lack of autonomy. Slade and Russell (1973) described an objective technique for determining bodily perception and used it to demonstrate that anorectic patients markedly overestimated their own body width.

For Crisp (1976), the core pathology was the development of a weight phobia "hingeing on and pivoting round puberty". The central phobia was not seen as being
directed towards an excess of weight but towards a "normal (functioning) adolescent weight". Crisp felt that for some individuals, fearful of adolescence, an association developed between the normal weight gain of puberty and the psychosexual and other maturational tasks expected by these changes. Carbohydrate starvation acted to halt the onset of adolescence and provided a means of returning to the safe and familiar state of childhood.

The personalities of patients who develop anorexia nervosa have also been the subject of considerable research. Based mainly upon the analysis of detailed clinical information, the results have at best been conflicting and must be interpreted in the light of their serious methodological limitations (Smart et al, 1976). Some authors have failed to find a uniform personality structure while others have emphasised traits such as obsessionality, conformity and goodness (Bruch, 1962; Crisp, 1976). Even those studies that have used objective personality assessments require careful interpretation because of methodological problems such as the use of nonvalidated scales and single assessments carried out at the time of illness. Overall, the results have continued to provide inconclusive evidence, although individual studies have reported personality profiles suggesting high neuroticism and introversion (Smart et al, 1976).

It was the work of Minuchin et al (1975) that first drew attention to the possibility that factors existing outside the individual may influence the onset of the disorder. In his development of an open systems family model, Minuchin outlined three conditions he believed to be necessary for the development and maintenance of severe psychosomatic disorders (including anorexia nervosa) in children. In addition to a child's physiological vulnerability, he described a type of family organisation that "encourages somatization". In particular, four characteristics ie. enmeshment, overprotectiveness, rigidity and a lack of conflict resolution were said to characterise the way in which families of anorectics operated. The final condition proposed in this family model was the tendency by the family to use the ill child to avoid family conflict.

In a study of fifty-six families of anorectic patients, Kalucy et al (1977) identified another set of factors, which he called "weight pathology". "Weight pathology" described "deviations in weight, shape, eating behaviour and activity" which he reported to be present in 40% of anorectic families. In later research, designed to test Kalucy's hypothesis, Hall et al (1986) was unable to demonstrate any significant differences in either weight history or attitudes towards weight-related matters between fifty-eight mothers of anorectic patients and a matched control group of 204 mothers of New Zealand schoolgirls. Garfinkel et al (1983) also failed to find differences between the parents of patient and control groups with respect to weight, Eating Attitudes Test scores (EAT) (Garner and Garfinkel, 1979) and on measures of body satisfaction or idealization of thinness.
While many of these earlier hypotheses, developed from case studies and heavily influenced by psycho-analytical and more recently, psychological theories, have their attractions from an intuitive viewpoint, none have been satisfactorily tested and no firm evidence exists to support them.

In the cognitive-behavioural model of anorexia nervosa, dysfunctional thoughts and attitudes are seen as central to the maintenance of the disorder. Most of these attitudes revolve around a core belief that thinness is not only highly desirable but comes to represent the sole measure of success. Weight loss, often positively remarked upon by others in the early stages of dieting, is said to become a positive self-reinforcer in itself as patients experience the pleasure of successful weight loss and develop a sense of control over their lives through the discipline dieting represents (Garner and Bemis, 1982). As anxiety about eating increases, a phobia towards food and weight gain develops, which is further reinforced in a negative way by the avoidance of feared situations (Garner and Bemis, 1982). The importance of cognitive factors in the maintenance of these avoidant behaviours has been emphasised by Bandura (1978), who stated that "behaviour is so powerfully controlled by bizarre internal contingencies that neither the beliefs nor the accompanying actions are much affected even by extremely punishing environmental consequences".

Evidence for a genetic contribution in the aetiology of anorexia nervosa comes from family and twin studies. Family studies have consistently demonstrated an increased prevalence rate of anorexia nervosa amongst first degree relatives of anorectic patients. Theander (1970) reported a 6.6% sibling risk for the disorder in his study of ninety-four anorectic probands while Crisp et al (1980) found that 7% of siblings had either had or were still suffering from anorexia nervosa. Fourteen percent of the mothers and 9% of the fathers were also reported to have histories consistent with the disorder, although some of these diagnoses were described as "marginal". Holland et al (1988), reporting only on female first-degree relatives found a rate of nearly 5%. Other studies have reported an increased risk of both eating and affective disorders amongst first degree relatives (Hudson et al, 1983a; Gershon et al, 1984).

The finding that a disorder occurs with increased prevalence within families of probands compared to the general population is consistent with either a genetic or environmental mode of transmission. Twin studies, however, provide confirmation of a genetic contribution to aetiology. Some of the first twin studies published were criticised on the basis of inadequate diagnostic clarification and inaccurate determination of zygosity. More recently Holland et al (1984) reported that of thirty female twin pairs, 55% of the monozygotic twins were concordant for anorexia nervosa compared to only 7% of the dizygotic pairs, although the question of selection bias with this particular group of patients was raised. A follow-up study, designed to minimise this problem was later
completed and reported a 56% concordance rate for anorexia nervosa in twenty-five monozygotic female twins compared to 5% of dizygotic pairs (Holland et al, 1988).

Interest in the possibility that a neuroendocrine abnormality may contribute to the aetiology of anorexia nervosa arose from the observation of amenorrhoea as a central feature and consistent findings that abnormalities of hypothalamic-pituitary and neurotransmitter function exist in patients suffering from the disorder (Garfinkel et al, 1975; Gerner and Gwirtsman, 1981; Halmi et al, 1987; Gwirtsman et al, 1989a; Kaye et al, 1990b). While earlier theories such as anterior pituitary insufficiency have now been largely discarded, debate continues over the significance of other neuroendocrine abnormalities, in particular, whether they represent abnormalities intrinsic to the disorder, or are best explained as consequences of the illness (Halmi et al, 1987; Gwirtsman et al, 1989a). To date almost all research in this area has been carried out on acutely ill patients, where the picture is complicated by the epiphenomena of starvation and weight loss, with or without purging effects, or on recently "recovered" anorectics who have just undergone refeeding. Research results must therefore be interpreted in this light.

Most endocrine abnormalities seen in anorexia nervosa are now thought to reflect starvation as indices frequently return to normal upon weight restoration (Halmi et al, 1987). Abnormalities include hypercortisolaemia, reduced gonadotrophin levels (luteinising hormone and follicle stimulating hormone), low serum tri-iodothyronine and delayed thyroid stimulating hormone responses to thyroid releasing hormone (TRH), elevated basal growth hormone (GH) levels, as well as incomplete suppression of adrenocorticotropic hormone (ACTH) and cortisol by dexamethasone (Garfinkel et al, 1975; Gerner and Gwirtsman, 1981; Halmi et al, 1987; Gwirtsman et al, 1989a).

Studies on neurotransmitter systems raise the possibility that abnormalities specific to anorexia nervosa may underlie the disorder as some changes persist with recovery. Evidence that the noradrenergic system may be involved comes partly from studies looking either directly at cerebral spinal fluid (CSF) concentrations of noradrenaline and its metabolite 3-methoxy-4-hydroxyphenylglycol or by studying peripheral 3-methoxy-4-hydroxyphenylglycol concentrations in the plasma or urine. In the acutely ill emaciated anorectic, consistently lower concentrations of plasma and urine 3-methoxy-4-hydroxyphenylglycol and CSF noradrenaline and 3-methoxy-4-hydroxyphenylglycol have been found compared to healthy controls suggesting reduced noradrenaline turnover (Halmi et al, 1978; Gerner and Gwirtsman, 1981; Halmi et al, 1987). While these findings may be secondary to starvation, as plasma noradrenaline and urinary 3-methoxy-4-hydroxyphenylglycol increase to near normal upon weight recovery (Halmi et al, 1978), one study found 50% lower CSF noradrenaline concentrations in their long-term weight recovered (twenty +/- seven months) anorectics (Kaye et al, 1984b). This raises the possibility that a "pervasive defect in noradrenaline metabolism may be a trait marker of
anorexia nervosa" (Fava et al, 1989). Other studies have examined noradrenergic function using neuroendocrine challenges or looked at lymphocyte β-adrenergic receptor binding. Again, while many of the abnormalities noted during the acutely ill phase normalise upon weight restoration, some do not. The clonidine-induced GH response, as a measure of postsynaptic sensitivity, was found to be normal in low-weight anorectics, but blunted following weight restoration (Kaye et al, 1985).

Abnormalities of other neurotransmitter systems have also been postulated. Findings on dopamine metabolism have been, at best, inconsistent (Fava et al, 1989) while some evidence for alterations in CSF opioid activity in underweight anorectics has been demonstrated compared to controls (Kaye et al, 1982). However, it is the serotonergic system that has been most implicated in the pathogenesis of the eating disorders and will be discussed in full later.

The influence of socio-cultural factors in the aetiology of anorexia nervosa was suggested by several lines of research. From very early studies an over-representation in upper social classes has been noted (Kendell et al, 1973; Crisp et al, 1976; Szmukler et al, 1986) and to some extent the disorder appears to be confined to areas where a "Western" style culture predominates. It has been suggested that the relatively recent shift in the idealized female shape from a curved figure to a thin, lean look has placed increasing pressure upon Western women to conform and may have contributed to the rising incidence of anorexia nervosa (Kendell et al, 1973; Garner et al, 1980; Willi and Grossmann, 1983; Szmukler et al, 1986). In support of this theory, Garner et al (1980) point to data from magazine centerfold and Miss American Pageant contestants which demonstrate a significant trend towards a thinner ideal. The average weight of the Miss America Pageant contestant has fallen over twenty pounds in the past thirty years. They further point out that these changes have occurred against a trend towards increasing standard body weight and size for North American women. The impact of this pressure to conform is said to be reflected in the high levels of reported body weight and shape dissatisfaction, and the fact that dieting behaviour is endemic amongst young females in our culture today (Johnson-Sabine et al, 1988; Whitaker et al, 1989).

To examine further the question that socio-cultural factors may influence the development of anorexia nervosa, Garner and Garfinkel (1980) examined a population of 183 professional dance students and fifty-six fashion models who, it could be argued by their career choice, would face intense pressure to maintain body weight and shape close to the currently accepted ideal. Seven percent of the modelling and 6.5% of the dance group met strict criteria for anorexia nervosa with all but one case in the dance group developing the disorder while studying dance. Furthermore, within the dance group, the risk for anorexia was higher amongst those students in programmes in which there was greater pressure to succeed as professionals. The authors argue that "these data suggest that both
pressures to be slim and achievement expectations are risk factors in the development of anorexia nervosa", although it would be difficult to separate out these two factors as for these students, achievement would depend upon slimness.

1.1.2.4 Treatment and Outcome

It was Sir William Gull (Gull, 1874) who provided the classic description of anorexia nervosa, highlighting the patient's denial of her illness and reluctance to cooperate in treatment as specific challenges. Since then, although many different treatments have been advocated, it remains true that no specific treatment for the disorder exists (Hsu, 1986). Most clinicians would agree, however, that improving patient functioning in certain key areas is central to a successful outcome (Hsu, 1986; Hall, 1987). Hall (1987) has emphasised the need "to facilitate the patient's recovery to normal physical functioning, including the loss of symptoms of starvation, maintenance of normal weight and endocrine functioning, loss of pathological attitudes to food and body fat and, at the same time, to facilitate the patient's emotional growth".

Few studies have rigorously compared the different treatments currently in use (Russell et al., 1987), despite claims by some authors for the superiority of their particular approach (Rosman et al., 1977). In particular, little comparison of the different psychotherapeutic approaches that dominate treatment has been done. More recently by employing a randomised, controlled trial design to compare family therapy with individual supportive therapy, Russell et al. (1987) have demonstrated some evidence that family therapy may be more effective for those patients whose illness has begun early (before the age of nineteen years) and has not yet become chronic. There was "a more tentative finding" that individual supportive therapy was of greater value in the older patient.

In their study, Hall and Crisp (1987) studied thirty out-patients with severe anorexia nervosa who were randomly allocated to either treatment regimes consisting of dietary advice or combined individual and family therapy. At twelve months follow-up, both groups showed significant overall improvement, with the dietary advice group making significant weight gain. Although the weight gain in the psychotherapy group did not quite reach significance, improvements in sexual and social functioning were also noted.

The use of drugs in the treatment of anorexia nervosa has remained limited and is usually seen as most effective when it forms part of a comprehensive management approach (Kennedy and Goldbloom, 1991). These authors state that the rationale for prescribing usually falls into three categories: a) drugs to promote food intake and weight gain, b) drugs to treat associated psychiatric disturbance and c) those that are used to treat any medical complications resulting from starvation. Controlled trials to date have shown little benefit in terms of weight gain following the use of cyproheptadine (Goldberg et al., 1979), amitriptyline (Halmi et al., 1986), clonidine (Casper et al., 1987), or the selective
dopamine antagonists pimozide (Vandereycken and Pierloot, 1982) or sulpiride (Vandereycken, 1984). Similarly, the use of chlorpromazine, a drug popular in the 1960s, is not backed by controlled trials in support of its efficacy (Vandereycken, 1984).

As depression is a common symptom in anorexia nervosa, the use of antidepressant drugs has been advocated for the promotion of both improved mood and food intake. While several uncontrolled case studies, such as that by White and Schnaultz (1977), using tricyclic antidepressants, have reported improvements in mood paralleling weight gain, only a few controlled trials have been reported. Both clomipramine (Lacey and Crisp, 1980) and amitriptyline (Biederman et al, 1985) were found ineffective for either weight gain or behavioural changes.

Overall, the treatment for anorexia nervosa remains eclectic in its approach. While the lack of a specific treatment may appear a disadvantage, many clinicians feel that the diversity of needs demonstrated by their patients and the patients' families seeking help, is best served by a flexible management plan (Hsu, 1986; Hall, 1987).

Most outcome studies into anorexia nervosa have shared multiple methodological problems. These include short periods of follow-up in highly selected patient populations, a failure to use standardised criteria for both diagnosis of the disorder and in evaluating outcome, along with high failure-to-trace rates. The differences in approach have resulted in findings that vary considerably, making it difficult to make comparisons between studies and to draw firm conclusions (Herzog et al, 1988). Two recent publications (Morgan et al, 1983; Hall et al, 1984), however, used essentially the same methodology as earlier research by Morgan and Russell (1975) and Hsu et al (1979) allowing comparisons to be made and identifying similar trends (Hsu, 1988). All four studies followed their patients for at least four years and used Morgan and Russell's outcome criteria, based upon body weight and restoration of menstruation. At follow-up, between 50% and 60% of patients were normal in weight and 47%-58% were menstruating regularly, findings in keeping with those of Theander (1970). The mortality rates varied between 1% (Morgan et al, 1983; Hall et al, 1984) and 5% (Morgan and Russell, 1975). Varying degrees of food and weight preoccupation remained common even in those given a "good" or "intermediate" outcome (Hall et al, 1984).

Several factors have been identified as poor prognostic indicators for recovery from anorexia nervosa: longer duration of illness, lower minimum weight, pre-morbid personality and social difficulties, disturbed relationships within the family and failed previous treatment (Morgan and Russell, 1975; Hsu et al, 1979; Morgan et al, 1983). Hall et al (1984) failed to demonstrate similar results, identifying only marriage at presentation as the one prognostic factor of a worse outcome. The latter authors warned in assessing the relevance of this finding that compared to the other three studies their married group was...
older (27.9 years vs 18.3 years) and had a significantly longer duration of time at weight below 15% of average body weight (70 months vs 21.3 months).

Mental state examination has also been utilised at follow-up to assess outcome. Although studies report high levels of symptomatology, especially affective symptoms, few studies have used standardized interviews enabling diagnostic criteria to be applied (Hsu, 1988). In a study by Morgan and Russell (1975), 45% of patients with anorexia nervosa reported the presence of affective symptoms (mainly depression) while 23% were troubled by symptoms of obsessive-compulsive disorder. The findings for Hsu et al (1979) were 38% and 22% respectively. Hall et al (1984) found that DSM-III criteria for a psychiatric disorder other than an eating disorder were fulfilled by 50% of her patients at follow-up. Dysthymic disorder proved the most common diagnosis, occurring in 34% (although exclusively in patients with continuing eating difficulties) while a further 6% were found to have major depression, 4% alcohol abuse and 2% each to have schizophrenia, bipolar disorder and personality disorder. Only 20% of the sample were free of any physical or mental abnormality at the time of interview.

A recent publication by Ratnasuriya et al (1991) looked at the twenty year outcome in a cohort of anorectic patients, reporting extended follow-up of the patients studied by Morgan and Russell (1975). The authors were interested in determining whether or not long-term findings confirmed those of the earlier study and if factors prognostically significant at five years were still predictors at twenty years. While predictors of outcome at five years remained fairly consistent, a later age of onset was also identified as being particularly predictive of a worse outcome at twenty years. The overall outcome of the cohort, however, was not as good as that expected from the intermediate term findings. In particular, the authors found a mortality rate (attributable to anorexia) of 15%, a finding in keeping with the 18% earlier reported by Theander (1985), but higher than the shorter term studies. The study by Ratnasuriya et al (1991) also demonstrated a high degree of ongoing morbidity, with one third of those alive at follow-up still leading a "socially isolated and restricted life" and reporting a high level of dependence on their families of origin. Concerns over sexuality, marriage and child-bearing were also present in 50% of patients.

Taken together, these studies point to the very serious nature of anorexia nervosa, especially if it takes a chronic course. While the findings of two studies (Theander, 1985; Ratnasuriya et al, 1991) suggest that full recovery is still possible after fifteen years or more of illness, it also appears that the hope of recovery gradually decreases with time, dropping off steeply after twelve to fifteen years.
1.1.3 BULIMIA NERVOSA

1.1.3.1 Epidemiology

Since bulimia nervosa (Russell, 1979) and bulimia (American Psychiatric Association, 1980), were first defined in the literature, numerous epidemiological surveys have been carried out in an attempt to estimate their true prevalence. To date, most of these studies have used only simple self-report questionnaire measures, often on selected populations, thus raising important methodological problems in the interpretation of the data, but some preliminary impressions have been gained.

Two American studies yielded high prevalence rates for bulimic behaviour with up to 60% of female college students reporting binge-eating, while 5% and 13% met DSM-III criteria for bulimia nervosa (Halmi et al, 1981; Hart and Ollendick, 1985). Other American authors have reported a three-fold increase in the prevalence of DSM-III bulimia nervosa in recent years (Pyle et al, 1986), a finding in keeping with those of Hall and Hay (1991), who report a six-fold increase in the annual referral rate amongst New Zealand women for the period 1977-1986 (from 6 to 44 per 100,000 females aged 15-29 years).

In contrast, two British studies, also using self-report questionnaires in a female population (attending a family planning clinic), revealed less alarming results in terms of binge-eating. Cooper and Fairburn (1983) found a prevalence of binge-eating (defined as excessive and uncontrollable eating) of 20.9% with 2.9% currently using induced vomiting as a means of weight control. Applying conservative criteria however, based upon Russell's definition, only 1.9% appeared to fulfil diagnostic criteria for bulimia nervosa per se. This study was replicated in 1986 with similar results ie. 27.1% women reporting binge-eating and 1.8% meeting criteria for bulimia nervosa (Cooper et al, 1987). In a New Zealand population based survey utilising a self-report questionnaire followed up by interview, Bushnell et al (1990) found a life time prevalence for females of 1.9% with 1.0% of women between the ages of eighteen and forty-four years currently meeting DSM-III criteria for bulimia, findings in keeping with those rates seen in the British studies.

At present it is unclear why the epidemiological research into bulimia nervosa continues to produce such different prevalence and incidence rates between the U.S.A. and Britain. One explanation is that it reflects genuine differences in disturbed eating behaviour in the two countries. Other authors suggest diagnostic variability, differing methods of assessment and the use of selected populations (such as college students) hold the key (Cooper et al, 1987). In the area of bulimia nervosa, more population based surveys using direct interviews are required.
1.1.3.2 Clinical Features

Central to the disorder of bulimia nervosa are recurrent episodes of binge-eating, defined as the rapid consumption of large amounts of food within a discrete period of time, with an associated feeling of loss of control over eating during the episodes (American Psychiatric Association, 1987). Weight gain is avoided either by the use of self-induced vomiting, laxative or diuretic abuse, strict dieting or fasting between binges or by the use of vigorous exercise. The frequency of binge-eating and vomiting varies, but can occur ten times or more each day (Fairburn, 1983). A persistent overconcern with body shape and weight is also present (American Psychiatric Association, 1987).

Patients with bulimia are usually of normal weight although a subgroup tend to be slightly overweight (Russell, 1979). The syndrome is seen predominantly in females with only 10-15% of cases affecting males (Carlat and Camargo, 1991). The age of onset is usually in late adolescence or early adulthood. Unlike anorexia nervosa, persistent amenorrhea is not present although menstrual irregularities have been reported in up to 50% of cases (Fairburn, 1983) and patients are more likely to be sexually active. Depressive features including low self esteem and feelings of worthlessness are common, as are symptoms of anxiety which can be severe and disabling (Cooper and Fairburn, 1986). In some patients, impulsive behaviours such as stealing, alcohol or drug abuse and sexual promiscuity are also seen (Fairburn, 1983).

Physical complications may result from bulimia, usually from self-induced vomiting or purging behaviours and may become life-threatening. These include electrolyte imbalances which may induce cardiac arrhythmias, urinary infections and renal failure, epileptic seizures, tetany, swollen salivary glands, dental decay and widely fluctuating weight levels (Russell, 1979).

1.1.3.3 Aetiological Theories

In common with anorexia nervosa, the aetiology of bulimia nervosa remains unknown although again a complex interaction of biological, psychological and social factors has been proposed. Methodological limitations of studies on bulimic patients include the confounding effects of ongoing bingeing and purging, possible underlying malnutrition effects despite patients remaining within the normal weight range, the presence of affective illness or other co-morbidity such as substance abuse and an almost complete lack of studies on long-term recovered patients.

Perhaps the most important predisposing factor so far identified is a past history of anorexia nervosa (Fairburn, 1983). While more than half of Russell's (1979) original group of bulimics had past histories of unequivocal anorexia nervosa (a rate now thought to reflect a unique referral population), a community study has suggested the true incidence
to be much less than 50%, including highly doubtful cases (Pyle et al, 1981). Indeed, Mitchell et al (1985) found that only 4.3% of his 275 bulimic patients had been treated in the past for anorexia nervosa or low body weight. Nevertheless, these rates are much higher than those seen in the general population.

The importance of the patient's beliefs and attitudes to weight and shape are emphasised in the cognitive-behavioural model of bulimia nervosa. It is these attitudes that are seen as central to the maintenance of the disorder (Fairburn et al, 1986) and must therefore be altered before lasting recovery can occur. Typical belief systems include a tendency to evaluate self-worth and success only in terms of slimness and personal attractiveness, a vehement dislike of fatness and a positive attitude to the maintenance of self-control (Fairburn et al, 1986). While Fairburn points out that such attitudes are widely held in Western societies, it is the rigidity and extremity of the bulimic patient's beliefs (which are also seen as having great personal significance) that lead to dysfunctional styles of reasoning. Such thinking styles include "dichotomous thinking, overgeneralisation and errors of attribution" and through their emphasis on the extreme importance of weight and shape are thought to explain the maintenance of much of the patient's behaviour (Fairburn et al, 1986). The value of a cognitive-behavioural model can to some extent be evaluated by its treatment success. While short-term outcome appears as good as other treatment approaches, studies on long-term follow-up, allowing a better evaluation of lasting change to be made, are still not available.

Some evidence now exists from family and twin studies for a genetic vulnerability in the development of the disorder, although similar methodological limitations outlined for the anorexia nervosa studies equally apply. Nevertheless, in an uncontrolled family study by Hudson et al (1983b) the prevalence of bulimia amongst relatives of bulimic probands was found to be 3.4%, while a controlled study by Kassett et al (1989), which included collecting information by direct interview, reported an increased risk of all eating disorders amongst relatives of bulimic probands compared to relatives of normal controls. Not all research groups, however, have demonstrated this increased risk for family members (Hudson et al, 1987; Logue et al, 1989). To date, only a few twin studies have been published but these also support the possibility of a genetic predisposition, with reported concordance rates for bulimia in monozygotic and dizygotic twins ranging between 22.9%-83% and 0%-26.7% respectively (Logue et al, 1989; Fichter and Noegel, 1990; Hsu et al, 1990; Kendler et al, 1991). Fichter and Noegel's (1990) study also reported a higher concordance rate for any eating disorder between his monozygotic and dizygotic twins.

A highly significant association has been found between bulimia nervosa and the affective disorders, especially depression, although the exact nature of the relationship remains uncertain (Russell, 1979; Hatsukami et al, 1984; Walters et al, 1992). While
depressive symptoms are commonly seen in patients suffering from bulimia nervosa and many patients meet diagnostic criteria for affective disorder at some stage in their lives (Pope et al, 1983; Mitchell et al, 1986; Fichter and Noegel, 1990), several studies have also reported higher rates of affective disorders amongst first-degree relatives of bulimic probands than amongst relatives of both psychiatric and normal controls (Hudson et al, 1983a; Hudson et al, 1987; Kassett et al, 1989; Logue et al, 1989). One study by Wilson and Lindholm (1987), looking at fifty-seven bulimic patients, suggested a correlation between the severity of the eating disorder and the degree of depression. These authors reported that the more severe the bulimia, "the more likely the bulimic was to be suffering from depression to a very high degree". Other studies have reported higher than expected rates of substance abuse, both alcohol and drugs (Kassett et al, 1989) and obesity (Fairburn and Hay, In press) amongst relatives of bulimic probands and have suggested that these may also represent risk factors in the development of the disorder.

Episodes of amenorrhoea or oligomenorrhoea are common features of bulimia nervosa (Fairburn and Cooper, 1984) in conjunction with other hormonal abnormalities (Gwirtsman et al, 1983; Levy et al, 1988; Coiro et al, 1992). Consequently, interest has developed in the concept of disturbances of the hypothalamic-pituitary-adrenal axis underlying bulimia nervosa or playing a role in its maintenance (Morley and Blundell, 1988; Leibowitz, 1990; Kaye and Weltzin, 1991a). A consistent finding is a failure to suppress cortisol following a dexamethasone suppression test in normal weight bulimic women (Gwirtsman et al, 1983; Mitchell and Bantle, 1983). Failure of suppression may be a feature of concurrent depression, malnutrition and/or low weight (Gwirtsman et al, 1983), a failure of bulimic patients to absorb or metabolise dexamethasone (Walsh et al, 1987a) or intrinsic neurobiological abnormalities distinct from the above epiphenomena.

Other abnormalities of the hypothalamic-pituitary-adrenal axis reported in normal weight bulimic women (although acutely ill) include delayed (Levy et al, 1988) or blunted thyroid stimulating hormone responses to TRH (Gwirtsman et al, 1983) and abnormal GH and cortisol responses to an intravenous glucose tolerance test (Coiro et al, 1992). Findings on hormone levels have been inconsistent, with twenty-four hour cortisol secretion reported as either normal (Walsh et al, 1987b; Weltzin et al, 1991) or elevated (Mortola et al, 1989); basal GH levels either normal (Kaye et al, 1989) or elevated (Kiriike et al, 1987) while twenty-four hour secretion of GH was found to be normal by Weltzin et al, (1991). Similar inconsistencies have been seen with prolactin (PRL) and ACTH (Mitchell and Bantle, 1983; Levy et al, 1988; Kaye et al, 1989; Mortola et al, 1989; Weltzin et al, 1991). In a more recent paper by Gwirtsman et al (1989a) some attempt to look at pituitary-adrenal function following abstinence of bingeing and vomiting was made by examining peripheral and central cortisol and ACTH levels. While indices of pituitary-adrenal function were found to be normal in acutely ill bulimics who were still bingeing and vomiting, CSF ACTH levels were significantly reduced following one month of
abstinence. As the declines in CSF ACTH levels were found to be negatively correlated to individual patient's energy intake during abstinence, the authors raised the possibility that their results reflected the relative caloric restriction observed during abstinence in all their patients, compared to the same patients' intake while actively ill (and still bingeing and purging).

Studies on neurotransmitter systems have consistently found abnormalities present in actively ill bulimics. Noradrenergic alterations have included reduced plasma and CSF noradrenaline levels (Kaye et al, 1990a; Kaye et al, 1990), while both lymphocyte and mononuclear leukocyte β-adrenoceptor activity have also been reported to be increased, but this finding has not been consistent (Lonati-Galligani and Pirke, 1986; Buckholtz et al, 1988). Interestingly, in the study by Buckholtz et al (1988) evidence for lymphocyte β-adrenergic hypersensitivity was also seen during the "controlled" phase of their patients' illnesses, defined as abstinence from bingeing and purging for three weeks, maintenance of a stable diet and weight stabilised within 15% of that expected. Other researchers have found increased platelet α2-adrenoceptor activity (Heufelder et al, 1985) but this finding is not consistent with that of Kaplan et al (1989) who reported no differences in either MHPG levels or GH responses to the α2-agonist clonidine (when used as a neuroendocrine challenge), in both bulimics and controls.

Few investigations have been reported on other neurotransmitter systems in bulimia, with the exception of the serotonergic system which will be discussed in detail later. CSF concentration of homovanillic acid, the major metabolite of dopamine has been reported as normal (Kaye et al, 1990a) or reduced (Jimerson et al, 1992) in acutely ill patients.

Some evidence exists for peptide neuromodulator disturbance in bulimia nervosa. The peptide cholecystokinin and neuropeptides such as neuropeptide Y have been shown to be important in mediating satiety and in stimulating carbohydrate feeding respectively (Gibbs et al, 1973; Smith and Gibbs, 1985; Stanley et al, 1986). Peripheral cholecystokinin is synthesised in the intestinal mucosa and is thought unlikely to cross the blood-brain-barrier so that its satiety promoting effects are mediated by vagal afferent fibres. Abundant cholecystokinin is also found in the brain and some evidence exists of a role for central nervous system cholecystokin in the promotion of satiety (Baile and Della-Fera, 1985). Geracioti and Liddle (1988) found an impaired cholecystokinin response to food intake, along with impaired post-prandial satiety, in a group of fourteen bulimic women. In this study, not only were plasma cholecystokinin responses to a meal found to be reduced in bulimic patients when compared to controls, but a statistical correlation was found between the cholecystokinin levels and measures of satiety. The plasma cholecystokinin concentration in the bulimic patients, however, may also have been influenced by abnormalities of gastrointestinal physiology, such as delayed gastric emptying which has been recognised in bulimic patients (Robinson et al, 1988). Elevation of the CSF
concentration of another neuropeptide, peptide YY, has been reported in normal-weight bulimics following one month's abstinence from bingeing and purging, compared to themselves when acutely ill (Kaye et al., 1990b). The authors suggested that increased peptide YY activity may contribute to the drive to over-eat in these patients.

In keeping with the studies on anorexia nervosa, no consistent personality type predisposes to the development of bulimia nervosa. Patients suffering from bulimia nervosa share the anorectic's discontent with their body shape and weight, tend to have low self esteem and a significant subgroup are reported as having impulse-control difficulties (Fairburn, 1983). A high rate of childhood sexual experiences has been reported by women with eating disorders (Palmer et al., 1990). Methodological problems existed with the early studies on sexual abuse and later, better designed research has not always confirmed the initial findings, raising the question of whether sexual abuse is indeed a specific risk factor (Fairburn and Hay, in press).

Socio-cultural factors thought to be important for the large increase in cases of anorexia nervosa probably apply equally to bulimia nervosa and have been previously outlined.

1.1.3.4 Treatment and Outcome

Evaluation of the different treatment approaches to bulimia nervosa has been made difficult by an almost complete lack of controlled trials. In a recent review of the controlled studies looking at psychological treatments, Fairburn (1988) concluded that for most patients, an outpatient approach utilising some form of cognitive behavioural therapy provided the best results. He also stated that many questions are still to be answered; in particular, the role of drug treatment remains uncertain, as does that of some of the other psychotherapies, such as family therapy.

Some attempts to determine the important components of psychotherapy that lead to a successful outcome have been made. In a study designed to evaluate the different components of cognitive behavioural therapy, Freeman et al. (1988) found a purely behavioural approach to be as effective as one incorporating cognitive restructuring. Fairburn and colleagues however, conducted a similar comparison and found the behavioural approach inferior on most outcome measures including drop out from treatment, with drop out rates of 32% and 12% respectively for the behavioural and cognitive behavioural groups (Fairburn, 1988). Final evaluation of these contradictory results is still awaited as follow-up data are not yet available, but central to the discussion is the cognitive view that lasting recovery from bulimia nervosa depends not only on behavioural change but also upon changes in attitudes to body weight and shape (Fairburn, 1988).
Preliminary evidence exists to suggest that psychotherapeutic treatments not incorporating a cognitive behavioural approach may be equally effective for bulimia nervosa. Kirkley et al (1985) compared group cognitive behavioural treatment with a form of non-directive group therapy and while at the end of the treatment phase the cognitive behavioural group were doing better, at three month follow-up, there were no longer differences between the groups. Seventy seven percent of the cognitive behavioural group and 78% of the non-directive group had achieved a 60% reduction in their vomiting frequency. Fairburn (1988) and colleagues have presented some preliminary results on a study looking at a three way comparison of behavioural therapy, cognitive behavioural therapy and modified interpersonal psychotherapy. This latter treatment "does not address the specific psychopathology of these patients: instead it concentrates exclusively on their mood and their relationships". To date, while the purely behavioural group has fared less well, the other two treatment groups have made equal progress, with decreases in both eating behaviour and in overall levels of general psychopathology.

One controlled trial has compared family therapy with non-specific individual supportive psychotherapy. Russell et al (1987) randomly allocated twenty-three patients who met his criteria for bulimia nervosa (Russell, 1979) to either of the above groups for one year's treatment. The outcome was poor for both groups with the majority of patients showing little improvement and no difference was seen between treatment approaches but the results must be interpreted in the knowledge that the patients were largely composed of treatment resistant cases at a tertiary referral centre. Nor was treatment designed specifically for bulimia nervosa, rather the study was part of a major family therapy treatment trial for anorexia nervosa.

Pharmacological treatment has been advocated because of its potential simplicity and cost effectiveness. Numerous controlled trials have now demonstrated antidepressant treatment to be superior to placebo in reducing abnormal eating behaviour, including binge-eating and vomiting, as well as improving the depressed mood that commonly accompanies bulimia nervosa. Amitriptyline (Mitchell and Groat, 1984), imipramine (Pope et al, 1983) and fluoxetine (Fluoxetine Bulimia Nervosa Collaborative Study Group, 1992) have all been found significantly effective in this way. Interestingly, the effects on eating do not appear to depend upon mood change per se. In other words, an anti-bulimic effect has been proposed although the best antidepressant effects appear to be seen in those patients who also meet criteria for major depression at the start of treatment (Mitchell and Groat, 1984). The value of antidepressant treatment will not be determined until adequate follow-up studies are carried out. Only one such trial has done this and results suggested that often complicated drug regimes were required and discontinuation of medication often led to a return of symptoms (Fairburn, 1988).
There is a need to directly compare drug treatment with psychotherapeutic approaches. Mitchell et al (1990a) recently reported a controlled trial comparing imipramine treatment, placebo treatment, imipramine treatment plus intensive group psychotherapy and the same group psychotherapy plus placebo. All three active treatments resulted in significant improvements in eating behaviour and mood when compared to placebo. The results also suggested that the improvements seen with intensive group psychotherapy were superior to drug treatment and the addition of drug treatment to group psychotherapy did not add to those gains made by therapy alone when looking at eating behaviour. Symptoms of anxiety and depression were further improved by the combined treatment approach.

It is clear from the published research that no single treatment for bulimia nervosa works for all patients. As is the case with anorexia nervosa, most clinicians adopt a flexible approach but usually incorporate some form of psychotherapy with or without antidepressant treatment. Subgroups of patients, such as those with histories of major weight fluctuations (either overweight or underweight), personality difficulties or multiple addictive behaviours will continue to have special needs (Fairburn, 1988). The results of long-term follow-up studies are needed to enable optimisation of treatment in terms of the maintenance of initial symptom improvement.

When Russell first drew attention to a subgroup of eating disordered patients with bulimic and purging behaviour in the presence of normal weight, he proposed that bulimia nervosa may represent an "ominous variant of anorexia nervosa" (Russell, 1979). In the absence of longer term follow-up data, he proposed that the prognosis was likely to be similar to the poor outcome seen in anorectics who were also habitual bingers and purgers. Theander (1970) and Hsu et al (1979) had also reported an association between severe self-induced vomiting and a more serious outcome in anorexia nervosa. Russell (1979) further based his pessimism upon the increased resistance to treatment, the more frequent and serious nature of physical complications and the "considerable" risk of suicide he had observed in his original group of patients.

Although long-term outcome studies are still awaited on bulimia nervosa, more recent literature has suggested that the prognosis for this disorder, at least in the intermediate term, is somewhat better than Russell first suggested. A dozen or so studies have been published with follow-up periods of one to five years, including those of Fairburn et al (1986), Swift et al (1987), Hsu and Sobkiewicz (1989), Herzog et al (1991) and Lacey (1983). While most have methodological short comings such as retrospective data collection, selected patient populations and differing diagnostic criteria and outcome measures, overall they lend support to the view that bulimia nervosa can follow a chronic, fluctuating course and that outcome has a somewhat heterogeneous pattern.

As might be expected, rates of recovery appear to vary depending upon patient selection criteria. The best rates have been reported for outpatient groups who have
completed treatment programs (Lacey, 1983; Fairburn et al, 1986) and the least promising results are seen with inpatient populations (Swift et al, 1987). Recovery rates of 30% to 70% and 13-40% (for at least twelve months follow-up), for outpatient and inpatient groups respectively have been reported, with significant improvements in symptoms such as vomiting and bingeing, along with enhanced social functioning, improved depressive features and menstruation (Swift et al, 1987).

Hsu and Sobkiewicz (1989) reported on a consecutive series of forty-five bulimic patients who had been followed for four to six years after termination of treatment. Of the thirty-five patients successfully followed, 16% still met criteria for bulimia nervosa while a further 16% had occasionally binged or purged in the previous six months and were therefore diagnosed as subclinical cases. These authors also compared patients' current status to that found at an earlier assessment taken at one to three years post treatment. Most patients who had received "good" outcomes at the earlier assessment maintained their improvement with sixteen of the twenty-three "good outcome" subjects having no bulimic features. In contrast, of the seventeen patients with bulimic features at the first follow-up, only five had recovered by the second assessment.

A recent study looking at mortality rates in bulimia nervosa has suggested lower figures than those originally thought probable by Russell (1979) in his initial report. Patton (1988) looked at a series of 460 consecutive patients with eating disorders seen between 1971 and 1981 and found a crude mortality rate of 3.1% for those with bulimia nervosa, although it should be noted that the minimum follow-up period was only four years.

1.1.4. DIETING BEHAVIOUR IN THE NORMAL POPULATION

1.1.4.1 Epidemiology

Most surveys looking at dieting behaviour amongst the normal population have studied those groups at high risk from developing the eating disorders, such as adolescent and school-age populations. Consequently, few data define the rates of dieting in the population at large. While caution must be applied in generalising findings from adolescent groups to the entire community, such studies are of particular interest as they reflect eating behaviours and attitudes occurring at the age of onset for the development of anorexia and bulimia nervosa.

The experience of feeling "too fat" is reported by most girls at some stage during their adolescence (Nylander, 1971; Leichner et al, 1986), an experience that increases with age (Nylander, 1971) and is more prevalent amongst those of higher body weight (Dwyer et al, 1967). Similarly, concerns about overweight and overeating are reported more often amongst girls than boys (Whitaker et al, 1989) with between 63-70% of high school girls reporting dissatisfaction with and wanting to reduce their weight (Huenemann et al, 1966). In contrast, most adolescent males report a wish to gain weight.
Although many methods of weight control are common amongst young women (Whitaker et al, 1989), dieting is said to be the most common behavioural response to the experience of feeling fat (Huenemann et al, 1966). It is not surprising, therefore, that dieting behaviour is also highly prevalent amongst adolescents. In an unselected English state-schoolgirl population, Johnson-Sabine et al (1988) found that 20% of fourteen to sixteen year olds were currently dieting and a follow-up study twelve months later (Patton et al, 1990) continued to demonstrate a dieting point prevalence rate of 21.3%. The prevalence of dieting amongst girls appears to increase with age, at least until mid-adolescence, and to be most common amongst those who are heavier (Dwyer et al, 1967; Nylander, 1971; Whitaker et al, 1989). Whitaker et al, (1989) surveyed 91% of an American county high school population and found the rate of dieting amongst girls increased from 13% per year for thirteen year-olds to 42% per year at aged sixteen. In contrast, dieting rates amongst boys remained steady at 8% per year between the ages of twelve and sixteen. Indeed, 50% of the entire female school population had initiated dieting by the age of 14.8 years while 50% of the males had still not dieted by the age of nineteen.

In his prospective follow-up study, Patton et al (1990) attempted to identify amongst schoolgirls those factors associated with attempts at weight control. He found that a childhood history of obesity, currently being heavier than average, having a family history of obesity or a mother perceived as thin, along with a personal history of amenorrhoea and current high levels of stress (either social, home life or financial in origin) were all factors predictive of attempts at weight control. The discrepancy between a girl’s perceived weight and her ideal weight also seems to be an important determinant of dieting, regardless of her actual body weight (Dwyer et al, 1967).

Weight concerns and dieting behaviour are not just confined to adolescence. Several recent community studies have clearly demonstrated that abnormal eating attitudes and dieting behaviours are common place in the general population, especially amongst young adults (Cooper and Fairburn, 1983; Cooper et al, 1987; Bushnell et al, 1990). A preoccupation with the desire to be thin and dissatisfaction with one’s own body weight and shape persist into adulthood, particularly amongst women (Bushnell et al, 1990). Both young adult males and females report binge-eating on a regular basis with a smaller percentage using behaviours such as self-induced vomiting, strenuous exercise, diet pills or laxatives as weight control measures (Halmi et al, 1981; Bushnell et al, 1990).

Jakobovits et al (1977) reported that 11% of the female college population he studied were on a diet, while a further 75% were consciously trying to limit their food intake. Other authors have linked dieting behaviour and the pursuit of thinness, at least in the North American population, to higher social class (Goldblatt et al, 1965) while Garner et al (1980) have reported on the more widespread sociocultural expectations placed upon
Western women to conform to the currently idealised thin female shape. Evidence is now emerging to suggest that such strong expectations are beginning to having effects across cultural boundaries, amongst women of immigrant groups living in Western societies (Dolan et al, 1990).

1.1.4.2 Psychological Effects of Dieting

Food restriction has clear implications for an organism in terms of survival and its ability to function well. Despite the importance of adequate nutrition, relatively little research has been carried out on the effects of dieting and food restriction in humans. Much of the existing literature on dieting in normal populations has developed from the eating disorders literature, and has therefore often been a series of analogue studies seeking to understand clinical eating disorders using populations of healthy volunteers. More recently, there has been a move towards examining the psychological consequences of dieting and food restriction, coupled with attempts to delineate eating behaviour patterns in healthy subjects.

Effects of Dieting on Psychological Functioning

Little work has been carried out in the area of how dieting may effect psychological functioning. The first direct evidence came from a Minnesota study (Keys et al, 1950) which identified a large number of psychological and physiological changes associated with weight loss over twenty-four weeks. The main findings were of subjective increases in lethargy, depression and irritability along with decreased ratings of energy.

More recently, it has been shown that female dieters perform less well on demanding cognitive tasks compared to non-dieters of both high and low restraint (Rogers and Green, 1993). This finding has since been replicated (Green et al, in press), demonstrating that the performance deficits encompass a range of behaviours including reaction times and memory (impaired recall).

Cognitive functioning in the eating disorders themselves have similarly been poorly studied. One group of researchers have reported impaired performance in a group of patients with bulimia nervosa compared to healthy controls (Laessle et al, 1990), with a correlation seen between metabolic signs of starvation and the size of impaired performance on tasks. General cognitive impairment has been demonstrated in restricting anorectics (Fox, 1981), while Hamsher et al (1981) have shown that deficits in attention also occur in this same subgroup.

What is not clear is whether cognitive defects are the result of food deprivation or form an intrinsic part of the eating disorder itself but they are thought to play a role in the maintenance of abnormal eating behaviours (Fairburn et al, 1986).
Evidence for an association between dieting and mood changes remains equivocal. Few studies have examined the effects of dieting on mood in healthy populations but those that have have yielded contradictory results. Keys et al (Keys et al, 1950) reported a decrease in mood following a twenty-four week semi-starvation diet and this finding was later replicated by Fichter et al (Fichter et al, 1985) studying five healthy volunteers placed on a three week starvation regime. In contrast, however, Pirke et al (Pirke et al, 1985b) found no change in mood across a six week diet and reported no change following a two week dieting period.

1.1.4.3 Dieting, Eating Disturbance and Eating Disorders

In recent years, interest has grown in the concept that dieting behaviour may act as a risk factor in the development of the eating disorders, thus moving away from the traditional clinical stance which viewed dieting simply as a symptom of the established illness. The concern that dieting behaviour plays a role in the establishment of disturbed eating and eating disorders comes from research encompassing mainstream psychology and more directly, clinical studies of eating disordered populations.

Much of our understanding of the link between dieting and disordered eating comes from the area of psychology. Wardle and Beinart (1981) and Polivy and Herman (1985) have proposed that an association, perhaps even causal, may exist between excessive dietary restraint (i.e. the conscious control of food intake in order to prevent weight gain or promote weight loss) and binge-eating. Although it is increasingly recognised that episodes of bingeing, food cravings and difficulties with stopping eating once started are behaviours that occur in unrestrained eaters and are in themselves probably not abnormal, the frequency of these activities is much greater in restrained eaters (Wardle and Beinart, 1981). Bingeing may represent a physiological response to short periods of starvation or to trying to maintain one's weight below a natural "set-point" for body weight which has been proposed by Nisbett (1972) to exist for us all.

Recent laboratory research in the area of "counter-regulation" has suggested that cognitive factors may also be important in the mechanism leading to bingeing. Counter-regulation refers to the phenomena whereby highly restrained eaters will eat more following a large energy preload compared to a no preload condition, a response in direct contrast to that seen in unrestrained eaters and to what might be expected in purely physiological terms (Hibscher and Herman, 1977). Counter-regulation has also been shown to occur in dieters, where Herman and Mack (1975) demonstrated that dieters ate more following a milkshake (preload condition) than after no milkshake (no preload condition). The importance of the belief regarding the amount of calories consumed, rather than the actual energy load ingested, was further demonstrated by Wooley (1972) and it
has been suggested that once the dieter thinks she has overeaten or exceeded her dietary allowance, she abandons all dietary restraint. A similar mechanism has been proposed to underlie binge-eating. No direct evidence exists that this cognitive process is necessary to the process of counter-regulation which occurs in dieters, or that it is indeed present (Jansen et al, 1988; Jansen, 1990).

Animal studies have provided further evidence of the link between food restriction and the subsequent development of abnormal eating. Young female rats will normally gain weight on an *ad libitum* diet of palatable food. An experiment by Coscina and Dixon (1983), however, demonstrated that a group of previously food deprived rats gained significantly more weight than controls, and that the weight gain was in direct proportion to the length of previous food deprivation. The differential weight gain was shown to be a result of difference in food consumption rather than metabolic alterations. This animal model suggests that prior deprivation influences the tendency to overeat even after weight loss has been restored.

For the substantial proportion of eating disordered patients who develop binge-eating, even where a past history of frank anorexia nervosa does not exist, it is usual for a period of food restriction or dieting to have preceded the onset of binge-eating. Additionally, the clinical picture frequently consists of alternate severe food restriction and compensatory bingeing (Fairburn, 1983). Clinical case reports suggest that bulimic anorectic patients often have marked periods of weight loss in the year preceding the onset of bulimia (Russell, 1979). Garfinkel *et al* (1980) demonstrated that in a group of sixty-eight bulimic anorectic patients, their bulimia developed a mean of 19.2 +/- 8 months after the onset of dieting. A similar pattern is also seen in normal weight bulimics and in obese subjects where Telch and Agras (1993) demonstrated that 62% of obese non-binge eaters reported an onset of binge-eating episodes in the three month period immediately after concluding a twelve week very low calorie diet.

Few data exist on dieting in normal populations. Most dramatic is the evidence from Keys *et al* (1950) who studied the effects of starvation on World War II conscientious objectors. These men were placed on a twenty-four week semi-starvation diet resulting in a weight loss equal to 26% of their body mass. Upon refeeding and a return to their normal body weight, they continued to exhibit a persistent tendency to binge, often to the limit of their physical capacity, a behaviour that had not been present premorbidly. More recently, Warren and Cooper (1988) found increased ratings of food preoccupation, increased urges to eat more frequently and feelings of lack of control over eating in fourteen people placed on a three week calorie controlled diet.

Evidence from several epidemiological studies has suggested that disordered eating patterns and the eating disorders themselves may arise out of "normal" dieting or follow periods of unintentional weight loss (Theander, 1970; Johnson-Sabine *et al*, 1988). Two
studies, following a prospective design in an "at risk" group (London schoolgirls), illustrate this first point (Johnson-Sabine et al, 1988; Patton et al, 1990). The point prevalence of dieting over the two years encompassed by the study remained steady at about 20%, with considerable movement occurring in and out of the dieting group over time. While Patton et al (1990) found that the majority of dieters either did not progress from "simple" dieting or had ceased dieting altogether within twelve months, 21% of the original dieters had in fact developed eating disorders during this interval and constituted 50% of the new cases found at second interview. In contrast, less than 1% of girls classified as non-dieters in the original survey were diagnosed as cases at follow-up. The authors concluded that "the relative risk in the total study population for a dieter of being diagnosed a case at follow-up was nearly eight times that of non-dieters".

As outlined above, evidence exists from a wide range of studies to suggest that dieting behaviour may predispose to the development of the eating disorders. What remains poorly understood is the mechanism underlying the transition from normal dieting to abnormal eating. In particular, little research has been carried out on the neurobiological consequences of food restriction which, it could be argued, mediate the behavioural changes accompanying dieting.

1.2 5-HYDROXYTRYPTAMINE

1.2.1 5-HYDROXYTRYPTAMINE BIOCHEMISTRY

1.2.1.1 Tryptophan and Transportation to the Brain

The mammalian brain utilises a number of monoamines as neurotransmitters. One of these, the indoleamine serotonin, is synthesised in the brain from the amino acid tryptophan (TRP) via the 5-hydroxyindole pathway. TRP cannot be synthesised by mammalian cells and is therefore an essential amino acid which must be obtained either from dietary sources, breakdown of tissues protein, or from lysis of neuronal proteins (Wurtman, 1978; Wurtman et al, 1981).

TRP is transported into the brain via the blood-brain-barrier. Here, as a large neutral amino acid (LNAA), it is in direct competition with other LNAAAs (ie. the aromatic amines such as tyrosine and threonine and the branch chain amino acids including isoleucine, leucine and valine) for the transportation carrier (Wurtman et al, 1981). Thus, it is the ratio of TRP to other LNAAAs that determines the amount of TRP being transported and consequently, it is argued that the entry of circulating TRP into the brain can be accelerated by either raising plasma TRP or by lowering plasma levels of other LNAAAs (Fernstrom and Wurtman, 1972). That this regulatory system is important in controlling levels of brain TRP is supported by animal studies in the rat that have shown that the major
metabolic consequence of TRP administration is to stimulate 5-hydroxytryptamine (5-HT) synthesis (Fernstrom, 1983).

In plasma, TRP in the L-isomer form binds to serum albumin and under normal physiological conditions, only ~10% remains unbound (free TRP). TRP is loosely bound, however, to its albumin carrier and is rapidly stripped off its binding site once inside the brain (Yuwiler et al., 1977). In plasma, non-esterified fatty acids will directly compete with TRP for albumin binding sites, displacing TRP, and thus increasing the availability of free TRP (Curzon et al., 1973).

Considerable debate exists in the literature as to whether it is the ratio of free or total TRP to other LNAAs that is important in determining the rate at which TRP will enter the brain. Wurtman (1978) has argued that it is the ratio of total TRP to competing LNAAs that is the determining factor, while Bloxam and Curzon (1978) suggest free TRP levels are more important. In a series of experiments in the rat, Fernstrom et al. (1976) compared brain TRP levels with plasma free TRP, plasma total TRP and the ratio of both free and total TRP to other LNAAs. They concluded from this work that the best correlations of brain function were with measures of total rather than free plasma TRP. The findings of Curzon (1988), however, are in direct conflict. His refeeding experiments in the rat have shown that the ratio of total plasma TRP to LNAAs remains constant while an overall fall in brain TRP, plasma free TRP and consequently, in the ratio of free plasma TRP to other LNAAs occurred. No alteration in the ratio of total plasma TRP to LNAAs was found, indeed, an increase in plasma total TRP was noted (Sarna et al., 1984). How the animal studies relate to changes that may occur in humans is unclear.

Methodological difficulties ensure that human studies directly measuring brain TRP levels are almost impossible to carry out, but one such study by Gillman et al. (1981) reported good correlations between serum free TRP and brain TRP levels in depressed human subjects undergoing psychosurgery. Poor correlations were seen between serum total TRP or the ratio of plasma TRP to LNAA and brain TRP. This study was complicated by the fact that its subjects were depressed. Another human study, utilising CSF TRP as an indirect index of brain TRP levels concluded that the relationship between plasma free TRP and brain TRP was not clear cut (Perez-Cruet et al., 1974), illustrating the complexity of the situation in vivo and the potential interaction between nutrient status and neurotransmitter regulation.

1.2.1.2 5-HT Synthesis and Metabolism

Monoamine neurotransmitter synthesis is controlled by the hydroxylase enzymes. In the formation of 5-HT, the essential amino acid TRP initially undergoes hydroxylation to form 5-hydroxytryptophan. The reaction is catalysed by the enzyme tryptophan hydroxylase, which is exclusively localised within 5-HT-producing neurones (Lovenberg
et al, 1968). Under normal physiological conditions, tryptophan hydroxylase is only 50% saturated with substrate and acts as the rate limiting step in the synthesis of serotonin. Early animal research established that alterations in levels of brain TRP directly affect the degree of enzyme saturation, in turn driving TRP hydroxylation and determining the rate of 5-hydroxytryptophan, and ultimately, 5-HT production (Ashcroft et al, 1965; Fernstrom and Wurtman, 1971b; Grahame-Smith, 1971). Therefore, the hypothesis is that substrate availability rather than end-product inhibition controls 5-HT synthesis in the brain, although some debate exists (Fernstrom, 1983). The final step in synthesis is conversion of 5-hydroxytryptophan to 5-HT by the enzyme aromatic L-amino acid decarboxylase (Lovenberg et al, 1962). Activity of the latter enzyme is much greater than that of tryptophan hydroxylase (Ichiyama et al, 1968) and normally, little 5-hydroxytryptophan is found within the mammalian brain (Fernstrom, 1983). Once formed, 5-HT is stored in granules at the presynaptic nerve terminals ready for release.

The main 5-HT metabolite in the brain is 5-hydroxyindoloacetic acid (5-HIAA) which is formed by a two step process. 5-HT is first oxidised to 5-hydroxyindoleacetaldehyde by monoamine oxidase (MAO) and then dehydrogenated to 5-HIAA by aldehyde dehydrogenase. A small amount of 5-hydroxyindoleacetaldehyde also appears to be reduced to 5-hydroxytryptophol by aldehyde reductase (Cheifetz and Warsh, 1980). Other minor metabolic pathways exist including a conjugation pathway to a sulphate derivative, and action by N-methyl transferase to produce methylated compounds such as bufotenin.

1.2.2 5-HT NEUROANATOMY AND CLASSIFICATION

1.2.2.1 5-HT Pathways

Serotonin is one of the most phylogenetically ancient neurotransmitters. 5-HT neurones mature early in fetal life and have widespread projections throughout the central nervous system. Serotonergic cell bodies are largely confined to the brain stem and in 1964, Dahlstrom and Fuxe (1964) described nine groups in the rat brain (designated B1-B9) corresponding largely to the raphe nuclei (figure 1.1; see over). Of these, groups B6 and B7 (the largest group of serotonergic neurones) are usually grouped together as comprising the dorsal raphe nucleus, while the B8 cell group comprises the median raphe nucleus (Molliver, 1987). A third cluster, known as B9, is located in the rostral brainstem and corresponds in part to the tegmental reticular nucleus of the pons (Sipe et al, 1978). It is these three raphe groups, ie. the dorsal raphe nucleus, median raphe nucleus and B9, that are thought to be of particular significance in that they give rise to most of the ascending serotonergic projections to the forebrain (Molliver, 1987).

Most serotonergic projections ascend to the forebrain via the medial forebrain bundle, which is situated in the lateral hypothalamus (Anden et al, 1966). They then fan out to reach the neocortex, septum, hippocampus, amygdala, cingulum, olfactory bulb and
ventrolateral cortical regions (Parent et al, 1981). These projections appear to be organised into two distinct systems with studies demonstrating that the dorsal and median raphe nuclei have different patterns of innervation within the forebrain, along with distinct morphology of their axon terminals (Kohler, 1982; Kohler and Steinbusch, 1982; O'Hearn and Molliver, 1984). Serotonergic projections from the median raphe nucleus are found preferentially in the hippocampus and septum. These axons are relatively coarse and beaded, with large spherical varicosities. Those from the dorsal raphe nucleus on the other hand, project primarily to the cortex and striatum, are very fine in structure and particularly sensitive to the neurotoxic effects of amphetamine derivatives (O'Hearn et al, 1988). Moreover, the two axon types have different pharmacological properties and are therefore also likely to be functionally distinct (Molliver, 1987).

Figure 1.1 Serotonergic pathways in the rat brain.

Recently, it was discovered that the fine 5-HT axon terminals (from the dorsal raphe nucleus) were most closely associated with 5-HT_2 receptors (Kosofsky and Molliver, 1987). This finding has further suggested that separate serotonergic projections arising from different raphe nuclei may terminate in relation to specific 5-HT receptor subtypes,
raising the possibility that separate projections may subserve different functions (Molliver, 1987).

Thus it would appear that 5-HT innervation of the cortex and limbic system does not function in a homogeneous way, but consists of distinct neuronal projections with high degrees of anatomical, morphological and pharmacological specialisation (Molliver, 1987; Cowen, 1991). Such an organisational pattern may well have major implications for our understanding of psychiatric illness. In particular, Cowen (1991) has suggested that, as evidence from animal studies supports the hypothesis that 5-HT pathways regulate many behavioural and affective states that are disturbed in psychiatric illness, the presence of distinct pathways provides us with an opportunity to investigate specific behaviours using selective pharmacological agents.

1.2.2.2 Receptor Subtypes

In 1957, it was first reported by Gaddum and Picarelli that 5-HT interacted with at least two distinct receptors in the guinea pig. Since then, our knowledge about this diverse system of neurotransmitter receptors has expanded rapidly, with the seminal delineation of 5-HT$_1$ from 5-HT$_2$ receptors by Peroutka et al (1979) and the subsequent classification of 5-HT receptors into three main families (5-HT$_1$, 5-HT$_2$ and 5-HT$_3$) based upon binding affinities and ligand studies by Bradley et al (1986). It was also shown that these three families could be separated largely by nature of their secondary messenger systems, ie. the mechanisms that mediate the intracellular responses to receptor activation (Cowen, 1991). More recent work, especially in the area of molecular biology, has served to confirm and extend the view of the distinct identity of these three groups (Humphrey et al, 1993), but has also led to the identification of an additional class of 5-HT receptors (designated 5-HT$_4$), as well as highlighting areas of dissatisfaction within the existing classification system. Through the cloning and sequencing of receptors, it has been realised that no single criterion, such as the action of specific ligands, should be used alone to classify receptors. Pertinent to this issue has been the debate surrounding the correct nomenclature of the 5-HT$_{1C}$ receptor, thought for many years to be more closely linked to the 5-HT$_2$ family. The result has been the introduction of a new classification by the Serotonin Club Receptor Nomenclature Committee (Humphrey et al, 1993) that has included the newly identified 5-HT$_4$ receptor family (Bockaert et al, 1990).

This thesis adopts the new classification system outlined in Table 1.1 (see over) alongside the traditional system.

5-HT$_1$ Receptor Family

Under the new classification, the 5-HT$_1$ family is subdivided into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ (Heuring and Peroutka, 1987; Humphrey et al, 1993), with the
5-HT<sub>1C</sub> receptor now transferred to the 5-HT<sub>2</sub> family, and renamed 5-HT<sub>2C</sub> (see Table 1.). The 5-HT<sub>1</sub> family shares nanomolar affinity for 5-HT and carboxamidotryptamine, and micromolar affinity for 5-HT antagonists such as ketanserin and mesulergine. The 5-HT<sub>1</sub> receptors also share the property of linkage to a G-protein as their secondary messenger system, the activation of which leads to the inhibition of adenylate cyclase (Peroutka, 1988).

Table 1.1 5-HT receptor family classification

<table>
<thead>
<tr>
<th>SUPERFAMILY</th>
<th>GROUP</th>
<th>SUBTYPE</th>
<th>PREVIOUS NAME</th>
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<tr>
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<tr>
<td></td>
<td></td>
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<td>5-HT&lt;sub&gt;6&lt;/sub&gt;, 5-HT&lt;sub&gt;IEβ&lt;/sub&gt;</td>
</tr>
<tr>
<td>G-Protein</td>
<td>5-HT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>&quot;classical&quot; 5-HT&lt;sub&gt;2&lt;/sub&gt;</td>
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<td></td>
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<td>5-HT&lt;sub&gt;2F&lt;/sub&gt;</td>
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<td></td>
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<td>5-HT&lt;sub&gt;1C&lt;/sub&gt;</td>
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<td>G-Protein</td>
<td>5-HT&lt;sub&gt;4&lt;/sub&gt;</td>
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From Humphrey et al (1993)

5-HT<sub>1A</sub> receptors are regarded as being the phylogenetically oldest members of this family. Recently, specific radioligands have been developed for the 5-HT<sub>1A</sub> receptor, the prototype of which is the aminotetralin, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) (Hoyer et al, 1985). 5-HT<sub>1A</sub> receptors also display high affinity for some pyrimidinylpiperazines (eg. ipsapirone and buspirone) (Traber and Glaser, 1987), and benzodioxanes (eg. spiroxatrine, MDL 72832). These receptors have a heterogeneous distribution in the brain with high concentrations found on the cell bodies of 5-HT neurones in the raphe nuclei and at post synaptic locations in the hippocampus, amygdala.
The 5-HT\textsubscript{1A} receptors on the cell bodies act as autoreceptors having an inhibitory effect upon 5-HT neuronal firing. Thus the administration of a selective 5-HT\textsubscript{1A} agonist such as 8-OH-DPAT leads to decreased raphe cell firing and a subsequent diminished release of 5-HT (Sharp \textit{et al}, 1989). Activation of post-synaptic 5-HT\textsubscript{1A} receptors on the other hand, mediates the effects of 5-HT released from the nerve terminals of which a typical response is post-synaptic cell hyperpolarisation due to entry of potassium ions (Bobker and Williams, 1990; Cowen, 1991).

5-HT\textsubscript{1B} receptors are species specific and so far have been found only in the rat, mouse, and hamster brain (Hoyer and Middlemiss, 1989). In the rodent they are present in high densities in the basal ganglia (Pazos \textit{et al}, 1988) and have high affinity for 5-HT, (-)-pindolol and relatively low affinity for 8-OH-DPAT or buspirone. The human equivalent is the 5-HT\textsubscript{1D} receptor (see Table 1.1), although this differs from the rat 5-HT\textsubscript{1B} receptor in displaying typical 5-HT\textsubscript{1D} characteristics (e.g. β-adrenergic antagonists have low affinity).

5-HT\textsubscript{1D} receptors are found in the striatum, substantia nigra, neocortex and hypothalamus (Pazos \textit{et al}, 1988), where in species lacking 5-HT\textsubscript{1B} receptors, (e.g. primates), they function as nerve terminal autoreceptors, regulating the release of 5-HT (Schlicker \textit{et al}, 1989). To date, few selective ligands for the 5-HT\textsubscript{1D} receptor exist, but recently sumatriptan (GR 43175) has been described as a selective 5-HT\textsubscript{1D} agonist (Schoeffter and Hoyer, 1989).

\textit{5-HT\textsubscript{2} Receptor Family}

The 5-HT\textsubscript{2} family consists of 5-HT\textsubscript{2A}, 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C} (previously 5-HT\textsubscript{1C}). These receptor subtypes share a common secondary messenger system linked to G-proteins which involves hydrolysis of phosphoinositides and mobilisation of intracellular calcium (Hoyer, 1988b; Hartig, 1989) and are located post-synaptically. 5-HT\textsubscript{2A} receptors are found in high concentrations in the cerebral cortex, certain regions of the amygdala, cingulate and hippocampus, while 5-HT\textsubscript{2C} receptors are concentrated in the choroid plexus, basal ganglia, cerebral cortex, hippocampus and hypothalamus (Pazos \textit{et al}, 1988).

\textit{5-HT\textsubscript{3} Receptor Family}

First identified in the peripheral nervous system, 5-HT\textsubscript{3} receptor binding sites have only recently been located in the central nervous system (Kilpatrick \textit{et al}, 1987). This family of receptors differs from that of the 5-HT\textsubscript{1} and 5-HT\textsubscript{2} classes in that they are directly coupled to ion channels without intervening G-proteins or secondary messenger systems (Peroutka, 1988). On the basis of binding studies with selective antagonists, it has been suggested that receptor subtypes exist, but further studies are required to confirm this (Peroutka, 1988). Within the central nervous system, 5-HT\textsubscript{3} receptors are found in highest concentration in the brain stem in the region of the nucleus tractus solitarius (Pratt \textit{et al},
1990) and in lesser numbers in the limbic system, including the hippocampus and amygdala, as well as regions of the entorhinal cortex (Kilpatrick et al., 1987).

5-HT$_4$ Receptor Family

Little has yet been published on the 5-HT$_4$ family of receptors. It appears that the receptors are G-protein linked (Humphrey et al., 1993) and positively coupled to adenylate cyclase (Dumuis et al., 1988).

1.2.3 THE ROLE OF 5-HT IN THE BRAIN

Evidence now exists to suggest that serotonin plays a significant role in the control of many aspects of human emotions and behaviour, including mood (Meltzer and Nash, 1988; Deakin and Graeff, 1991; Power and Cowen, 1992), appetite and food intake (Blundell, 1984; Leibowitz and Shor-Posner, 1986; Silverstone and Goodall, 1986) and aggressive and impulsive behaviour (Shaw et al., 1967; Linnoila et al., 1992). While abnormalities are seen within all these areas during episodes of clinical eating disorders, a full review is beyond the scope of this thesis. Emphasis will, therefore, be given to two areas most directly implicated in the pathogenesis of eating disorders, that is the role of serotonin in feeding behaviour and mood.

1.2.3.1 5-HT and Feeding Behaviour

It is now firmly established that brain serotonin plays a central role in the control of feeding behaviour in both animals and humans (Samanin, 1983; Blundell, 1984; Leibowitz and Shor-Posner, 1986; Curzon, 1990). One of the most striking and consistent findings in this area of research has been the suppression of food intake by experimental conditions that either directly, or indirectly, enhance brain 5-HT neurotransmission. The converse of this effect, i.e. an increase in food consumption accompanying reduced brain 5-HT has also been demonstrated but appears to be a less robust phenomenon more sensitive to experimental design. More recent experiments have further suggested that 5-HT may also be important in controlling circadian patterns of feeding and in determining macronutrient choice through satiety mechanisms involving the termination of feeding behaviour (Leibowitz, 1990).

Animal Studies

The earliest studies to provide evidence that brain 5-HT mechanisms were important in the control of food intake came from research in rats. Peripheral administration of TRP (Fletcher and Burton, 1984) and 5-hydroxytryptophan (Joyce and Mrosovsky, 1964), precursors of serotonin, were both found to suppress food consumption. Similar results were then demonstrated utilising a wide range of peripherally administered drugs which all had the net effect of enhancing brain 5-HT function. 5-HT agonists such as quipazine
(Samanin et al., 1977), meta-chlorophenylpiperazine (mCPP) (Samanin et al., 1979), the 5-HT releasing drug d-fenfluramine (Samanin et al., 1980), and 5-HT reuptake inhibitors including fluoxetine and zimelidine (Leibowitz, 1990) were all shown to produce hypophagia. Evidence for a major role by serotonergic receptors in mediating the action of these peripherally administered drugs was supplied by studies demonstrating that centrally acting, but not peripherally acting, 5-HT antagonists reversed the fenfluramine-induced suppression of feeding (Carruba et al., 1986). In addition, fenfluramine-induced anorexia was found to be attenuated by neurotoxin (Clineschmidt, 1973) or electrolytic lesions (Samanin et al., 1972) which depleted brain 5-HT stores.

Other experiments in the rat have looked at the effects of central nervous system manipulations on anorexia. Bilateral intraventricular injections of the neurotoxin p-chlorophenylalanine, which causes marked depletion of presynaptic brain 5-HT, was found to produce clear hyperphagia with transient weight gain (Breisch et al., 1976). Similar findings followed 5-HT depletion in the septum, hippocampus and hypothalamus by another neurotoxin, 5,7-dihydroxytryptamine (Saller and Stricker, 1976), although these findings were not universal (Hoebel et al., 1978). In a study by Waldbilling et al. (1981), chemical depletion of hippocampal and hypothalamic serotonin led to an increase in food intake but this was only sustained in those rats on a high fat (highly palatable) diet, suggesting serotonin depletion augmented the capacity of a highly preferred diet to increase food consumption. It has been suggested that this interaction between central manipulation and diet composition may account for some of the negative findings following brain serotonin depletion (Blundell, 1984). Brain 5-HT depletion has also been achieved by surgical lesions. Lesions of the media forebrain and midbrain tegmentum have been found to cause hyperphagia (Grossman et al., 1977; McDermott et al., 1977), while those of the B8 raphe nuclei (but not B7 or B9) led to weigh gain (Geyer et al., 1976).

Studies using central nervous system microinjections of serotonergic agents have highlighted the importance of the hypothalamus as playing a critical role in the regulation of feeding behaviour (Leibowitz et al., 1988; Leibowitz, 1990). The hypothalamus is known to receive and integrate input from a wide range of sources reflecting the organism's nutritional status, and three nuclei (the paraventricular nucleus (PVN), ventromedial nucleus and the suprachiasmatic nucleus) situated in the medial hypothalamus, are now known to be especially sensitive to direct serotonergic stimulation (Rogacki et al., 1989).

Injections of 5-HT directly into the PVN inhibit feeding without any general effects upon arousal (Leibowitz and Papadakos, 1978), with doses of between 1 µg and 10 µg producing reliable dose-dependent decreases in food consumption of up to 50% in food deprived rats. Furthermore, 5-HT injected into the PVN immediately prior to injections of noradrenaline (which stimulates feeding), was found to inhibit the feeding response to
noradrenaline by 90% (Leibowitz and Papadakos, 1978). Similar results have been reported for 5-hydroxytryptophan, the precursor of 5-HT (Leibowitz and Papadakos, 1978). Studies with the drug d-fenfluramine, which is believed to act through release of endogenous 5-HT, demonstrated that it too mimicked the feeding-inhibitory action of exogenous serotonin when injected into these medial hypothalamic regions (Leibowitz et al, 1988; Weiss et al, 1989). 5-HT reuptake inhibitors such as fluoxetine also suppress noradrenaline-induced and deprivation-induced feeding when injected into the PVN (Blundell, 1984).

Examination of the way serotonin influences meal patterns led to the hypothesis that it has specific effects in controlling temporal aspects of feeding (Blundell, 1984). Administration of serotonin or d-fenfluramine directly into the PVN was found to affect meal patterns by significantly reducing the size and duration of individual meals in association with a reduced rate of food consumption (Shor-Posner et al, 1986; Leibowitz et al, 1988). In contrast, these drugs had no effect upon the latency to meal onset (ie. the length of time between meals) or upon meal frequency, suggesting 5-HT is acting primarily through satiety mechanisms, or in other words, serotonin influences the termination of feeding rather than the initiation of eating. Similar effects have been demonstrated in animals receiving peripheral injections of serotonergic agents (Blundell, 1984; Leibowitz et al, 1988).

Evidence from both hypothalamic and peripheral studies suggests a role for serotonin in macronutrient choice (Wurtman and Wurtman, 1979; Blundell, 1984; Leibowitz et al, 1988). Serotonin appears to be primarily concerned with the control of carbohydrate and protein intake, with serotonergic stimulation leading to a reduction in the proportion of carbohydrate consumed in the diet. In animals tested in a two-diet self-selection paradigm, both peripheral injections of 5-HT agonists (Wurtman and Wurtman, 1979) and central injections of serotonin and norfenfluramine directly into the PVN, (Shor-Posner et al, 1986) were found to reduce the intake of energy-rich carbohydrate while sparing protein ingestion. The 5-HT precursor, TRP, was also found to affect macronutrient choice with both acute and chronic administration to rats between meals leading to a subsequent reduction in carbohydrate consumption with a corresponding increase in protein intake (Li and Anderson, 1984; White et al, 1988). Similar findings have been observed after hypothalamic serotonergic stimulation in freely-feeding rats. Serotonin injected directly into the PVN results in a dose-dependent decrease in carbohydrate consumption with no effect (or a slight enhancing effect) on protein or fat intake (Leibowitz et al, 1988; Weiss and Leibowitz, 1988). Direct d-fenfluramine and fluoxetine injections into the PVN, suprachiasmatic and ventromedial nuclei are effective in suppressing carbohydrate intake, presumably through their 5-HT enhancing actions (Weiss and Leibowitz, 1988; Weiss et al, 1989). Peripheral administration of d-fenfluramine and fluoxetine suppresses food intake, but drug dosage and the nutritional status of the animal appear important, while the
effects upon macronutrient choice usually involve suppression of fat as well as carbohydrate (Blundell, 1986; Shor-Posner et al, 1986; Leibowitz et al, 1988). With peripheral administration of 5-HT antagonists such as cyproheptadine and metergoline, the opposite effect is found, i.e. an enhanced intake of carbohydrate and fat (Shor-Posner et al, 1986; Leibowitz et al, 1988; Dourish et al, 1989; Stallone and Nicolaidis, 1989). A mechanism underlying the control of carbohydrate intake has been proposed, whereby carbohydrate ingestion through enhancing brain uptake of TRP (see section 1.2.1), may directly increase 5-HT synthesis thus inhibiting further carbohydrate intake and promoting protein intake (Li and Anderson, 1984; Wurtman and Wurtman, 1979).

Taken together, these experiments demonstrate the key role serotonin depletion and enhancement play in the development of hyperphagia (and in some situations weight gain) and hypophagia respectively. They further suggest that the site of 5-HT depletion is crucial, and highlight the hypothalamic region as an important zone of action for 5-HT in the mediation of feeding behaviour (Blundell, 1984; Leibowitz, 1990). Studies on meal patterns have demonstrated significant and specific effects of serotonin on temporal aspects of feeding through its action on satiety mechanisms, while also suggesting a role in macronutrient selection through the control of carbohydrate and protein intake.

The introduction of specific 5-HT ligands and their subsequent availability as challenge drugs has led to an interest in the role of receptor subtypes in mediating feeding behaviour. Findings from studies with 5-HT antagonists have suggested that 5HT_1 receptors may mediate centrally acting serotonergic-induced feeding suppression. This proposal is supported by the lack of effect of either the selective 5-HT_2 receptor antagonist ritanserin or the 5-HT_3 receptor antagonists ICS205-930 and MDL 72222 upon food intake in freely feeding rats (Massi and Marini, 1987; Dourish et al, 1989). In contrast, however, the 5-HT_1 antagonists metergoline and methylsergide, were both effective in abolishing PVN responsiveness to serotonin stimulation (Weiss et al, 1986). More recently it has been demonstrated that metergoline produces a dose-dependent increase in food intake and carbohydrate preference in food-deprived rats (Stallone and Nicolaidis, 1989). Further evidence for a role by 5-HT_1 receptors comes from a study by Weiss et al (1986) who demonstrated that intrahypothalamic injections of β-adrenergic blockers, which also possess 5-HT_1A/1B binding properties, significantly attenuated serotonin-induced feeding inhibition.

Attempts to further delineate the receptor subtypes involved in feeding behaviour have highlighted the role of the 5-HT_1B receptor in the rat, which is thought to be selectively involved in serotonin-induced hypophagia. Direct 5-HT receptor stimulation by PVN injections of the 5-HT_1B agonists quipazine, RU 24969 (Weiss et al, 1986), and 1-[3-(trifluoromethyl)-phenyl] piperazine (TFMPP) (Hutson et al, 1988), in common with other drugs that enhance brain 5-HT function, have been shown to suppress food intake. In
contrast, 8-OH-DPAT, the selective 5-HT$_{1A}$ agonist, while having no significant effects upon feeding when administered into the PVN, causes significant hyperphagia when injected into the midbrain raphe nuclei (Dourish et al, 1986; Hutson et al, 1988). 8-OH-DPAT-induced hyperphagia is thought to result from 5-HT$_{1A}$ autoreceptor activation in the brainstem, reducing endogenous serotonin release, and thereby reversing the natural inhibitory action of serotonin on feeding (Leibowitz, 1990). Evidence also exists to suggest that the 5-HT$_{1C}$ receptor is important in the control of eating behaviour in the rat. Both m-chlorophenylpiperazine (mCPP) and TFMPP, which have high affinities for the 5-HT$_{1C}$ receptor subtype, are known to suppress food intake following intraventricular or PVN administration (Hutson et al, 1988; Kennett and Curzon, 1988b), an effect blocked by mianserin, a potent 5-HT$_{1C}$ antagonist (Kennett and Curzon, 1988b). In keeping with these findings, binding studies in the rat have demonstrated high densities of 5-HT$_{1B}$ and 5-HT$_{1C}$ receptors in the hypothalamus, with the highest concentrations of 5-HT$_{1B}$ receptors situated in the medial hypothalamic nuclei, precisely where endogenous serotonin is believed to be acting to modulate nutrient intake (Rogacki et al, 1989; Weiss et al, 1989).

**Human Studies**

Human feeding behaviour is thought to be more complex than that of animals, with significant influences from psychological, emotional and cognitive factors as varied as past experiences and associations, through to the hedonic aspects of food presentation such as taste, colour and smell (Silverstone and Goodall, 1986). As eating in humans can be driven by different motivations, it is important to identify these differing influences when considering the neurobiological basis of feeding. One important distinction that must be drawn is between hunger and appetite. Silverstone and Goodall (1986) define "hunger", when applied to humans, as those psychological sensations that accompany significant food deprivation, while "appetite" is said to refer to a person's desire for a particular food at a given time, regardless of the reason. These distinctions complicate the picture, but such influences can be teased out in human studies by carefully worded questions, usually presented to subjects in the form of visual analogue rating scales (Silverstone and Goodall, 1986). While such influences are acknowledged as playing an important role in human feeding behaviour, the following review will focus on the effects of serotonin.

There is considerable evidence that serotonin also plays a crucial role in determining aspects of human feeding. Peripheral administration of drugs which stimulate serotonin function have been found to suppress aspects of food intake. Oral TRP causes a dose-dependent suppression of food intake in healthy male volunteers, a finding that reaches significance with 2g of TRP in terms of both total calories consumed and reduced carbohydrate intake (with a smaller effect on protein) (Silverstone and Goodall, 1986). A study by Hrboticky et al (1985) similarly demonstrated that 2g and 3g of oral TRP had
short term anorexigenic effects when given to lean healthy young males prior to a meal. In this experiment, total food intake was significantly reduced as evidenced by a 18-20% reduction in energy consumption.

The effects of 5-HT antagonists on food consumption have also been studied. Early clinical observations that cyproheptadine, when given to asthmatic children caused weight gain, led to the suggestion that the drug may be acting as an appetite stimulant and increasing caloric intake (Lavenstein et al, 1962). Several controlled studies in healthy but underweight adult volunteers confirmed a significant increase in both appetite ratings (as determined by daily hunger rating scales) and weight gain secondary to an increased caloric intake in subjects taking cyproheptadine (Pawlowski, 1975; Silverstone and Goodall, 1986). These effects were thought to be mediated by the drug's 5-HT receptor antagonist activity. Methysergide, when used to treat migraines has similarly been reported to cause weight gain (Graham, 1967). The effects of the 5-HT antagonist metergoline on fenfluramine-induced anorexia were examined in another double-blind, placebo-controlled trial. While no effects upon food intake or hunger were observed for the eight subjects overall, fenfluramine significantly lowered food intake in four out of the eight volunteers. Furthermore, in three of these subjects, this anorectic effect was blocked by metergoline pretreatment (Goodall et al, 1984). Metergoline alone has also been shown to significantly increased food intake when compared to placebo (Silverstone and Goodall, 1986). In a later study by the same group, d-fenfluramine-induced anorexia in healthy male volunteers was attenuated by pre-treatment with the 5-HT$_{2A/2C}$ antagonist ritanserin (Goodall et al, 1993).

Clinical experience with antidepressant drugs further supports the hypothesis that 5-HT influences food intake in humans. The tricyclic antidepressant amitriptyline, which possesses potent 5-HT antagonist properties, is thought to cause weight gain through increasing carbohydrate cravings (Paykel et al, 1973), findings in keeping with animal studies showing increased carbohydrate intake with reduced 5-HT neurotransmission (see section 1.2.3.1). In contrast, the selective 5-HT reuptake inhibitor, zimelidine, is known to cause weight loss, presumably through its ability to enhance serotonergic neurotransmission (Gottfries, 1981), while fluvoxamine (Abell et al, 1986) and fluoxetine (Fuller and Wong, 1989) also decrease food intake in humans although some debate exists as to whether the anorectic effect in fluoxetine is 5-HT mediated.

Serotonin effects on temporal aspects of feeding behaviour have similarly been elicited. As seen in the rat, administration of serotonergic drugs such as d-fenfluramine, lead to a reduction in both the rate of eating and the size of individual meals through their effects upon satiety (Hill and Blundell, 1986). Influences on macronutrient selection are also seen. In one study, oral administration of 1g TRP to healthy volunteers as part of a high protein meal, led to a decrease in carbohydrate intake at a later free choice meal
(Blundell and Hill, 1987). Wurtman and Wurtman (1984) demonstrated that d-fenfluramine, when given to obese subjects, suppressed carbohydrate intake while sparing protein consumption, a finding in keeping with that of the Goodall et al. (1991) study where both d- and l-fenfluramine were found to exert an anorectic effect (as measured by total energy intake) on male volunteers, with d-fenfluramine producing a significant reduction in carbohydrate consumption while sparing protein. Of particular interest from this latter study was the finding that the effect of d-fenfluramine was not seen upon carbohydrate per se, but only upon nonsweet carbohydrate. The specificity of these effects may help account for studies with d-fenfluramine that have shown inconsistent results (Hill and Blundell, 1986).

The technique of indirectly lowering human brain TRP through dietary manipulation is being utilised increasingly and usually involves the administration of either a low TRP diet or a TRP-free amino acid drink. In the rat (Biggio et al., 1974) and non human primates (Young et al., 1989), low TRP diets have been found to significantly lower plasma and tissue TRP as well as brain serotonin, while in humans, the ingestion of a 50 or 100g TRP-free amino acid drink has been shown to lower plasma TRP by 70-80%, a fall thought also to be accompanied by reduced brain TRP levels (for full discussion see section 2.2). In a study carried out by Young et al. (1988), the effects of a low TRP drink on food intake was examined in healthy male subjects. TRP depletion was found to have no effect upon total calorie or carbohydrate intake but resulted in a small but significant reduction in protein selection at a subsequent buffet style meal. A more recent study, however, utilising a 50g TRP-free amino acid drink paradigm failed to elicit any effects of TRP depletion on appetite and food intake in terms of either calories consumed or macronutrient choice in a group of twelve healthy female volunteers (Van de Kar, 1991).

To date little is known about the role different 5-HT receptor subtypes play in the control of human feeding behaviour. The available data outlined above suggest 5-HT_1 and possibly 5-HT_2 receptors are involved in mediating serotonin-induced hypophagia while some preliminary results have suggested that 5-HT_1A agonists such as gepirone and buspirone cause hyperphagia (Dourish, 1992). The lack of specific ligands available for use as 5-HT receptor antagonists in humans has meant that receptor subtype roles have yet to be determined, however, the failure to detect 5-HT_1B receptors in humans suggests that 5-HT_1C or possibly, 5-HT_1D and 5-HT_2 receptors may be involved in mediating serotonin-stimulatory feeding effects (Leibowitz, 1990).

### 1.2.3.2 Effects of 5-HT on Mood

While the literature on 5-HT and the role it plays in the control of mood is somewhat contradictory many researchers believe there now exists strong evidence for a direct link between the two (Young, 1986; Deakin and Graeff, 1991; Power and Cowen, 1992). Evidence comes from a number of sources including research into the action of
antidepressant drugs, neuroendocrine challenge studies in depression and TRP depletion studies. These will be briefly reviewed.

A central role for 5-HT in the control of mood was originally proposed in the serotonin hypothesis which postulated that a functional deficit of 5-HT was a causal factor in the pathogenesis of depression (Coppen, 1967). This theory was in part based upon the observation of the therapeutic efficacy in the treatment of depression of drugs that enhanced serotonergic neurotransmission (DeMontigny and Blier, 1984). Furthermore, several early studies found low CSF 5-HIAA levels (the major metabolite of 5-HT) in depressed patients. While CSF metabolite results have often been conflicting, overall the data suggests that for a subgroup of patients, especially those prone to impulsive, violent suicide, 5-HIAA levels are indeed low. Reduced levels of 5-HT and/or 5-HIAA and increased numbers of 5-HT2 receptor binding sites have been reported from postmortem examinations of the brains of suicide victims (Meltzer and Nash, 1988; Mann et al, 1989). Neuroendocrine challenge studies have also provided evidence suggesting that dysregulation of this neurotransmitter occurs during episodes of depression. PRL and GH responses to TRP (Heninger et al, 1984) and d-fenfluramine (O'Keane and Dinan, 1991), and PRL responses to the 5-HT reuptake inhibitor clomipramine (Golden et al, 1990) have all been found to be blunted in depression, although again inconsistencies exist in the literature (Power and Cowen, 1992).

While clinical trials suggest that precursors of 5-HT, including TRP, are not generally effect as antidepressants on their own, TRP will potentiate the action of antidepressant drugs such as monoamine oxidase inhibitors, effects thought to be the direct result of enhanced 5-HT synthesis and decreased 5-HT breakdown (Grahame-Smith, 1971). Furthermore, in some studies mildly depressed patients have been found to benefit from the antidepressant effects of TRP alone (Thomson et al, 1982).

The results of TRP depletion studies in normal subjects have been somewhat contradictory. In an initial study by Young et al (1985), administration of a 100g TRP-free amino acid drink was found to have a significant mood lowering effect upon healthy young male subjects, a finding later replicated by the same group (Smith et al, 1987). More recent work has failed to replicate these findings in healthy female subjects (Van de Kar, 1991), although some methodological issues exist that may account for these differences. Patient groups have also been examined. Delgado et al (1990) administered a 100g TRP-free amino acid drink to a group of twenty-one recently recovered depressed patients maintained on antidepressants. This led to a significant lowering of plasma TRP and was accompanied by a return of full clinical depression within five to seven hours of receiving the drink. Reversal of antidepressant activity secondary to TRP depletion has been suggested as the underlying mechanism. In contrast, drug-free depressed patients reported no alteration in mood during TRP depletion, achieved by a combination of a low
TRP diet for twenty-four hours followed by TRP-free amino acid drink, but had a bimodal change in mood on the day following the amino acid drink procedure (Miller et al, 1992). Twenty-three percent of patients reported a worsening of their depression while 37% showed an improvement, as reflected in a fall in the Hamilton Rating Scale for Depression of greater than ten points. The mood response was highly correlated with ultimate treatment response as patients who relapsed after TRP-depletion proved highly treatment resistant, and those that improved showed a good response to antidepressant medication. While these results suggest different pathophysiological mechanisms may be operating in different subgroups of depression, the reason for mood enhancement following TRP-depletion remains uncertain. It has been suggested that acute depletion may be followed by a rapid return of TRP concentrations to normal, enhancing overall 5-HT neurotransmission and therefore improving mood (Miller et al, 1992).

1.2.4 MEASURES OF BRAIN 5-HT FUNCTION IN HUMANS

In animals, brain 5-HT and metabolites such as 5-HIAA can be measured directly. This is usually carried out using methods of receptor binding and electrophysiological techniques. Direct measures for central 5-HT are not yet possible in the living human brain, and a variety of indirect methodologies have therefore been developed.

1.2.4.1 CSF 5-HIAA

CSF can be obtained from the lumbar space and the concentrations of 5-HIAA measured. This is an invasive technique and has been criticised as not necessarily reflecting events occurring in higher regions of the central nervous system (Bulat, 1984).

1.2.4.2 Post Mortem 5-HT Receptor Binding Studies

Receptor binding studies offer an index of receptor numbers in particular brain regions. The imipramine binding site is thought to be located on the 5-HT nerve terminals associated with the 5-HT uptake site or transporter (Paul et al, 1984). Studies of $[^3]H$-imipramine binding may therefore provide an index of 5-HT uptake site numbers and/or function.

1.2.4.3 Neuroendocrine Challenge Studies

The measurement of anterior pituitary hormone responses to 5-HT drugs provides an indirect measure of brain 5-HT function (see section 2.1 for full explanation). The basis for this approach is the finding that alterations in brain 5-HT function leads to a corresponding alteration in the levels of certain plasma hormones, including PRL, cortisol, ACTH and GH (Tuomisto and Mannisto, 1985). Thus, if a standardised pharmacological challenge of 5-HT pathways is employed, the size of the resulting hormonal response provides an index
of the functional state of the 5-HT pathways mediating that endocrine response (Cowen et al, 1992).

1.2.4.4 Peripheral Measures

Platelet 5-HT

Measures of platelet 5-HT uptake sites offer a model for the functional status of central 5-HT neurones. The human platelet expresses a functional 5-HT receptor which resembles the 5-HT₂ subtype (Geaney et al, 1984). It is controversial, however, whether platelet and 5-HT neuronal function can be equated. For example, a recent study of central 5-HT neurone lesioning in rats demonstrated a profound change in brain 5-HT levels and receptor binding, but had no effect on paroxetine binding (Moret and Briley, 1991), suggesting that peripheral mechanisms, rather than central one may be responsible for platelet abnormalities.

Plasma Tryptophan

Measurement of plasma TRP is more fully discussed in sections 1.2.1 and 2.2. It is considered that plasma TRP levels and the ratio of TRP to other LNAAs determine uptake of TRP into the brain, and hence determine the synthesis of brain 5-HT. The effect has been well documented, and has been shown in examples of TRP depletion and supplementation in both rats and non-human primates (Biggio et al, 1974; Gibbons et al, 1979b; Young et al, 1989).

1.3 5-HT IN EATING DISORDERS AND DIETING

The balance of pharmacological evidence to date, from both animal and human studies, supports the hypothesis that serotonergic dysfunction occurs in the eating disorders (Leibowitz, 1990). Research has escalated over the past two decades and findings from basic animal work have been applied to human studies in an attempt to understand the exact nature of the serotonergic abnormalities. In addition, research suggests that dieting alters brain 5-HT function, findings that may have implications for the development of the eating disorders.

1.3.1 ANOREXIA NERVOSA

1.3.1.1 Precursor and Metabolite Studies

Early studies of serotonergic function in anorexia nervosa measured peripheral concentrations of the precursor amino acid TRP and the major metabolite of 5-HT, 5-HIAA, in the urine and plasma. Inconsistent results were found in the acutely ill anorectic
patient. While Coppen et al (1976) reported reduced free and bound plasma TRP levels, Russell (1967) did not find any alterations in plasma concentrations amongst his patients. Kaye et al (1984b) compared the plasma TRP/LNAA ratio in anorectic patients, before and following short- and long-term recovery, with a group of controls and also found no difference. Such results argued against the hypothesis of reduced plasma TRP availability, secondary to reduced dietary intake, to the brain for 5-HT synthesis in the acute phase of anorexia nervosa. Evidence, however, from the small number of studies is by no means conclusive.

The rationale behind CSF neurotransmitter and metabolite studies is that they provide a more direct measure of central neurotransmitter function compared to peripheral studies. Alterations in CSF 5-HIAA concentrations, however, may not necessarily reflect alterations in serotonergic neurotransmission; as Kaye et al (1991) points out factors such as alterations in the metabolism of 5-HT or metabolic clearance of the metabolites may also be implicated. Despite these limitations, valuable information has been gained from in vivo human studies.

Studies of CSF 5-HIAA concentrations have consistently found low levels in malnourished and underweight anorectic patients (Gillberg, 1983; Kaye et al, 1984b; Kaye et al, 1988). Refeeding and short-term weight restoration appear to normalise CSF 5-HIAA levels (Gerner et al, 1984; Kaye et al, 1988), with weight restoration defined as up to two months target weight maintenance. Many patients, despite reaching target weight remained significantly underweight compared to the normal population and were still amenorrhoeic. The reduced CSF 5-HIAA concentrations seen in acutely ill patients are thought to reflect starvation, as even partially refeed patients who have made only minimal weight gain have been shown to have normal levels (Gerner et al, 1984). In an attempt to avoid the complicating factors of malnutrition and recent refeeding, Kaye et al (1991) studied 5-HIAA levels in long-term weight restored patients who had maintained a stable weight above 85% average body weight for at least six months. These authors found increased 5-HIAA concentrations in the CSF of their subjects compared to controls. Although some methodological difficulties exist with this paper, such as studying a mixed restrictor/bulimic population, the inclusion of patients who were continuing to purge and binge on a regular basis and/or were still amenorrhoeic, it raises the possibility of increased brain 5-HT function as a trait marker in long-term recovered anorexia nervosa.

Some differences between restrictor and bulimic subgroups can be demonstrated. Kaye et al (1984a) measured CSF 5-HIAA levels in anorectics after an intravenous probenecid infusion, which blocks the active transport of 5-HIAA out of the CSF and thus allows the metabolite to accumulate. The restrictor type anorectic patients were found to have consistently higher levels of 5-HIAA compared to the bulimics, regardless of short- or long-term recovery status. The hypothesis that restrictor type anorectics have greater
serotonergic activity is further supported by the treatment study of Halmi et al (1986) using the serotonergic antagonist cyproheptadine, in which restrictor type anorectics were found to respond significantly better than the bulimic subgroup. The exact significance of this difference in CSF 5-HIAA levels is not yet understood. It is possible that it reflects differences in weight and nutrition, membrane transport or other differences in central probenecid effects between restrictor and bulimic subtypes (Kaye et al, 1984a), but more excitingly, it may represent a trait marker for the two subgroups reflecting 5-HT turnover rates that are independent of starvation or other central effects (Brewerton et al, 1990). Indeed it has been suggested that greater serotonergic activity amongst restrictor anorectics may account for their more obsessional, ritualistic, perfectionistic and rigid personality traits which, it has been argued, persists even with long-term weight recovery and may contribute to the high relapse rates seen in this disorder (Kaye and Weltzin, 1991b).

1.3.1.2 Platelet Studies

Blood platelets have been shown to take up, store and release 5-HT by mechanisms similar to that of central monoamine neurones (Zemishlany et al, 1987). Consequently, platelet 5-HT uptake and imipramine binding, a measure of 5-HT receptor site numbers, have been used as models of studying central serotonergic function in many psychiatric illnesses (Zemishlany et al, 1987). Studies in anorexia nervosa, however, have been few, making it impossible to draw firm conclusions at this stage. Those studies that have been carried out have reported decreased imipramine binding suggesting reduced 5-HT activity in the acute phase of the illness (Weizman et al, 1986). A difference in 5-HT uptake was not found between anorectic patients and control subjects (Weizman et al, 1986; Zemishlany et al, 1987). Neither of these studies reported on bulimic verses restrictor subgroups.

Platelet MAO activity similarly has been studied as a measure of serotonergic function in different psychiatric populations. To date only one paper has reported on MAO activity in anorexia nervosa. Biederman et al (1984) found reduced MAO activity amongst a subgroup of anorectic patients with depression but normal activity in the non-depressed anorectics and controls.

1.3.1.3 Neuroendocrine Studies

Neuroendocrine challenge tests have become increasingly recognised as valid, if indirect, measures of central serotonergic function (Checkley, 1980; Cowen et al, 1990b; Yatham and Steiner, 1993). To date however, few studies using neuroendocrine challenges have been carried out in anorexia nervosa.

Brewerton et al (1990) have reported blunted PRL responses (peak minus baseline) to both TRP and the 5-HT agonist mCPP in underweight anorectic patients when compared to
healthy controls. Furthermore, responses to TRP and mCPP challenges carried out in patients four weeks after achieving their goal weights were also found to be blunted. Ten out of twelve in the TRP group and eight out of twelve in the mCPP group had higher PRL responses to the challenge drug at goal weight than when underweight. The authors could not account for the findings in this subgroup in terms of plasma drug concentration differences, baseline oestradiol levels, Hamilton Rating Scale or Beck Depression Inventory scores or number of years of illness. One possible explanation is that blunted PRL responses reflect malnutrition and low weight, and could be expected to normalise once weight is within normal range. This is supported by finding a trend towards higher PRL responses in most patients achieving their goal weights. The fact that the PRL responses remain somewhat blunted at goal weight could simply reflect the patients' ongoing low body weights which were only 85%+/- 3% of average body weight. Blunted responses can also occur secondary to low PRL stores or impairment of the pituitary lactotroph function. However, as PRL responses to TRH, which acts directly at pituitary level to release PRL, have been found to be largely (Beumont et al, 1976; Kiriike et al, 1987; McBride et al, 1991) but not always (Waldhauser et al, 1984), normal in underweight anorectic patients this explanation also seems unlikely.

An alternative explanation, that cannot be proven one way or the other on the current available literature, is that serotonergic receptor responsivity of the pathways mediating the PRL responses to these challenge drugs are blunted in anorexia nervosa, regardless of patients' weights and nutritional status at the time of testing (Brewerton et al, 1990).

Goodwin et al (1989), however, failed to find blunted PRL responses to a TRP infusion in thirteen acutely ill anorectic patients compared to controls. In this paper a significantly blunted GH response to the same TRP infusion was reported, suggesting different effects for the two hormones in anorexia nervosa. The authors suggested that the blunted GH response may be related to reduced dopamine function (Kaye et al, 1984b) secondary to dietary factors.

1.3.2 BULIMIA NERVOSA

1.3.2.1 Precursor and Metabolite Studies

It has been suggested that bulimic behaviour is consistent with hyposerotonergic activity but evidence for this occurring in bulimia nervosa is inconsistent (Kaye and Weltzin, 1991b). In one of the few peripheral studies published looking at 5-HT function Lydiard et al (1988) found no baseline plasma TRP/LNAA ratio differences in normal weight bulimic patients compared to healthy controls, suggesting normal TRP availability. In an uncontrolled laboratory study of nine actively bingeing bulimic patients, Kaye et al (1988) found an inverse relationship between the development of satiety (defined as cessation of bingeing and vomiting) and the plasma TRP/LNAA ratio. In other words,
"bulimic patients who developed an increased plasma TRP ratio during bingeing and vomiting had fewer cycles of bingeing and vomiting....than did subjects who did not develop an increase in the plasma TRP ratio" (Kaye et al, 1988).

The authors raise the possibility that an increase in plasma TRP/LNAA ratio may be associated with the termination of a binge episode through its effects upon brain 5-HT metabolism. This suggestion is further supported by their finding that those patients who exhibited increased plasma TRP/LNAA ratio, were also found to have greater increases in plasma PRL, suggesting enhanced brain 5-HT neurotransmission had indeed occurred.

A number of studies have reported normal CSF 5-HIAA concentrations in normal weight bulimic patients (Jimerson et al, 1988; Kaye et al, 1990a) although in Jimerson's study patients with a binge frequency of twice daily or above were found to have lower CSF 5-HIAA levels than both less symptomatic patients and controls. The lower 5-HIAA levels did not appear to relate to differences in body weight or to a history of depression and suggest that higher frequencies of bingeing may be "associated with relatively persistently reduced central serotonin turnover" (Jimerson et al, 1990). Whether or not the negative correlation between binge frequency and CSF 5-HIAA concentrations represent serotonergic dysregulation intrinsic to the development of the disorder, or occurs as a consequence of persistent bingeing and purging behaviour and/or nutritional disturbances has still to be determined.

1.3.2.2 Platelet Studies

Goldbloom et al (1990) recently reported on twenty-two normal weight bulimic patients, who were still actively bingeing and purging, and found increased platelet 5-HT uptake compared to controls. No significant correlations were found between platelet 5-HT uptake results and depression scores, body weight, or frequency of recent bingeing and vomiting behaviour, nor were there significant menstrual or seasonal effects, both of which are known to influence platelet 5-HT uptake. Goldbloom et al (1990) suggest that increased platelet 5-HT uptake could reflect decreased availability of 5-HT at postsynaptic receptors at the hypothalamic level, thus reducing satiety signals and, possibly, altering macronutrient choice towards increased carbohydrate preference. In contrast, Hallman et al (1990) reported no difference in platelet 5-HT uptake, but lower platelet MAO activity, between controls and a group of sixteen women who currently met DSM-III criteria for bulimia nervosa.

In a single preliminary report on platelet 3H-imipramine binding, a group of non-depressed bulimic patients were found to have significantly reduced density of binding sites compared to controls (Marazziti et al, 1988).
1.3.2.3 Neuroendocrine Studies

Neuroendocrine studies in bulimia nervosa are few in number, but those that have been reported provide evidence for serotonergic dysregulation. Peak PRL responses to mCPP were blunted compared to controls in bulimic patients meeting both DSM-III and DSM-IIIR criteria, regardless of the presence or absence of major depression, while the PRL response to a TRP challenge in the same group of patients was also found to be blunted, but only in those bulimics concurrently meeting criteria for major depression (Brewerton et al, 1992). The blunted PRL responses to mCPP were not related to age, body weight, medication, plasma drug levels or baseline oestradiol levels. The possibility that the blunted PRL responses were due to alterations in pituitary lactotroph activity is not supported by other work demonstrating that bulimic patients have normal PRL responses to a TRH challenge (Levy et al, 1988; McBride et al, 1991). An inverse correlation was found between PRL responses following the mCPP challenge and baseline cortisol levels in the bulimic patients, paralleling animal work where PRL responses to mCPP were found to be blunted following long-term cortisol treatment (Bagdy et al, 1989a) and human studies in depression where a similar inverse relationship has been observed (Hsu, 1990; Power and Cowen, 1992).

Brewerton et al (1992) suggest that the different hormonal responses to the two serotonergic drugs could reflect a number of factors including differential involvement of presynaptic (TRP) and postsynaptic (mCPP) mechanisms, different 5-HT receptor subtypes mediating the responses of the two drugs or different anatomical loci of action (Brewerton et al, 1992). While careful controls for recent dieting, bingeing and purging behaviour (over the previous four weeks prior to testing) were made in the study, it is still not possible to conclude whether the blunted PRL responses reflect 5-HT dysregulation consequent on long-term bingeing and purging behaviour that had not normalised by the time of testing, or whether it reflects a "premorbid state that predisposes these patients to bulimia nervosa" (Brewerton et al, 1992).

McBride et al (1991) have recently reported on dl-fenfluramine challenges in a group of eating disordered women. Unfortunately, numbers were too small to allow subgroup responses to be individually analysed, but they reported a trend towards reduced PRL responses to dl-fenfluramine in seven patients of whom four currently met criteria for bulimia nervosa. There was also preliminary results from three patients retested at six to twelve weeks after successful treatment suggesting that responses remained blunted.

Using the 5-HT precursor 5-HTP, Goldbloom et al (1990) has examined central 5-HT function in bulimia nervosa. In keeping with the other 5-HT neuroendocrine challenge studies they found evidence suggesting reduced serotonergic activity. Both the GH and
PRL responses to intravenous 5-hydroxytryptophan were found to be blunted in the bulimic patients compared to controls.

**1.3.3 EFFECTS OF DIETING ON 5-HT**

Examining the effects of altered nutrition on brain 5-HT function has important implications for our understanding of psychiatric illness, especially the eating disorders. Such research will enable us to separate those neurobiological abnormalities that are the result of food deprivation from those that are intrinsic to the aetiology of the disorders themselves. The following review focuses on studies carried out in relation to 5-HT function. The effects of food intake and dietary restriction on levels of neurotransmitter precursors, and on the synthesis of brain neurotransmitters will be examined. With respect to human functioning, studies examining the neurochemical effects of dieting will also be covered.

**1.3.3.1 Amino Acid Precursor Supply in Relation to Dietary Restriction**

Most research looking at the effects of food deprivation on amino acid supply have focused upon tyrosine and TRP, the amino acid precursors for the neurotransmitters, dopamine and catecholamines, and serotonin respectively. However, studies of the effects of fasting have encompassed most amino acids.

Short periods of fasting (24 to 120 hours) have been shown to have little effect on plasma concentrations of most amino acids in the rat (Enwonwu, 1987), with levels of plasma phenylalanine increasing but alanine, arginine, glutamine and threonine all showing moderate decreases. Brain concentrations of most amino acids in the rat also remain unaltered, although the modest plasma changes are reflected by increases in brain phenylalanine and decreases in alanine and arginine (Knott et al, 1973). For two amino acids, however, the impact of fasting on brain concentrations may not be reflected in changes in plasma concentrations. Plasma threonine falls significantly following a twenty-four hour fast (Enwonwu, 1987), but concentrations remain unaltered in the brain. In contrast, it has been argued that plasma TRP levels are unaltered in response to fasting, yet brain levels significantly increase (Curzon et al, 1972). Fernstrom and Wurtman (1971a) further demonstrated that brain TRP levels increase when rats are given a large carbohydrate meal free of TRP.

One possible explanation for these apparently paradoxical results has been put forward by Curzon (1988) who hypothesised that fasting increases plasma unesterified fatty acids (released from fat stores), which directly compete with TRP for binding to plasma albumin. This results in increased concentration of free plasma TRP available for transport across the blood-brain-barrier (Knott and Curzon, 1972; Curzon et al, 1973). A high carbohydrate meal causes insulin release, resulting in an increased uptake of LNAA into
muscle and a reduction in the ratio of LNAA/TRP, again enhancing transportation of TRP to the brain (see section 1.2.1.1).

A complex relationship appears to exist between TRP intake, plasma levels and brain concentrations in the rat following short term fasting. It is not clear how well these findings can be applied to the human situation, in particular to sustained food restriction as opposed to total fasting. One study has examined this issue in a healthy human population and found no alteration in the ratio of plasma TRP/LNAA over a six week diet compared to controls (Schweiger et al, 1986).

1.3.3.2 Effects of Dieting on Brain 5-HT Function

To date, only a handful of published studies have looked at the effects of dieting on brain 5-HT function in humans. These have utilised the indirect neuroendocrine challenge paradigm which will be outlined in detail in section 2.1.

The first evidence that dieting may effect human brain 5-HT function came from a study by Goodwin et al (1987b) who placed a group of twelve healthy men and women on a calorie controlled diet for three weeks. Although no effect upon plasma TRP levels were found, an enhanced PRL response to a subsequent TRP challenge was demonstrated in the women but not the men. This same group of researchers (Anderson et al, 1990a) later replicated these results in another group of volunteers, with the additional finding that dieting led to a fall in total plasma TRP in both male and female dieters. (The significant reduction in total plasma TRP following dieting replicated an earlier finding Anderson et al, 1989). The enhanced PRL response to the TRP challenge after dieting was again demonstrated as occurring in women alone, despite greater absolute and percentage body weight loss in males (Anderson et al, 1990a).

It was hypothesised (Goodwin et al, 1987b; Anderson et al, 1990a) in light of these findings that moderate dieting decreases plasma TRP sufficiently in women to reduce brain 5-HT synthesis (see section 1.2.1). The reduced TRP would lead to an overall reduction in 5-HT neurotransmission across the synapse, and in turn, produce an adaptive supersensitivity of the post-synaptic 5-HT receptors, as reflected in the enhanced PRL response to TRP. Thus, although dieting increased the PRL response to a TRP challenge, the underlying change was a functional decrease in brain 5-HT neurotransmission. Support for this theory comes from a recent study by Delgado et al (1989) who demonstrated that a ten day low-TRP diet in healthy volunteers significantly reduced plasma TRP levels in both males and females, and in females alone this change was associated with an enhanced PRL response to a subsequent TRP challenge. Consistent with the dieting work, the plasma TRP fall was greater in the women than in the men.
Two reasons, not necessarily mutually exclusive, were suggested for the effect being female specific. Firstly, plasma TRP levels fell further in the women than in the men (Anderson et al, 1990a) so that brain 5-HT synthesis could have been more severely affected. Secondly, it is well established that rates of 5-HT turnover in both human (Young et al, 1980) and non-human primates (Young and Ervin, 1984) are greater in females compared to males, again making female brain 5-HT function more likely to be compromised.

In order to examine the specificity of these diet induced changes on 5-HT-mediated brain function, Anderson et al (1989) carried out a series of experiments in which they demonstrated that dieting had no effect upon the PRL response to the dopamine antagonist metaclopramide. These results suggested that the enhanced PRL response seen in women following dieting was not the result of changes in dopaminergic neurotransmission, a possible alternative explanation as PRL release is under the inhibitory control of dopamine (Quigley et al, 1980). Furthermore, Anderson et al (1989) showed that these changes were not secondary to direct stimulatory effects upon the pituitary lactotrophs as dieting had no effect upon the TRH-induced release of PRL. Dieting was also found to have no effect on the physiological release of PRL, as measured by sleep-induced changes, nor upon basal PRL measurements. A further study by Goodwin et al (1988) has failed to find dieting-induced effects upon the hypothalamic-pituitary-adrenal axis as measured by alterations in the rate of non-suppression of cortisol by an oral dose (1mg) of dexamethasone.

Taken together, these results suggest an effect of dieting upon brain 5-HT-mediated PRL release, but this suggestion rests on findings utilising TRP as the challenge probe. The findings further suggest that such dieting induced changes are probably restricted to women. In view of the central role that brain 5-HT plays in the control of mood and appetite (see sections 1.2.3 and 1.1.4.3) and the widespread evidence now existing to suggest that dieting behaviour acts as a risk factor in the development of eating disorders (see section 1.1.4.3), these findings are of considerable interest, particularly as it is well recognised that the vast majority of eating disorders occur in females. The question therefore arises as to whether or not women have a neurobiological vulnerability to developing the eating disorders. Indeed one possible mechanism would be through differing biological responses to alterations in food intake, compared to men, as the above evidence suggests may be the case for TRP and subsequent brain 5-HT synthesis.

1.3.4 CURRENT STATUS OF 5-HT RESEARCH IN EATING DISORDERS AND DIETING

1.3.4.1 Anorexia Nervosa

Overall, research findings suggest that 5-HT dysregulation does occur in anorexia nervosa although the precise nature of the dysregulation remains unclear. While it is
reasonably clear that the low CSF 5-HIAA concentrations seen in acutely ill patients probably reflect starvation, it is also possible that restrictor- and bulimic-type anorectic subgroups can be separated on the basis of central 5-HT turnover rates, the former having higher rates than the latter. The finding by Kaye and Weltzin (1991b) that CSF 5-HIAA concentrations are elevated in long-term recovered anorectics further raises the possibility of increased 5-HT neurotransmission in individuals with this disorder. Increased serotonergic activity is theoretically consistent with many features seen in anorexia nervosa and raises the intriguing possibility that anorectic patients may reduce food intake in order to lower brain 5-HT activity.

The blunted hormonal responses to mCPP, TRP and dl-fenfluramine may appear at first glance to contradict the CSF metabolite results, in that they suggest reduced 5-HT neurotransmission. All the neuroendocrine studies published so far, however, have been carried out either on acutely ill patients, or on patients who have only recently recovered and in whom body weight often remains low and menstrual functioning impaired. As with the metabolite studies, effects of starvation, low body weight and/or partial refeeding could still be having a significant influence at this stage.

1.3.4.2 Bulimia Nervosa

In bulimia nervosa there is also evidence for a disturbance in 5-HT function. Precursor and metabolite studies, platelet re-uptake findings and experiments with three separate 5-HT neuroendocrine challenge probes suggest that, at least during the acute and early recovery phases of the disorder, 5-HT function is abnormal. Long-term recovery studies are still awaited. To date most of the evidence, although indirect, supports a state of relative hyposerotonergic activity, which is theoretically consistent with many of the features and behaviours typical of bulimia nervosa.

While the initial results on 5-HT function in the eating disorders are exciting, they need to be interpreted in the light of several major research limitations. Perhaps the most important is the difficulty in determining when an individual patient's nutritional status and weight has been restored to a level at which normal functioning can be assumed to have resumed (Goldbloom et al, 1991), thus removing the confounding factors of starvation and low body weight. Starvation is known to bring about significant cognitive, affective and behavioural changes (Garfinkel and Kaplan, 1985) along with metabolic alterations in its own right (Sharp and Freeman, 1993). While starvation effects have obvious application to anorectic patients, it is increasingly recognised that they probably play an major role in bulimia nervosa as well (Pirke et al, 1985a; Gwirtsman et al, 1989b). It has been proposed in light of the "set-point" theory, that for many bulimic patients, "normal" body weights as determined by standard population measures may in fact be below their "natural" weight (Russell, 1979; Garner et al, 1985). Thus, a weight within the standard range for such a
patient may in fact represent a chronic state of starvation, even after apparent clinical recovery.

In addition to weight changes and nutritional effects, some behaviours seen in the eating disorders such as bingeing or purging through vomiting, laxative abuse or diuretics also have biological consequences and must therefore be carefully controlled for.

In terms of research, the methodological issues outlined above become important when trying to separate out biological abnormalities that occur as a consequence of the disorder or are important in symptom maintenance (such as starvation effects), from those that might predispose an individual to the development of eating difficulties and hence are important in furthering our understanding of the pathogenesis of the disorders. While prospective research would be ideal in enabling us to tease out these different factors, major practical and financial problems exist with carrying out such studies. Long-term recovered patients offer the most fruitful alternative in that confounding factors can be minimised, allowing a more accurate assessment of underlying brain 5-HT activity. Such studies are still awaited for both anorexia and bulimia nervosa. Further benefit would be gained by examining the confounding factors themselves (such as the effects of weight loss on brain 5-HT function) thus enabling a more accurate interpretation of the currently available research.

1.3.4.3 Dieting

While strong evidence now exists for an effect of dieting upon brain 5-HT function, the available data suggest the effects are confined to women. Whether or not females have a neurobiological vulnerability to developing the eating disorders remains unclear as further dieting studies are required to replicate earlier work demonstrating female-specific brain 5-HT changes. The exact mechanism by which dieting produces changes in brain 5-HT function is also still to be elicited. TRP is a rather non-specific challenge (Yatham and Steiner, 1993) and no direct evidence exists from the above studies to support the supersensitivity hypothesis. An enhanced PRL response to a TRP challenge would also be consistent with the hypothesis that dieting leads to increased brain 5-HT function, as proposed by Curzon (1988) although it would be difficult to explain the reduced plasma TRP findings in terms of his hypothesis.

Anorexia and bulimia nervosa are associated with high morbidity and significant mortality. In view of the significant implications clarification of the issues outlined above would have, in terms of aetiology and furthering our understanding of the factors that lead to symptom maintenance, it would be important to pursue these issues further. The main body of work carried out for this thesis addresses these points.
Chapter 2

EXPERIMENTAL APPROACH AND GENERAL METHODS

In this thesis the effects of dieting on brain 5-HT function and the role of serotonergic pathways in the control of appetite have been examined. Broadly, three experimental approaches were utilised; neuroendocrine challenge tests, TRP depletion studies and a test meal procedure. In this chapter the rationale for use of the three approaches is considered and general experimental techniques are outlined.

2.1 NEUROENDOCRINE TESTS OF 5-HT FUNCTION

In a recent publication, Cowen et al (1990b) stated that "measuring brain 5-HT function in the living human brain presents obvious difficulties but endocrine responses to increased brain 5-HT function (5-HT neuroendocrine challenge tests) offer a possible approach".

2.1.1 RATIONALE FOR USE OF NEUROENDOCRINE CHALLENGE TESTS

Although an indirect measure, the rationale behind neuroendocrine testing is the role monoamine pathways play in the control of anterior pituitary hormone secretion (for a full review see Tuomisto and Mannisto, 1985). Neurones utilising monoamine neurotransmitters innervate hypothalamic nuclei which contain the cell bodies of anterior pituitary hormone releasing (and inhibitory) factor neurones. The anterior pituitary hormone releasing and inhibitory neurones project to the median eminence where their nerve terminals end adjacent to capillaries of the hypothalamo-hypophyseal portal system. Stimulation of the neurones results in secretion of either releasing or inhibitory factors which enter the portal system and are transported to the anterior pituitary. Here the hormone releasing or inhibitory factors interact with specialised anterior pituitary cells to either stimulate or inhibit the release of specific hormones. Neuroendocrine testing involves giving a drug to selectively stimulate one of the monoamine pathways and then measuring the resultant change in hormone release, with the change in hormone output
providing an index of the functional activity of the monoamine pathway with which the
drug interacts (Checkley, 1980). Two assumptions are implicit: firstly, that the hormone
response results from the stimulation of one type of neurotransmitter releasing system and
secondly, that these receptors are situated within the brain and not within the pituitary or
peripheral nervous system (Checkley, 1980).

2.1.2 INNERVATION OF THE HYPOTHALAMUS AND PITUITARY
AND THE RELATIONSHIP TO NEUROENDOCRINE FUNCTION

5-HT innervation of the hypothalamus arises principally from brainstem median and
dorsal raphe nuclei (Ungerstedt, 1971; Kellar et al, 1977; Palkovits et al, 1977). The
hypothalamus as a whole is one of the most densely 5-HT innervated structures in the
central nervous system with the greatest concentration of 5-HT neurones situated within
the ventromedial and mamillary nuclei (Takeuchi, 1989). Animal work suggests that
individual hypothalamic nuclei receive differential 5-HT innervation but species
differences do exist and must be taken into account. 5-HT projections to the
suprachiasmatic nucleus and the anterior hypothalamic region arise principally from the
median raphe nucleus (Van de Kar and Lorens, 1979). In contrast, serotonergic neurones
from the dorsal raphe nucleus do not project exclusively to specific hypothalamic
structures. The anterolateral hypothalamic region and the arcuate nucleus receive 5-HT
neuronal projections originating from both the dorsal and median raphe nuclei (Van de Kar
and Lorens, 1979).

Release of the hypothalamic releasing and inhibitory factor hormones is regulated by
the hypothalamic nuclei. For example, the arcuate and ventromedial hypothalamic nuclei
are involved in regulating the secretion of luteinizing hormone (Halasz and Pupp, 1965;
Voloschin et al, 1968), while cells containing corticotropin-releasing hormone are located
within the PVN and are known to have nerve terminals that extend to the external zone of
the median eminence (Olschowka et al, 1982).

Autoradiography and immunocytochemistry techniques in the rat have demonstrated
the presence of 5-HT axon terminals and neurones containing vasoactive intestinal
polypeptide (thought to be a strong candidate as a physiological prolactin releasing factor)
in the suprachiasmatic nucleus (Bosler and Beaudet, 1985), suggesting a role by this
structure in the regulation of PRL secretion. Hokfelt et al (1982) demonstrated the
presence of peptide histidine isoleucine amide, a vasoactive intestinal peptide homologue
capable of releasing PRL (Werner et al, 1983), in the PVN, dorsomedial, ventromedial and
premamillary nuclei, but again the greatest density of fibres were found concentrated in the
suprachiasmatic nucleus. Watts et al (1987), however, were unable to demonstrate the
presence of efferent fibres from the suprachiasmatic nucleus to the median eminence,
raising the question as to whether or not the suprachiasmatic nucleus was directly involved in 5-HT-mediated PRL release. It is possible that the suprachiasmatic nucleus exerts its effects via regulation of hormonal circadian rhythms (Watts et al., 1987). The PVN has also been implicated in 5-HT-mediated PRL release. In the rat, the PVN’s parvocellular region is moderately innervated by 5-HT fibres originating from both dorsal and median raphe nuclei (Sawchenko et al., 1983). Lesions of the PVN abolish the PRL response to 5-hydroxytryptophan and stress (Minamitani et al., 1987), and a parvocellular paraventricular neuronal system, extending to the external layer of the median eminence and containing the PRL releasing agent, peptide histidine isoleucine amide, has been demonstrated by Hokfelt et al. (1982). The arcuate nucleus receives both 5-HT and dopaminergic innervation (Kiss and Halasz, 1986) while 5-HT innervation in the median eminence is largely confined to the external layer (Soghomonian et al., 1988).

2.1.3 5-HT REGULATION OF PROLACTIN RELEASE

While PRL is secreted by cells in the anterior lobe of the pituitary gland, secretion is predominantly under inhibitory hypothalamic control (Tuomisto and Mannisto, 1985). Conditions that disconnect the anterior pituitary from the hypothalamus, such as hypothalamic lesions (Arimura et al., 1972) are known to increase PRL release. The control of PRL release is complex, regulated by a family of multiple PRL-releasing and PRL-inhibiting factors which are in turn dependent upon physiological conditions.

Dopamine, originating in the tuberoinfundibular system in the arcuate nucleus exerts a tonic inhibitory control so that any increase in dopamine secretion will result in a corresponding decrease in PRL release. In contrast, animal studies have suggested hypothalamic and PVN peptides, including vasoactive intestinal peptide, neuropeptide Y, histamine, TRH polypeptide histidine isoleucine-27, oxytocin and arginine vasopressin are important in stimulating the release of PRL (Kjaer et al., 1994; Arey and Freeman, 1992). For example, the neurohormone oxytocin is thought to increase PRL secretion following 5-HT stimulation (Bagdy and Makara, 1993), while the hypothalamic neurotransmitter histamine appears to participate in stress-induced PRL release, an effect perhaps mediated via the hypothalamic factor arginine vasopressin (Kjaer et al., 1994).

The regulatory peptides vasoactive intestinal peptide and neuropeptide Y are influenced by the thyroid hormones. Thyroid hormones have an inhibitory action upon vasoactive intestinal peptide and neuropeptide Y-producing cells of the pituitary. In clinical conditions of hypothyroidism there is an increase in gene expression and hence peptide levels, an effect abolished following anterolateral deafferentation of the hypothalamus, suggesting a role for some hypothalamic factor in the mediation of the
effects of hypothyroidism on pituitary vasoactive intestinal peptide and neuropeptide Y levels (Michalkiewicz and Suzuki, 1994).

Oestrogen is similarly thought to be a stimulatory factor for PRL, acting at multiple sites to control the release of PRL from the anterior pituitary. Animal studies have suggested that oestrogen increases the number of PRL-secreting cells in the pituitary gland and inhibits the feedback loop on PRL by suppressing hypothalamic DA secretion (Leibenluft et al, 1994).

A wide range of drugs, including antipsychotic and antidepressant medications are capable of inducing stimulation or suppression of PRL secretion, mainly through their effects upon central neurotransmitter pathways. Direct (e.g. dopamine and apomorphine) and indirect dopamine (e.g. amphetamine and nomifensine) receptor agonists decrease PRL release, as do drugs which impair 5-HT neurotransmission (e.g. metergolone and methysergide), γ-aminobutyric acid-mimetic drugs (e.g. sodium valproate, histamine H3 receptor agonists and cholinergic (muscarinic and nicotinic) receptor agonists. In contrast, the main classes of PRL-stimulating drugs include dopamine receptor antagonists and drugs that indirectly impair dopamine neurotransmission (e.g. both typical and atypical antipsychotics, carbidopa), drugs that enhance 5-HT neurotransmission including 5-HT precursors (e.g. L-tryptophan and 5-hydroxytryptophan), 5-HT agonists (e.g. quipazine) and indirect 5-HT agonists including 5-HT reuptake inhibitors (e.g. fluoxetine) and releasers of 5-HT such as d-fenfluramine (Muller et al, 1983).

While the importance of these factors in the regulation of PRL secretion must be acknowledged, a full overview of PRL regulation is beyond the scope of this thesis. Thus, the brief review below will focus on the role 5-HT plays in PRL release.

2.1.3.1 Animal Studies

Animal studies have demonstrated that 5-HT containing cells in the dorsal raphe nucleus stimulate PRL release (Van de Kar and Bethea, 1982; Barofsky et al, 1983). One study suggested that cells from the median raphe nucleus may be involved, but because of methodological limitations, the authors' conclusions remain tentative (Fessler et al, 1984). PRL release induced by 5-hydroxytryptophan, restraint, or other stress is inhibited by electrolytic lesions of the PVN (Minamitani et al, 1987). Physiological release of PRL occurs secondary to suckling. Suckling induces a significant increase in plasma vasoactive intestinal peptide concentration and a concomitant increase in plasma PRL concentration (Rolandi et al, 1987). Injections of the neurotoxin 5,7-dihydroxytryptamine into the anterior pituitary inhibits suckling-induced PRL release in the female rat (Parisi et al, 1987), as do lesions of serotonergic neurones in the dorsal raphe nucleus (Barofsky et al,
1983) and depletion of central 5-HT stores with p-chlorophenylalanine (Kordon et al., 1973). These studies suggest involvement of serotonergic neurones in either, or both, the PVN and anterior hypothalamus in the regulation of PRL secretion (Van de Kar, 1991), although other hypothalamic sites may also be involved (Van de Kar, 1991).

Administration of 5-HT precursors, including TRP and 5-hydroxytryptophan increase plasma PRL in the rat (Lu and Meites, 1973), as do several 5-HT receptor agonists, along with the 5-HT releasing drug fenfluramine (Van de Kar et al., 1985; Serri and Rasio, 1987). Administration of RU 24969, a 5-HT$_{1A/1B}$ agonist, and MK-212, a nonselective 5-HT$_{1/2}$ agonist, increase plasma PRL in a dose dependent manner, in contrast to the selective 5-HT$_{1A}$ agonists, 8-OH-DPAT and ipsapirone, which inconsistently elevate PRL (Van de Kar et al., 1989). More recently, the 5-HT$_{2A/2C}$ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has been found to increase plasma PRL, an effect inhibited by ritanserin, a 5-HT$_{2A/2C}$ antagonist (Rittenhouse et al., 1990; Rittenhouse et al., 1993). The non-selective 5-HT agonist mCPP similarly increases PRL in the rat (Aulakh et al., 1988b). Pre-treatment with the 5-HT$_2$ receptor blocker LY53857 failed to block either RU 24969 or fenfluramine-induced PRL release (Van de Kar et al., 1989a; Van de Kar et al., 1989b) and ketanserin, a selective 5-HT$_2$ antagonist, failed to block the PRL response to the 5-HT$_2$ agonists 5-methoxy-N,N-dimethyltryptamine and quipazine or the PRL response to the 5-HT precursor, 5-hydroxytryptophan. Pre-treatment with the 5-HT$_2$ receptor antagonist ritanserin, however, has been reported to block quipazine and d-fenfluramine-induced PRL release (Di Renzo et al., 1989). The role of 5-HT$_3$ receptors in the control of PRL secretion has also been examined. In a study by Jorgensen et al. (1993), the 5-HT$_3$ agonist 2-methyl-5-HT was found to have little or no effect in increasing PRL on its own but was found to enhance the PRL releasing activity of other 5-HT$_1$ receptor agonists such as RU 24969. Pre-treatment with ICS 205930, the 5-HT$_3$ antagonist, was found to have no effect on RU 24969-induced PRL release. In contrast, the 5-HT$_3$ antagonists ICS 205930 and GR 38032F have been reported to block the PRL response induced by 5-HT and combined fluoxetine and 5-hydroxytryptophan (Jorgensen et al., 1992). In summary, no consistent receptor subtype has been found to induce PRL release in the rat and evidence to date suggests involvement of 5-HT$_1$, 5-HT$_2$ and 5-HT$_3$ receptors in the mediation of the PRL response.

The mechanism underlying serotonergic-induced PRL secretion is thought to be release of a prolactin releasing factor (Clements et al., 1978), possibly the polypeptide vasoactive intestinal peptide, which stimulates PRL secretion (Shimatsu et al., 1982). The finding that vasoactive intestinal peptide antiserum attenuates 5-hydroxytryptophan-induced PRL release in the rat, strengthens the hypothesis that vasoactive intestinal peptide may act as an important mediator of serotonergic-induced PRL secretion (Ohta et al,

2.1.3.2 Human Studies

Although hampered by a lack of 5-HT challenge drugs suitable for human studies, the available evidence supports a significant role for serotonergic neurones in the release of PRL in man. Administration of the 5-HT precursors TRP (MacIndoe and Turkington, 1973; Charney et al., 1982; Cowen et al., 1985) and 5-hydroxytryptophan (Meltzer et al., 1982) increases plasma PRL in humans, although the increase in PRL following 5-hydroxytryptophan appears to be less consistent. The PRL response to TRP is enhanced by pretreatment with 5-HT uptake inhibitors including clomipramine (Anderson and Cowen, 1986) and fluvoxamine (Price et al., 1989). Clomipramine alone, when given intravenously, will produce a reliable increase in plasma PRL (Laakmann et al., 1984; Golden et al., 1989) but less consistent effects follow oral administration (Laakmann et al., 1984). mCPP, thought originally to be a selective 5-HT1 agonist, stimulates PRL secretion (Mueller et al., 1985; Kahn et al., 1990; Kahn and Wetzler, 1991) but it is now clear that this compound also binds to other 5-HT receptor subtypes (Hamik and Peroutka, 1989). The 5-HT1 partial agonist gepirone (Anderson et al., 1990) and the 5-HT releasing drug d-fenfluramine (Quattrone et al., 1983; O'Keane et al., 1991) also increase plasma PRL concentrations. Furthermore, subacute and chronic pre-treatment with lithium has been shown to enhance the PRL response to both TRP (Glue et al., 1986) and clomipramine (McCance et al., 1989), effects probably mediated through the drugs' capacity to facilitate pre-synaptic 5-HT release (Treiser et al., 1981; Walsh et al., 1991). In contrast, an increased PRL response is not seen following administration of the 5-HT2 agonist quipazine (Parati et al., 1980).

Studies with 5-HT antagonists have yielded more conflicting results (Tuomisto and Mannisto, 1985). Serotonin antagonists are known to inhibit suckling-induced PRL release in puerperal women (Crosignani et al., 1979), while the non-selective 5-HT antagonist m metergoline will either block or attenuate the prolactin response to TRP (McCance et al., 1987), mCPP (Mueller et al., 1986; Kahn et al., 1990) and fenfluramine (Quattrone et al., 1983). Oral methysergide, another non-selective 5-HT antagonist, inhibits sleep-induced PRL release in man (Tuomisto and Mannisto, 1985) and ergot alkaloids with 5-HT blocking properties, are known to decrease PRL in a number of pathological conditions (Chiiodini et al., 1976; D'Agata et al., 1977) as well as in normal puerperium (Delitala et al., 1977). Pindolol, the β-blocker with 5-HT1 antagonist properties has been reported to attenuate the PRL response to TRP (Smith et al., 1991) and the 5-HT2 agonist ritanserin attenuated the mCPP-induced PRL response (Seibyl et al., 1991). In contrast, BRL 43694 a 5-HT3 antagonist, failed to block the TRP-induced PRL response in healthy
volunteers. The decrease in PRL response seen with 5-HT antagonists may result from the non-specificity of the drugs (Tuomisto and Mannisto, 1985). Many of the drugs have mixed dopaminergic agonist/antagonist properties which may in themselves explain PRL secretion (Creese et al., 1975; Cocchi et al., 1978; Ellis et al., 1991).

Overall, data from both animal and human studies support the hypothesis that 5-HT acts as a stimulatory transmitter in the secretion of PRL (Tuomisto and Mannisto, 1985; Cowen and Anderson, 1986). Serotonergic neurones arising in the dorsal raphe nucleus stimulate PRL release in the rat by activation of 5-HT$_1$ and/or 5-HT$_2$ receptors located on the putative prolactin releasing factor neurones (Van de Kar, 1991). A similar mechanism involving 5-HT neurones is proposed for man, but the small number of antagonist drugs available for human work has meant that identification of the exact 5-HT receptor subtype mediating PRL secretion has yet to be determined (Van de Kar, 1991). Data outlined above, however, suggests a role by 5-HT$_1$ and possibly 5-HT$_{2A/2C}$ receptors although unidentified 5-HT receptor subtypes cannot be ruled out (Van de Kar, 1991).

When plasma PRL levels are measured throughout the morning a fall in concentration is seen across time, reflecting the circadian fall in PRL release that occurs in humans at this time of day (Figure 2.1). All neuroendocrine challenge tests in this thesis were carried out during the morning.

![Figure 2.1](image.png)

Figure 2.1. Mean ± sem PRL response in twelve healthy female volunteers following placebo administration at 0900 hours. PRL levels fell across time reflecting the circadian fall in PRL release seen throughout the morning.
2.1.4 5-HT CHALLENGE DRUGS IN HUMANS

The recent discovery of multiple biochemical forms of the 5-HT receptor (Peroutka et al., 1979; Peroutka, 1988) has been associated with a growing awareness of the distinct functional properties mediated by each receptor subtype (see section 1.2.2). In man, the post synaptic effects of 5-HT appear to be mediated by multiple 5-HT receptor subtypes. 5-HT1A receptors are found presynaptically on the 5-HT cell bodies where they function as autoreceptors (Cowen et al., 1990b). A similar autoreceptor function is carried out by 5-HT1D receptors located on the nerve terminals (Hoyer, 1988a). Outlined in Figure 2.2 is a schematic diagram of a typical 5-HT neurone and synapse, with receptor subtypes indicated.

![Schematic diagram of 5-HT neurone and synapse](image)

Figure 2.2 Schematic diagram of 5-HT neurone and synapse
The delineation of 5-HT receptor subtypes has in turn led to an interest in the development of selective agonist and antagonist drugs that can be utilised safely in man. A wide range of drugs, including 5-HT precursors, releasing agents, reuptake inhibitors, receptor agonists and antagonists have been used as 5-HT neuroendocrine challenge probes (Yatham and Steiner, 1993). Some of these are outlined in Table 2.1.

Table 2.1

<table>
<thead>
<tr>
<th>Drugs Used to Evaluate Human Serotonergic Function</th>
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<tr>
<td>Precursors in Serotonin Synthesis</td>
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<tr>
<td>TRP 5-Hydroxytryptophan</td>
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<td>Serotonin Releasing Agents</td>
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<td>( d)-Fenfluramine</td>
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<td>( dl)-Fenfluramine</td>
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<tr>
<td>Serotonin Reuptake Inhibitors</td>
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<td>Clomipramine</td>
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<td>Fluoxetine</td>
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<td>Fluvoxamine</td>
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<tr>
<td>Serotonin Agonists</td>
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<td>( m)CPP (5-HT(_{1A/1D/1C}))</td>
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<td>Buspirone (5-HT(_{1A}))</td>
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<tr>
<td>Gepirone (5-HT(_{1A}))</td>
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<tr>
<td>Ipsapirone (5-HT(_{1A}))</td>
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<tr>
<td>Quipazine (5-HT(_{1}))</td>
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<tr>
<td>Serotonin Antagonists</td>
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<td>Methysergide (5-HT(_{1/2}))</td>
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<tr>
<td>Metergoline (5-HT(_{1/2}))</td>
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<tr>
<td>(-) Pindolol (5-HT(_{1A/1B}))</td>
</tr>
<tr>
<td>Ritanserin (5-HT(_{2A/2C}))</td>
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<tr>
<td>Ketanserin (5-HT(_{2}))</td>
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</tbody>
</table>

Most drugs utilised as 5-HT challenge probes share the problem of lack of serotonergic specificity. For example, the 5-HT precursors, including TRP and 5-hydroxytryptophan, are not 5-HT specific as they affect catecholamine metabolism (van Praag et al, 1986) and the 5-HT releasing drug, \( dl\)-fenfluramine is also known to effect dopaminergic neurotransmission at high concentrations (Invernizzi et al, 1986). Drugs acting as 5-HT antagonists also lack 5-HT specificity. Methysergide and metergoline have dopaminergic agonist properties (Krulich et al, 1981) and a recent study by Ellis et al (1991) demonstrated that the PRL response to the dopamine agonist haloperidol was blocked by metergoline, suggesting that metergoline's PRL inhibitory action may be dopaminergic rather than serotonergic-mediated. Another 5-HT antagonist, cyproheptadine, has antagonist properties at both muscarinic and histaminic receptors sites (Leysen et al, 1981). More selective antagonist drugs are beginning to become available such as the selective 5-HT\(_{2}\) antagonists ritanserin and ketanserin.
The development and availability of specific 5-HT agonists that stereo-selectively interact with specific receptor subtypes has equally been slow, although the recent introduction of the azapirones (5-HT<sub>1A</sub> partial agonists including gepirone, ipsapirone and buspirone) and the non-selective 5-HT agonist mCPP, is said to have improved the situation (Yatham and Steiner, 1993). These new drugs are not without their own problems however. For example, at least one of the azapirones, buspirone, has recently been reported to affect dopaminergic transmission (Cowen <i>et al</i>, 1990a). A comprehensive review of all 5-HT drugs utilised in man is beyond the scope of this thesis but a brief overview of the challenge drugs used in the following experimental work will be outlined below.

### 2.1.4.1 Fenfluramine

<em>Chemistry, Pharmacokinetics, Receptor Binding and Behavioural Studies</em>

Fenfluramine exists in two stereo-isomer forms; the racemic isomer, dl-fenfluramine and the dextro-rotatory isomer, d-fenfluramine (Campbell, 1991; McTavish and Heel, 1992). Fenfluramine's main action appears to be on the serotonergic system where it brings about an enhancement of 5-HT neurotransmission. Early studies demonstrated that enhancement of serotonergic neurotransmission took place indirectly by stimulation of pre-synaptic 5-HT release from nerve terminals. Hence, depletion of 5-HT stores was believed to be necessary before the drug could exert its pharmacological effect (Fuller <i>et al</i>, 1988). More recent studies have demonstrated that fenfluramine also exerts effects on serotonergic neurotransmission through inhibition of 5-HT re-uptake (Campbell, 1991) and possible direct post-synaptic agonist activity at 5-HT<sub>2C</sub> receptor sites by the metabolite, norfenfluramine (Invernizzi <i>et al</i>, 1982). Specific binding studies of d-fenfluramine in the mouse, rat and guinea pig (Mennini <i>et al</i>, 1988) have demonstrated that the highest binding is to 5-HT<sub>1A</sub> > 5-HT<sub>1C</sub> > 5-HT<sub>1B/1D</sub> but as affinity to all these receptor subtypes is low, it is unlikely to contribute to the pharmacological activity (Campbell, 1991). An indirect action on 5-HT through stimulation of 5-HT<sub>1</sub> receptors is further suggested by studies demonstrating attenuation of fenfluramine-induced anorexia in both rats (Neill and Cooper, 1989) and man (Goodall and Silverstone, 1988b) by the 5-HT<sub>1</sub> antagonist metergoline but failure to block the food inhibitory effect of fenfluramine by either the 5-HT<sub>2</sub> antagonist ketanserin or the 5-HT<sub>3</sub> antagonist, ICS-205-930 (Neill and Cooper, 1989) and only partial blockade by the 5-HT<sub>2C</sub> antagonist ritanserin (Samanin <i>et al</i>, 1989).

Both isomers are capable of inducing 5-HT release, but d-fenfluramine is more potent in this respect (Campbell, 1991) and has the added advantage of being more serotonin specific in that it does not effect catecholamine neurotransmission (Consolo <i>et al</i>, 1980; Invernizzi <i>et al</i>, 1986). In the rat, administration of d-fenfluramine at plasma
concentrations of 5µmol/l leads to a 25% stimulation of serotonin release, which is accompanied by increased brain 5-HT concentrations (McTavish and Heel, 1992). The higher specificity and potency of the dextro-isomer has led to it become the preferred drug for use as a 5-HT challenge probe.

In humans d-fenfluramine remains detectable in plasma for up to forty-eight hours after a single oral dose, while multiple administration leads to the development of steady-state plasma concentrations in four to eight days (McTavish and Heel, 1992). The drug is highly lipophilic and is, therefore, widely distributed throughout the body. However, it does not appear to accumulate within the tissues and is virtually entirely eliminated via the kidneys. d-Fenfluramine has one main active metabolite, d-norfenfluramine, which is detectable in plasma one to two hours after administration of the parent drug. At 31.2 hours, the elimination half-life of d-norfenfluramine is longer than that of d-fenfluramine (18.2 hours) (McTavish and Heel, 1992).

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In the rat, dl-fenfluramine (Van de Kar et al, 1985) induces a dose-dependent increase in plasma PRL. The dl-fenfluramine-induced PRL response is potentiated by pretreatment with the 5-HT precursor, TRP, and blocked by pretreatment with 5-HT re-uptake inhibitors including fluoxetine and indalpine (Van de Kar et al, 1985). Inhibition of fenfluramine’s action by 5-HT re-uptake inhibitors is thought to take place by a mechanism in which the 5-HT re-uptake inhibitors prevent entrance of fenfluramine into the serotonergic nerve terminals. Stimulation of 5-HT release is therefore prevented. Similar results were obtained by Serri and Rasio (1987) using d-fenfluramine at doses thought to ensure fenfluramine’s relative specificity in interacting with serotonergic pathways. A dose-dependent increase in PRL followed d-fenfluramine administration which and this response was partially blocked by fluoxetine pre-treatment. Human studies further support the hypothesis that the fenfluramine-induced PRL response is 5-HT mediated. Plasma PRL is significantly increased by fenfluramine in healthy volunteers (Quattrone et al, 1978; Quattrone et al, 1983; Lewis and Sherman, 1985), depressed patients (Siever et al, 1984; Maes et al, 1989; O’Keane and Dinan, 1991) and in patients with other psychiatric illnesses (Lerer et al, 1988; McBride et al, 1991). The effect is dose-dependent and attenuated by pretreatment with the 5-HT antagonists, metergoline (Quattrone et al, 1978; Quattrone et al, 1983) and cyproheptadine (Lewis and Sherman, 1984). In a study by Goodall et al (1993), PRL responses to d-fenfluramine were abolished by pretreatment with the 5-HT2A/2C antagonist ritanserin.

In summary, administration of d- or dl-fenfluramine leads to a dose-dependent increase in plasma PRL concentrations. Evidence from both animal and human studies suggest, that
at least for \textit{d}-fenfluramine, the PRL response is 5-HT mediated. Furthermore, the most likely mechanism of action is indirect enhancement of serotonergic neurotransmission through 5-HT$_1$ receptors secondary to pre-synaptic 5-HT release and re-uptake inhibition. Direct post-synaptic receptor activity by the metabolite \textit{d}-norfenfluramine is also possible. Thus, \textit{d}-fenfluramine is a relatively specific 5-HT drug, well tolerated by humans (Campbell, 1991), that can be utilised to examine brain serotonergic function.

2.1.4.2 \textit{mCPP}

\textit{Chemistry, Pharmacokinetics, Receptor Binding and Behavioural Studies}

\textit{mCPP} belongs to the piperazine group of drugs and is a metabolite of the antidepressant trazodone (Caccia \textit{et al}, 1982). Early animal work suggested that the effects of \textit{mCPP} were most consistent with direct agonist activity at central 5-HT receptors (Samanin \textit{et al}, 1979), findings in keeping with \textit{in vitro} studies. Receptor binding in the rat has demonstrated a potent interaction by \textit{mCPP} with 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$ and 5-HT$_{2C}$ receptors (Hoyer, 1988a; Hamik and Peroutka, 1989), with the most potent agonist activity occurring at 5-HT$_{1B/2C}$ receptor sites. In the rodent, \textit{mCPP} also binds to other 5-HT$_2$ and 5-HT$_3$ receptors where it displays antagonist activity (Conn and Sanders-Bush, 1987; Kilpatrick \textit{et al}, 1987). Less potently, \textit{mCPP} binds to $\alpha_1$-adrenergic and $\beta$-adrenergic receptors (Invernizzi \textit{et al}, 1981).

Rat behavioural responses to \textit{mCPP} include decreased food intake (Samanin \textit{et al}, 1979; Bagdy \textit{et al}, 1989b) and reduced locomotion (Vetulani \textit{et al}, 1982; Kennett and Curzon, 1988c; Bagdy \textit{et al}, 1989b), effects that are blocked by pretreatment with 5-HT antagonists. Kennett and Curzon (1988c) were able to block \textit{mCPP}-induced hypoactivity with the 5-HT$_{1/2}$ antagonists metergoline and cyproheptadine, and with the 5-HT$_{2C}$ antagonist, mianserin but not with either the 5-HT$_2$ receptor antagonists ketanserin or ritanserin, or the 5-HT$_3$ antagonist ICS 205-930. Furthermore, blockade of \textit{mCPP}-induced hypoactivity was not achieved with the 5-HT$_{1A/1B}$ antagonists (-) pindolol, (-) propranolol and (+/-) cyanopindolol, or following pretreatment with the 5-HT$_{1A/2}$ and dopamine receptor antagonist spiperone. Pretreatment with metergoline, cyproheptadine, mianserin and the 5-HT$_{2C/2}$ antagonist 1-naphthyl-piperazine, but not ketanserin, ritanserin or ICS 205-930, were found to oppose the \textit{mCPP}-induced reductions in feeding (Kennett and Curzon, 1988c). Furthermore, administration of \textit{mCPP} in the rat produces a dose-related hyperthermic response which is abolished by pretreatment with the 5-HT antagonist metergoline, but not the dopamine antagonist haloperidol, nor (-) pindolol, propranolol, or ritanserin. Taken together, the results from the above experiments strongly suggest that \textit{mCPP} exerts its behavioural and hyperthermic effects via 5-HT receptors. More specifically, they suggest a role by 5-HT$_{2C}$ receptors in mediating hypoactivity, 5-HT$_{1B/2C}$
receptors in feeding behaviour, while debate continues as to whether or not 5-HT$_1$ or 5-HT$_2$ receptors mediate the hyperthermic effects of mCPP (Pawlowski, 1984; Wozniak et al, 1989).

Binding studies on human brain cortex have also demonstrated potent activity by mCPP at central 5-HT receptor sites. In a study by Hamik and Peroutka (1989) mCPP demonstrated equal and high potency at all 5-HT receptor subtypes, while displaying less potent activity at $\alpha$-adrenergic, dopamine D$_1$ and D$_2$, and $\beta$-adrenergic receptors. When administered to humans, mCPP produces marked psychological effects including increased ratings in anxiety and decreases in happiness and calmness (Charney et al, 1987). 5-HT agonist activity by mCPP to induce an anxiogenic effect in humans is consistent with current hypotheses about the central role of 5-HT in the aetiology of anxiety disorders (Kahn et al, 1988) and is in keeping with findings from animal anxiety models. Although findings with 5-HT antagonists are somewhat inconsistent (Kahn et al, 1988), treatments which decrease 5-HT neurotransmission are known to have anxiolytic effects (Geller and Blum, 1970; Winter, 1972). In a study by Pigott et al (1993), intravenous administration of mCPP (0.1mg/kg) to ten patients with Obsessive Compulsive Disorder led to a significant exacerbation of their symptoms, including anxiety ratings. Pretreatment by the 5-HT antagonist metergoline however, completely blocked the exacerbation of symptoms, suggesting that these behavioural responses are also 5-HT$_{1A}$ receptor mediated. This study replicated findings of an earlier one by the same group in which oral mCPP-induced anxiety in six Obsessive Compulsive Disorder patients was blocked by metergoline pretreatment (Pigott et al, 1991).

When administered to humans, oral mCPP (0.25mg/kg) produces peak plasma concentrations after 2.5 hours (Kahn et al, 1990), and at a dose of 0.5mg/kg has a half life of approximately three hours (Murphy et al, 1989). Gender differences in absorption appear to exist. mCPP plasma concentrations in both healthy volunteers and patients in one study were twice as high in the men as in the women (Kahn et al, 1991). Oral administration produces wide variability in mCPP blood levels and the response is not clearly dose-related (Kahn and Wetzler, 1991). Intravenous mCPP results in more consistent plasma concentrations, but has the disadvantage of a greater number and severity of side effects including nausea, sweating, light headedness, palpitations, anxiety and chest pressure (Murphy et al, 1989). The physical effects of mCPP, such as nausea and light-headedness, appear to be dose-related, while mCPP-induced anxiogenic effects and panic attacks have a more complex relationship with the drug's administration. The onset of panic attacks and of anxiety-related symptoms appears dose-dependent following oral administration, but is dependent upon duration of infusion time after intravenous administration. A shorter infusion time has been found to induce panic attacks in greater numbers of patients and healthy volunteers (Kahn and Wetzler, 1991). mCPP elevates
blood pressure and pulse rate but not consistently (Mueller et al., 1985; Murphy et al., 1989; Kahn et al., 1990).

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mCPP consistently enhances PRL release in both rats (Aulakh et al., 1988a; Bagdy et al., 1989b) and non-human primates (Aloi et al., 1984). In the rat the mCPP-induced PRL response is blocked by pretreatment with the 5-HT$_{1A}$ receptor antagonist metergoline (Aloi et al., 1984; Bagdy et al., 1989b), but not the 5-HT$_{2C}$ receptor antagonist ritanserin or 5-HT$_{3}$ antagonist MDL 72,222 (Bagdy et al., 1989b). Ritanserin pretreatment in the rhesus monkey also leads to only partial blockade of the mCPP-induced PRL response (Henninger et al., 1988).

Human neuroendocrine studies in healthy volunteers have demonstrated consistent mCPP-induced PRL responses at oral doses of 0.5 and 0.75mg/kg, with marginal effects seen at 0.25mg/kg (Mueller et al., 1985; Murphy et al., 1989; Kahn et al., 1990). mCPP-induced PRL responses have also been reported in patient populations (Zohar et al., 1987; Kahn et al., 1992). The dose-response relationship does not appear to be linear, with 0.5mg/kg of mCPP releasing five times more PRL than the 0.25mg/kg dose (Kahn et al., 1990). Peak hormone responses across the three doses were reached at approximately two hours after drug administration (Kahn et al., 1990). Intravenous administration within the dose range of 0.06-0.1mg/kg also produce reliable and consistent elevations in PRL when compared to placebo, with a smaller PRL response reported at 0.05mg/kg (Charney et al., 1987; Lawlor et al., 1989; Murphy et al., 1989). Time to peak PRL response appears variable following intravenous mCPP and may occur as late as ninety to 120 minutes (Kahn et al., 1990). Gender differences have been reported for mCPP-induced PRL responses. Although mCPP plasma concentrations are lower in women compared to men at a given dose, PRL release is greater (Charney et al., 1987; Kahn et al., 1991). It has been suggested that the gender difference is due to circulating oestrogens in women potentiating the mCPP-induced PRL response as has been reported with other 5-HT challenge drugs (O'Keane et al., 1991). Blockade of oral mCPP-induced PRL responses have been reported following pretreatment with both metergoline (4mg) and methysergide (4mg), the 5-HT$_{1A}$ antagonists (Kahn et al., 1990). In a study by Pigott et al. (1991), pretreatment with metergoline was found to block the PRL response to mCPP in a group of twelve patients with Obsessive Compulsive Disorder.

In a drug capable of inducing panic attacks and anxiety symptoms, the question must be raised as to whether or not the hormone responses are secondary to its behavioural and physical effects. The finding that hormonal responses followed the onset of physical and behavioural responses would support this theory. Following intravenous administration of
mCPP, this sequence of events has been reported with peak hormonal responses reported to occur fifteen to thirty minutes after peak anxiety levels (Charney et al, 1987; Murphy et al, 1989). However, following oral administration, the picture is much less clear. One study reported peak anxiety as preceding peak PRL response by ninety minutes (Murphy et al, 1989), but another found the peak physical symptoms coincided with peak PRL levels (Kahn et al, 1990). No increases in anxiety were reported to have occurred.

In summary, considerable evidence exists from both animal and human studies to support the use of mCPP as a highly selective probe of serotonergic function (Kahn and Wetzler, 1991). It appears most potent as a 5-HT agonist and in humans, induces reliable PRL responses following oral and intravenous drug administration. Animal studies suggest mCPP-induced PRL is probably mediated via direct agonist activity at post-synaptic 5-HT \textsubscript{1B} (rat) or 5-HT \textsubscript{2C} (rhesus monkey) receptors. The limited antagonists studies so far carried out in humans confirm mCPP-induced PRL release via agonist action at 5-HT receptors, but the subtype(s) involved remains unknown. While caution must be taken in the interpretation of mCPP-induced PRL responses where significant behavioural effects have occurred, careful study design should minimise potential confounding factors.

2.1.5 METHODOLOGICAL CONSIDERATIONS

The need for careful control of neuroendocrine challenge tests has been emphasised by Checkley (1980), who outlined specific criteria that must be satisfied before it can be assumed that a hormone response to a particular drug results from stimulation of a specified receptor. The criteria are:

i) The same hormone response should result from the administration of all drugs which act as agonists for the specified receptor.

ii) The hormone response to receptor stimulation should be inhibited by drugs which act as selective antagonists for the receptor.

iii) The hormone response should not be inhibited by drugs which are antagonists at other receptors.

iv) Further evidence is provided if antagonists of the receptor also inhibit hormone responses to physiological stimuli such as sleep or exercise.

In order to establish that the receptor being stimulated is located within the brain, two further considerations are required:

v) If the hormone response is only produced by agonist drugs that cross the blood-brain-barrier, then it can be assumed that the drugs must be working within the brain.
vi) Hormone responses produced by agonist drugs that do not cross the blood-brain-barrier may still be acting at receptors within the brain. This assumption is accepted if animal experiments provide evidence that the receptors lie within the median eminence, a structure without the usual blood-brain-barrier.

The above criteria apply to drugs acting directly at the site of the receptor. For precursors of the challenge drug or indirect agonists, alterations in hormone responses may also result from changes in neurotransmitter release or reuptake.

Accurate interpretation of monoamine function using a neuroendocrine challenge is only possible if careful controls are made of all other biological variables that may in themselves alter the hormone responses under examination (Checkley, 1980). Such controls are applicable to both healthy and patient populations and those seen as most important are outlined below.

i) For patient populations, the concurrent use of psychotropic drugs is a major source of artefact in neuroendocrine testing as most alter monoamine function (Checkley, 1980). Some psychotropic drugs act directly at the level of the pituitary to alter hormone secretion (Meltzer et al, 1978) while others may interact with the psychoactive drugs (eg. amphetamine or clonidine) being employed as the neuroendocrine challenge. Other psychotropic medication is known to alter receptor sensitivity (Friedhoff and Alpert, 1978). Consequently, it is usual to exclude the concurrent use of antidepressant and neuroleptic drugs with a washout period of at least three weeks, although debate exists as to whether or not this length of time is sufficient (Corn et al, 1984; Schittecatte et al, 1989). Night sedation is often permitted but again caution is required as evidence exists that, at least acutely, benzodiazepines may alter hormone responses (Nutt and Cowen, 1987).

ii) For all subject groups, the use of recreational drugs such as alcohol, caffeine and nicotine must be considered. Excessive intake of alcohol leads to suppression of pituitary hormone responses (Chalmers et al, 1977), while excessive caffeine, which is pharmacologically related to amphetamines, may stimulate hormone release (Checkley, 1980). Subjects partaking in excess of either are, therefore, best excluded.

iii) Age and sex must be controlled for as both influence hormone secretion.

iv) Hormone responses such as GH and PRL appear to alter at different phases of the menstrual cycle. Studies utilising the 5-HT1A partial agonist buspirone, have shown marked variations in PRL response across the menstrual cycle with a three fold increase.
seen premenstrually (Yatham et al, 1989; Dinan et al, 1990). In a more recent paper, d-phenfluramine-induced PRL responses also varied with the phase of menstrual cycle and were found to be greatest at mid-cycle (O'Keane et al, 1991). Such variations in hormone responses are thought to be primarily oestrogen effects. Careful control of the menstrual cycle phase during which the neuroendocrine challenge is carried out is therefore essential.

v) The secretion of ACTH, GH and PRL are all increased by stressful situations (Mason, 1968; Greene et al, 1970), including procedures such as venipuncture. A baseline period of 30-60 minutes following insertion of the venous cannula (before the challenge drug is given and hormone sampling begins) is usually incorporated into the neuroendocrine procedure. The challenge drug itself may produce unwanted side effects such as nausea and anxiety, which again will influence the hormone response.

vi) Nutritional status is likely to be important. Malnutrition is known to directly affect hormone secretion (see sections 1.1.2.3 and 1.3.1.3), and moderate weight loss has been shown to influence PRL responses to TRP in women (Goodwin et al, 1987b; Anderson et al, 1989).

vii) A number of other variables are thought important in influencing hormonal responses. These include severe physical activity (Casmore et al, 1977), the presence of chronic physical illness (Krieger, 1975) and pregnancy (Burns, 1976). Standardization of the time of day at which tests are carried out is necessary due to the pulsatile and circadian rhythm of secretion that governs most anterior pituitary hormones.

Three other points not covered by Checkley (1980) are emphasised by Anderson (1992).

viii) The hormone response depends not only upon the activity of the monoamine synapse with which the drug interacts, but on a series of steps beginning with the monoamine neurone and ending with secretion of the hormone from the anterior pituitary. Other variables such as the rate at which the hormone is cleared from the body will also influence the final response. Thus, any given challenge tests the function of the whole neuroendocrine system and an abnormality in any one of the steps could account for an abnormal finding in a given patient population.

ix) Consideration must be given to the pharmacokinetics of the challenge drug being used. Oral administration of drugs such as tricyclic antidepressants are known to result in variable absorption rates (Preskorn, 1993) leading to inconsistent plasma levels which in turn may affect the hormone response.
Finally, great inter-individual variations in hormone responses to a given challenge drug exist, with the consequence that small changes may be obscured by the size of the variance, leading to false negatives (type II errors). Anderson (1992) points out that while no easy solution exists for this problem, study replication and the use of appropriate statistical analyses will lessen the possibility of small but important changes being missed.

### 2.2 TRYPTOPHAN DEPLETION STUDIES OF 5-HT

TRP depletion studies provide an alternative method of studying 5-HT function in the living human brain (Young et al, 1985; Delgado et al, 1990; Oldman et al, 1994). The rationale behind these studies is the role TRP availability plays in the control of brain 5-HT synthesis (see section 1.2.1 for a full explanation).

#### 2.2.1 ANIMAL STUDIES

Animal work has demonstrated that dietary manipulations which acutely lower plasma TRP produce decreases in brain 5-HT turnover in both rodents and non-human primates (Young et al, 1989; Gartside et al, 1992). Increases (Fernstrom and Hirsch, 1975) and decreases (Gal and Dreses, 1962) in dietary TRP lead to corresponding changes in brain TRP and 5-HT levels in rodents. In a study by Biggio et al (1974), a TRP-free diet given to rats produced a 90% reduction in plasma free TRP accompanied by a 85% reduction in brain TRP, a 58% fall in brain 5-HT and a 76% fall in brain 5-HIAA within two hours of depletion. Furthermore, dietary TRP depletion in rodents enhances behavioural indices thought to be mediated by decreased brain 5-HT function. TRP depletion in rats increases sensitivity to pain (Lytle et al, 1975), increases muricidal behaviour i.e. mouse killing (Gibbons et al, 1979a) and reduces rapid eye movement sleep (Moja et al, 1979), behaviours that are all reversed by TRP repletion. 5-HT depletion secondary to a low TRP diet has also been shown to result in enhanced PRL responses to a 5-hydroxytryptophan infusion, presumably through the development of post-synaptic receptor supersensitivity secondary to reduced brain 5-HT synthesis (Clemens et al, 1980). The administration of TRP-free amino acid drinks to rats also leads to a rapid reduction in plasma TRP and brain 5-HT concentrations (Moja et al, 1989) while Gartside et al (1992) were able to show that administration of a similar amino acid treatment decreased the 5-HT release evoked by electrical stimulation of the dorsal raphe nucleus of rats in vivo. In a study by Young et al (1989) the effects of TRP depletion in non-human primates i.e. vervet monkeys was examined. Using a TRP-free amino acid drink paradigm, a smaller but highly significant reduction in plasma TRP was seen compared to those changes obtained in the rodent. The reduced plasma TRP levels were accompanied by reductions in CSF 5-HIAA concentrations (Young et al, 1989), suggesting corresponding reductions in central 5-HT function.
2.2.2 HUMAN STUDIES

In humans, brain 5-HT can be reduced either by blocking synthesis or by restricting dietary TRP (Miller et al, 1992). Two clinical studies published in the 1970s utilised the 5-HT synthesis inhibitor p-chlorophenylalanine, which blocks synthesis of 5-HT by inhibition of the rate limiting enzyme tryptophan hydroxylase. The authors used p-chlorophenylalanine to examine the role of 5-HT in depression and found that inhibition of 5-HT synthesis by p-chlorophenylalanine reversed the antidepressant effects of both the tricyclic imipramine (Shopsin et al, 1975) and the monoamine oxidase inhibitor tranylcypromine (Shopsin et al, 1976). However, the use of p-chlorophenylalanine is associated with side effects (Shopsin et al, 1976) including a high incidence of allergic eosinophilia, rendering its use as an investigative agent in humans highly problematic (Miller et al, 1992). Diets free or low in TRP have been administered to healthy volunteers as an alternative method of reducing brain 5-HT. In a study by Delgado et al (1989) subjects were given diets containing two different magnitudes of TRP restriction, i.e. 200mg and 700mg TRP respectively for ten days. Both diets led to a reduction in total plasma TRP of between 15% and 20% with greater falls observed in the women compared to the men. No significant fall in plasma free TRP was seen but a trend towards a decrease was noted. Of further interest were the responses to an intravenous TRP infusion given in a neuroendocrine challenge paradigm, both before and following the ten day low TRP diet. An enhanced PRL response was seen following the low TRP diet, in keeping with the animal data. Several practical issues including the complexities of administering a low TRP diet and the modest falls in plasma TRP obtained as a result, limit dietary restriction as a method of 5-HT depletion (Miller et al, 1992).

A number of studies have been published which utilise a mixture of amino acids deficient in TRP to achieve an acute reduction in transportation of plasma TRP to the brain, and hence a reduction in brain 5-HT synthesis (Young et al, 1985; Delgado et al, 1990). This method of amino acid loading reduces brain TRP in two ways. Firstly, TRP transportation into the brain occurs by an active transport system, which is shared by five other amino acids. Competition for the carrier site can lead to a deficiency of any of these amino acids relative to the others (Gessa et al, 1974). Thus, if a large amino acid load deficient in TRP is given, the ratio of TRP to other large neutral amino acids will fall, reducing the amount of TRP transported across the blood brain barrier. As outlined in section 1.2.1, TRP availability to the brain acts as the rate limiting step in the synthesis of 5-HT, hence any reduction in TRP entering the brain will be accompanied by a fall in 5-HT synthesis. Secondly, and perhaps more importantly, an amino acid load deficient in TRP appears to increase protein synthesis in the liver leading to a subsequent fall in tissue and plasma TRP stores (Biggio et al, 1974). The drive for protein synthesis depletes
plasma TRP, thereby diminishing TRP transportation into the brain through increased competition with other amino acids. This method has been shown to effectively reduce levels of plasma total and free TRP in humans (Young et al., 1985; Delgado et al., 1990). For example, in the study of Young et al (1985), a 100g amino acid drink deficient in TRP led to a 60% reduction in plasma free TRP five hours after administration. Acute TRP depletion has been shown to reverse the analgesic effects of morphine in the cold-pressor test in healthy males (Abbott et al., 1992) in keeping with the animal literature that suggests 5-HT mediated behaviours are also altered by the amino acid drink paradigm. Furthermore, as outlined above, animal data strongly supports the hypothesis that reduced plasma TRP leads to reduced brain TRP and a fall in 5-HT synthesis.

In conclusion, this methodology provides an acute manipulation of brain 5-HT which can be safely carried out in the human population. Most studies in humans have used doses of 100g of amino acids (Young et al., 1985; Smith et al., 1987; Delgado et al., 1990) but such high doses are associated with a high incidence of nausea. A pilot study carried out by our group examined the effects of a 52g dose of amino acids given to nine healthy volunteers (Oldman et al., 1994). The 52g dose led to a fall in both total and free plasma TRP 4.5 hours after administration. The magnitude of the fall was comparable to that seen with the higher dose of 100g and the drink well tolerated by subjects in terms of unwanted side effects. On the basis of these findings, the 52g amino acid drink appeared to offer the most effective method of acutely lowering plasma TRP allowing an accurate assessment of the effects of acute brain 5-HT depletion on appetite and mood in the absence of confounding side effects.

2.2.3 THE MENSTRUAL CYCLE: EFFECTS ON APPETITE AND TRYPTOPHAN METABOLISM

2.2.3.1 Menstrual Cycle Effects on Appetite

Any study that aims to examine appetite in women must take into account the effects of hormone changes across the menstrual cycle. The past few years have brought an increased awareness of the changes in mood and appetite that accompany the phases of the menstrual cycle in both normal women and in women that report pre-menstrual symptoms (Bancroft et al., 1988; Warner and Bancroft, 1990; Goodall and Silverstone, 1993). Most published studies have simply divided the menstrual cycle into follicular (defined as the first ten days of the cycle from the onset of menses) and luteal (defined as the last ten days of the cycle before the onset of menses) phases, but two studies have further differentiated the periovulatory phase (Gong et al., 1989; Lyons et al., 1989). Methods of measuring food intake have also varied. Food diaries, recording daily intake over the two ten day periods corresponding to the follicular and luteal phases of the menstrual cycle and then "averaged
out", have been the most common approach. However, some researchers have utilised a test meal method, again given at times to correspond with the follicular and luteal phases. While criticism has been levelled at the methodological limitations of both study approaches (Goodall and Silverstone, 1993), consistent findings have been reported across the menstrual cycle with respect to both total energy intake and macronutrient selection.

In a population survey carried out by Warner and Bancroft (1990), 5,457 women completed a menstrual health questionnaire that had been published in a women's magazine. Of the women who completed the questionnaire, 36% reported "severe" cravings for sweet foods around the time of menstruation, 7% reported "severe" cravings for salty foods and 11% reported cravings for other types of food perimenstrually. A prospective study was then carried out on seventy-six women who had reported premenstrual cravings in the magazine survey (Bancroft et al, 1988). The study confirmed a premenstrual pattern of food craving in 72% of the women, with cravings confined to the menstrual phase in 13%. No consistent association between food cravings and mood change, in terms of either severity or timing of symptoms, or between food cravings and breast tenderness were found. The authors concluded, therefore, that perimenstrual food craving was a cyclical phenomena in its own right and not dependent upon mood or other physical changes. In support of this hypothesis, Cohen et al (1987) found no relationship between mood scores, reported food cravings and eating scores amongst his group of healthy undergraduate women. However, Both-Orthman et al (1988) reported a significant positive correlation, premenstrually, between mood change and increased appetite in women reporting severe cyclical mood swings. This finding did not extend to those women who did not report significant premenstrual mood changes.

Other studies have examined total energy intake between the follicular and luteal phases. In two out of three studies using daily food diaries over ten days of the follicular and luteal phases, mean energy intake was found to be higher in the luteal phase compared to the early follicular phase (Dalvit-McPhillips, 1983; Giannini et al, 1985; Manocha et al, 1986) and appeared to be accompanied by greater food cravings (Cohen et al, 1987). A higher mean energy intake during the luteal phase compared to the follicular phase has been confirmed by three further studies using single day food measurements (Pliner and Fleming, 1983; Leiter et al, 1987; Bowen and Grunberg, 1990), but Wurtman et al (1989) were unable to replicate these findings using a test meal procedure given in the follicular and luteal phases. Measurements of appetite have been found to be increased in the premenstrual week (Both-Orthman et al, 1988), while Lyons et al (1989) reported decreases in energy intake in the periovulatory period compared to premenses, postovulatory and menses phases.


Macronutrient preference across the menstrual cycle has been examined. Increased carbohydrate (Dalvit-McPhillips, 1983; Leiter et al, 1987), sweet food (Leiter et al, 1987; Bowen and Grunberg, 1990) and fat (Anantharaman-Barr et al, 1988; Tarasuk and Beaton, 1991) consumption has been reported in the luteal phase compared to the follicular, while a significant decreased protein intake has been found in the periovulatory phase (Lyons et al, 1989), and perimenstrually (Abraham et al, 1981).

2.2.3.2 Menstrual Cycle Effects on Tryptophan Metabolism

One study has examined the effects of the menstrual cycle on TRP loading. Hrboticky et al (1989) studied the metabolism of TRP during the follicular and luteal phases of the menstrual cycle in eight healthy women. Three grams of TRP or placebo was given during the follicular and luteal phases across two menstrual cycles in a cross over design. Three hours after TRP administration, plasma kyneurine concentrations were 40% higher, and urinary kyneurine excretion was 28% greater in the luteal compared to the follicular phase. The results strongly suggest that rate of catabolism of TRP, via the kyneurine pathway in the liver, is altered across the menstrual cycle and is higher in the luteal phase.

2.2.3.3 Appetite Research Design

As can be seen from the above brief review, consistent changes in appetite occur across the menstrual cycle and must, therefore, be taken into account in any study design. The most consistent change occurs in the late luteal phase and involves increased energy intake and enhanced carbohydrate consumption. Increased metabolism of TRP during the luteal phase is also suggested from the findings of one paper. On the basis of these results, it would be important to exclude the premenstrual phase from both appetite and endocrine studies.

2.3 GENERAL METHODS

2.3.1 MEASURES OF VITAL SIGNS AND SIDE EFFECTS

Adverse reactions and side effects from challenge drugs were monitored by a combination of physiological readings to observe changes in the subject's vital signs, and visual analogue (VA) scales.

2.3.1.1 Vital Signs
layers, the hexane extract was transferred to a tapered 10ml tube which contained 1ml of 0.05M sulphuric acid for back extraction. To the remaining aqueous phase, 50µl of butyl acetate and 500µl of 5M ammonia was added and vortexed for twenty-five seconds. The solution was centrifuged at 2000g for five minutes and the lower aqueous phase aspirated and discarded. The upper solvent phase was then ready for injection into the gas liquid chromatograph (Varian 3400; Varian Ltd., Wotton-on-Thames, Surrey).

Results were interpolated from standard curves and linear calibration curves were obtained for both d-fenfluramine and d-norfenfluramine in plasma with high correlation coefficients (r=0.98). The intra-assay CV was between 3.3% and 12% for both d-fenfluramine and d-norfenfluramine, while the inter-assay CV was 6.9% and 27.5% for d-fenfluramine and d-norfenfluramine respectively. The limit of detection for d-fenfluramine was 0.5ng/ml and for d-norfenfluramine, 2ng/ml.

### 2.3.2.4 mCPP Assay

Plasma mCPP was assayed by high performance liquid chromatography as described by Franklin (1992). This procedure, based on solid-phase sorbent extraction of mCPP from the plasma followed by isocratic reversed-phase high performance liquid chromatography with coulometric detection, utilises gepirone was as the internal standard. Standard curves were prepared fresh daily and consisted of five concentration points over the range 1-25ng/ml of mCPP in drug free plasma. Using the Vac-Elut Manifold system (Jones Chromatography Ltd.; Hengoed, Mid-Glamorgan), 50ng of the internal standard gepirone was added to each 1ml volume of sample or standard. Cyanopropyl sorbent columns (Bond Elut, Analytichem International, Hbour City, USA) were conditioned with single column volumes of methanol and water, the vacuum being diverted to keep the columns from drying out. The sample or standard was transferred to the column and the vacuum applied to allow the materials to pass completely through. Each column was washed with two column volumes of distilled water and taken to full dryness under vacuum. The vacuum was again diverted and the manifold needles wiped dry and a collection tray containing 10x75mm glass tubes inserted into the Vac-Elut Manifold system. The compounds were eluted with one column volume of methanol and the methanolic eluates evaporated to dryness under vacuum at 40°C. Samples were reconstituted in mobile phase (0.04M potassium phosphate buffer adjusted to pH6.45 with 2M KOH, acetonitrile and methanol [600:250:150 v/v/v]), vortexed and injected into the high performance liquid chromatograph (Coulochem II, ESA, Bedford, Mass., USA).

Results were interpolated from standard curves, determined by assaying pooled drug-free plasma spiked with known amounts of mCPP. Peak heights rather than peak areas in the chromatograms were normally measured. Concentrations of mCPP were assessed by
using the slope of the standard curve for peak-height ratios for the analyte and the internal standard. The intra-assay CV was between 3-8.5% and the inter-assay CV was 5.9%. The detection limit was 0.07ng and plasma concentrations of mCPP are quoted in nanograms per millilitre (ng/ml).

### 2.3.2.5 Oestradiol Assay

Plasma oestradiol concentrations were measured using a highly specific radioimmunoassay kit from Diagnostic Products Corporation, Los Angeles, California. The kit contained all necessary reagents and tubes for the procedure, including oestradiol antibody-coated tubes, iodinated oestradiol ($^{125}$I-oestradiol) and oestradiol calibrators (i.e. 0, 20, 50, 150, 500, 1800 or 3600pg/ml of oestradiol in processed human plasma) from which the standard curve was constructed.

Four plain uncoated polypropylene tubes were labelled "total" and "non specific binding" in duplicate. 100µl of each calibrator solution, the control solution and unknown plasma sample were pipetted into duplicate sets of antibody-coated tubes. 1ml of $^{125}$I-oestradiol was added to all tubes, vortexed, and the resultant solution incubated for three hours at room temperature. The contents of each tube, except "total" tubes, were decanted and allowed to drain on absorbent paper for two to three minutes. The tubes were placed in the gamma counter to be counted for five minutes.

Results were calculated by standard curve interpolation. Plasma oestradiol concentrations are quoted as pg/ml. The intra-assay and inter-assay CVs varied from 4-7% and 4.2-8.1% respectively over the range of the standard curve. The limit of detection was 8pg/ml.

### 2.3.2.6 TRP Assays

#### Total Tryptophan

Plasma total TRP was assayed according to the method described by Bloxam and Warren (1974). 10µl volumes of plasma were added to 2ml of ice-cold 10% (W/V) trichloroacetic acid contained in 4ml polystyrene tubes. The tubes were centrifuged at 2,500g for fifteen minutes at 4°C to remove the protein precipitate. Standards in the range 0-20 ng/ml TRP were prepared similarly. For very high values of plasma TRP (following TRP infusion) the deproteinised sample was further diluted with 10% TCA to give values within the range of the standard curve. To each sample/standard on ice was added 0.2ml of 2% (W/V) formaldehyde and 0.1ml of 6mM ferric chloride in 10% TCA, the tubes vortexed and immediately transferred to a heated dry block (temperature 100°C) for one
hour. The fluorescence in each tube was measured in a Hitachi model 2000 spectrofluorimeter (excitation wavelength 300nm, emission wavelength 440nm).

Results were interpolated from standard curves constructed of fluorescence versus TRP concentration. Plasma TRP concentrations are quoted as micrograms per millilitre (µg/ml). Intra- and inter-assay coefficients of variation were 4.4% and 9.4% respectively and the limit of detection was 0.1ng/l.

Free Tryptophan

Plasma free TRP was assayed according to the method described by Bloxam et al., (1977) 200µl of plasma was pipetted into the reservoir of a Millipore ultrafree 10,000 NMWL filter (Millipore UK Ltd.; Watford, UK). The sample was gassed with a 95% oxygen/5% carbon dioxide mixture by inserting a 19 gauge syringe needle into the reservoir above the sample for thirty seconds before quickly closing the top of the centrifuge tube to maintain the gas atmosphere. This prevents plasma pH increasing during centrifugation due to loss of carbon dioxide (which would result in a change in the proportion of TRP bound to albumin). The tubes were then inverted for ten minutes before being centrifuged for thirty minutes in an Eppendorf benchtop micro-centrifuge. The resultant filtrate was analysed as described for total TRP.

2.3.3 MEASURES OF APPETITE

Two methodologies were combined to measure appetite. Total calorie intake and macronutrient intake (carbohydrate, protein and fat) were calculated from the test meal procedure, while self-rating scales were used to measure appetite related parameters and macronutrient preferences.

2.3.3.1 Test Meal Procedure

The test meal is designed to measure total calorie intake (calculated in kilojoules [kJ]) and the calorie intake of each macronutrient (carbohydrate, protein and fat).

Selection of Items

As part of the initial screening procedure, subjects completed a food item checklist designed to determine food preferences from which the test meals were later based (see Appendix Four). Each volunteer was asked to rate a list of food item on a scale from one to nine, where one equated with "extremely dislike" and nine with "extremely like". Seven different food categories were included in the list and the test meal made up to include
items from each category plus margarine and tomatoes, thus ensuring a good selection of food from each type of macronutrient. Where ever possible, the favourite foods from each category was chosen but where some or all of the food items in one category were rated equally, set rules for selection were followed. If no clear favourite food item existed, the first item on the list was chosen eg. Rich Tea biscuits for the biscuit category.

For sandwich fillings the same rules were applied, with four choices provided. Two protein items were always included in the final selection, and if this was not achieved on preference alone, the least preferred non-protein item was swapped for the highest rated remaining protein food. The inclusion of two protein items within the sandwich fillings ensured a balanced selection of macronutrients and included cheddar cheese, cottage cheese, ham, tuna and chicken roll.

Amounts

In order to ensure that no constraints were placed on subjects in terms of the amount of a particular food, and hence macronutrient consumed, excess portions of each item was presented. The quantities with the approximate weights are set out in Appendix Five. Weights of particular items varied slightly because quantities were kept constant eg. two tomatoes.

Presentation

Test meals were presented in a standardised manner to minimise any confounding factors, such as visual cues that might help the volunteer determine how much of a particular item had been consumed. All excess packaging was removed (eg. crisp packets, yoghurt pots) and food presented on plates and in dishes that were kept identical for all test meals. Items of food were presented in a standardised layout on two trays which had been set by the author. Drinking water was also made available during the meal.

Instructions to Subjects

Volunteers were given a standard set of instructions before eating. They were asked "to ignore any dietary restrictions that you may have placed upon yourself with regards the types of foods that you would/would not eat, or the quantities of food that you would/would not eat, and to eat until you have had enough".

Calculation of Food Consumed
During preparation of the meals, all food items were pre-weighed to the nearest 0.1g using electronic scales (Ohaus, model CT1200-S, sensitive to the nearest 0.01g) and the weights entered onto a record sheet (see Appendix Six). The prepared food was then kept in the refrigerator covered with clingfilm to minimise water loss, which might alter the weight. Immediately the volunteer had completed the meal, food items were again weighed to the nearest 0.1g and the final weights entered onto the record sheet.

Calculation of Calorie Consumption

Food consumption was calculated by subtraction of the final weight for each item from its original weight. Data were then analysed to determine total calories consumed, total calories of each macronutrient i.e. fat, carbohydrate and protein, and total calories of sweet and savoury foods. Analysis of data was carried out on "Direct", a specifically designed software programme (for Goodall and Silverstone; London; UK). The programme is designed to analyse each meal separately and to provide a printout of the breakdown data for that meal.

2.3.3.2 Visual Analogue Scales

Visual analogue scales were used to measure parameters of appetite and satiety and included ratings such as "wish to eat" and "fullness". Each VA scale consisted of a ten centimetre line with a scale from zero to one hundred marked out in increments of ten across the top (see Appendices Three and Seven). Subjects were instructed to complete the VA scales by either placing a mark on the line or encircling the number that most closely reflected how they felt. For the test meal, the VA ratings were related to normal aspects of appetite and satiety including wish to eat, feel full, find/found the meal satisfying and could eat a large amount of food, and subjects were required to rate how they felt at that precise moment.

2.3.3.4 Presentation of Appetite Rating Scales

All VAs were given in a standardised order ten minutes before and ten minutes after the test meals.

2.3.4 MEASURES OF MOOD

A combination of VAs and the Profile of Mood States Questionnaire (POMS) were used to assess mood. In keeping with the appetite scales, subjects were instructed to complete the mood scales according to how they felt at that moment. Mood scales were included in the VAs as outlined in Appendices One, Two, Three and Eight.
2.3.4.1 Profile of Mood States Questionnaire

Mood was assessed using the sixty-five item version of the POMS (see Appendix Nine). Designed as a standardised adjective rating scale, this version measures six mood or affective states i.e. anxiety/tension, depression, anger/hostility, vigour, fatigue and confusion, and is recognised as a sensitive measure for use in normal, non psychiatric populations. Subjects are instructed to rate their current mood state by circling the number which best reflects to how they feel on a scale from zero ("not at all") to four ("extremely"). The scores are then tallied to provide six subscale totals.

2.4 SELECTION AND ASSESSMENT OF SUBJECTS

2.4.1 SCREENING QUESTIONNAIRES

2.4.1.1 Beck Depression Inventory

The Beck Depression Inventory (Beck et al, 1961) was used to screen volunteers for the presence of depressive illness and is outlined in Appendix Ten. The inventory is a twenty-one item, self-rated questionnaire in which each item describes a specific behavioural manifestation of depression. Volunteers were asked to rate each item, made up of four to six self-evaluative statements, by selecting the statement that best fitted them over the preceding week. Completion of the scale resulted in a total score (maximum of sixty) that provided a measure of the depth of depression. The Beck Depression Inventory has been validated for both psychiatric in- and outpatients and is provides a sensitive measure of change in depressed mood. It is also a useful tool in screening the normal population for the presence of depressive illness.

2.4.1.2 Eating Attitudes Test

The forty item Eating Attitudes Test was used to screen for the presence of eating disorders amongst normal volunteers, and to help elicit the continuing presence of an eating disorder amongst recovered bulimics. The Eating Attitudes Test (Garner and Garfinkel, 1979) is a self-rating scale with satisfactory reliability and validity for use as a screening device and is outlined in Appendix Eleven. The questionnaire is designed to help quantify disordered attitudes towards eating and food, and to address a broad range of symptoms of anorexia nervosa in three subscales i.e. dieting, bulimia and food preoccupation. Each question is accompanied by a six point scale in columns marked "never, rarely, sometimes, often, very often and always". Subjects were instructed to mark
the column for each question that most closely applied to how they had been over the previous four weeks. The completed questionnaire provided a total score, out of possible maximum of 120, that could be used as a guide to the extent of the presence of disordered eating patterns. A follow-up clinical interview enabled a firm diagnosis to then be made.

2.4.2 VOLUNTEER SELECTION

2.4.2.1 Normal Volunteers

Normal volunteers were recruited from hospital staff, research colleagues and by newspaper and poster advertisements. Potential subjects received a semi-structured clinical interview, physical examination, electrocardiogram (for all neuroendocrine challenge studies), and underwent routine blood screening (full blood count, platelets, liver function test, creatinine and urea and electrolytes). They also completed the Beck Depression Inventory, the Eating Attitudes Test and food preference forms from which acceptable test meals could later be prepared (see section 2.3.3.1). Subjects were excluded if they had any of the following: a history of cardiac or endocrine abnormality or any potentially life threatening illness (e.g., cancer), present or past history of psychiatric illness or substance abuse; alcohol intake above twenty-one units for men and fourteen units for women; drug treatment within the preceding two months. For the dieting studies, volunteers were required to be of normal weight, or no more than one and one-half stone overweight, as determined by a body mass index (weight in kg divided by height in metres$^2$) of between twenty and twenty-seven for females and twenty and thirty-two for males. All volunteers were required to be free of any eating disorder as determined by an abnormal Eating Attitudes Test score greater than twenty-five, or greater than fifteen when the presence of abnormal eating or an eating disorder was confirmed by clinical interview.

2.4.2.2 Recovered Female Bulimic Patients

Ex-patients were recruited mainly from newspaper advertisements, but a number of other sources were used including psychology outpatient waiting listings and medical records at the Warneford Hospital in Oxford and direct approaches to general practitioners within the area. All subjects had, at some stage in the past, met criteria for DSM-III-R Bulimia Nervosa (American Psychiatric Association, 1987) but were now recovered.

Recovery was determined by a semi-structured clinical interview administered by the author and the EAT and EDE-Q3 (Cooper and Fairburn, 1987) self-rating scales on eating behaviour. Criteria for recovery were: i) no episodes of self-induced vomiting, excessive exercise or laxative use within the past six months; ii) no more than one objective binge (a discrete episode of eating in which an abnormally large amount of food is consumed
accompanied by a sense of loss of control over eating) in the same period; iii) no more than one subjective binge per week (a discrete episode of eating in which the subject feels they have eaten an excessive amount for them, but the amount consumed would not be regarded by the average person to be excessive, accompanied by a sense of loss of control over eating). In addition, subjects were required to be of normal weight as determined by a body mass index between twenty-one and twenty-eight, to have regular eating habits and following a normal diet and their weight stable in the previous three months. Potential subjects scoring above twenty on the Beck Depression Inventory or currently undergoing treatment for major depression were excluded, as were those subjects who had been on medication in the previous three months (other than benzodiazepines) or who were judged to have a significant current physical illness, or a history of alcohol or drug abuse.
Chapter 3
EFFECTS OF DIETING ON BRAIN
5-HT FUNCTION

3.1 INTRODUCTION

The literature reviewed in Chapter One strongly suggests that "normal" dieting is in itself a risk factor in the development of the eating disorders. Dieting behaviour now appears to be endemic amongst the adolescent and young adult population in Western-style societies, and for the majority it remains predominantly a harmless pursuit. Recent evidence from animal and human studies, however, suggests that some physiological changes accompanying food restriction may contribute to the development of clinical eating disorders in predisposed individuals (see sections 1.1.4.3, 1.2.3 and 1.3.3).

Previous work, outlined in section 1.3.3, demonstrates that moderate weight loss through dieting leads to an enhanced PRL response to a subsequent TRP challenge in women but not men (Goodwin et al., 1987b; Anderson et al., 1990a). Animal and human data suggest that the PRL response to TRP is probably 5-HT mediated (Marsden and Curzon, 1976; Cowen and Anderson, 1986; Price et al., 1990), and work carried out by Anderson et al. (1989) further suggests that dieting-induced changes in the PRL response to TRP may also be 5-HT mediated. On the basis of these findings, it was suggested that moderate weight loss through dieting decreases plasma TRP sufficiently in women to reduce brain 5-HT synthesis. A subsequent reduction in 5-HT neurotransmission across the synapse results, which in turn produces an adaptive supersensitivity of post-synaptic 5-HT receptors, as evidenced by an enhanced PRL response to a TRP challenge after dieting (Goodwin et al., 1987b; Anderson et al., 1990a). As the enhanced PRL response has been reported only in women, it raises the possibility that females may be particularly vulnerable to dieting-induced changes in brain 5-HT function. This possibility is of interest in view of the consistent finding that clinical eating disorders such as anorexia and bulimia nervosa are largely confined to women. Additionally, mood and appetite become severely disrupted during established eating disorders (see section 1.1), behaviours in which brain 5-HT pathways have a central role.
The hypothesis that dieting alters brain 5-HT function in women (but not men) is however, based on only a small number of studies. Furthermore, the studies published to date have all utilised intravenous TRP as the neuroendocrine challenge, which may in itself be problematic. Some researchers have raised doubts as to whether or not TRP-induced PRL release necessarily reflects changes in brain 5-HT neurotransmission (van Praag et al, 1986), as TRP is known to interact with dopaminergic pathways which may in turn influence PRL release (Price et al, 1990). In addition, the earlier studies demonstrated that dieting reduces plasma total TRP, raising the possibility that alterations in TRP disposition may occur following infusion (if TRP is used as the 5-HT challenge drug). Finally, as a pre-synaptic challenge of 5-HT pathways, TRP infusion is unable to provide direct evidence to support the presence of post-synaptic 5-HT receptor supersensitivity. While the finding of an enhanced PRL response to a TRP infusion is consistent with the development of post-synaptic receptor supersensitivity, it is also consistent with pre-synaptic enhancement of 5-HT neurotransmission (eg. through increased 5-HT release). Enhanced 5-HT neurotransmission across the synapse would in itself lead to an increased PRL response, even if post-synaptic receptor sensitivity was unaltered.

As has been previously outlined, d-fenfluramine administration in humans is well tolerated, has minimal side effects, and leads to a dose-dependent increase in plasma PRL concentration (section 2.1.4.1). Substantial evidence from animal and human studies suggests that the d-fenfluramine-induced PRL response is 5-HT mediated, and that the most likely mechanism of action is indirect enhancement of serotonergic neurotransmission through 5-HT₂ receptors, secondary to pre-synaptic release and re-uptake inhibition. An enhanced PRL response to d-fenfluramine would, therefore, provide additional evidence in support of the hypothesis that dieting-induced changes in the PRL response are 5-HT mediated.

Similarly, animal and human studies suggests that mCPP is also a highly selective probe of serotonergic pathways. It too is well tolerated and produces a reliable and consistent increase in PRL secretion when administered to humans (section 2.1.4.2). In contrast to d-fenfluramine, mCPP appears most potent as a 5-HT agonist, acting directly at post-synaptic 5-HT receptors. The mechanism of action of mCPP would, therefore, allow the hypothesis of post-synaptic receptor supersensitivity to be directly tested and the finding of an enhanced PRL response to mCPP, would provide strong evidence in support of this theory.

Few studies have examined whether or not order effects on PRL response occur as a result of repeat challenges with 5-HT test drugs. By necessity, dieting studies involve individual subjects undergoing two challenge tests, before and after dieting. Two d-
fenfluramine challenges carried out one month apart to correspond with the dieting challenge tests would enable any order effects on PRL response to be elicited.

In order to firmly establish the hypothesis that dieting alters brain 5-HT function and that such changes are confined to women, replication of the earlier work is required. Replication utilising more specific 5-HT challenge drugs, such as d-fenfluramine and mCPP, would also provide further evidence that the dieting-induced changes are indeed 5-HT mediated. Using a neuroendocrine approach, the aim of the present study was threefold: firstly, to attempt to replicate earlier findings of sex-specific (women only) dieting-induced increases in the PRL-response to a 5-HT challenge drug (d-fenfluramine); secondly, to examine more closely the mechanism underlying diet-induced changes in brain 5-HT function by using the combination of a pre-synaptic (d-fenfluramine) and post-synaptic (mCPP) challenge probe; and thirdly, to investigate the possibility of an order effect of repeat d-fenfluramine administration on PRL responses by carrying out two d-fenfluramine challenge tests one month apart.

3.2 METHODS

3.2.1 DETERMINATION OF DRUG DOSAGE

3.2.1.2 Intravenous mCPP (0.04mg/kg Intravenous mCPP Pilot Study)

Introduction

Human neuroendocrine studies have demonstrated that intravenous administration of mCPP enhances PRL release in healthy volunteers. Within the range of 0.06-0.1mg/kg, a reliable and consistent elevation in PRL is seen when compared to placebo, while smaller responses have been reported at 0.05mg/kg (Charney et al., 1987; Lawlor et al., 1989; Murphy et al., 1989). Most studies in the literature, however, have been carried out on men. One study that did include female subjects found a clear gender difference in terms of drug absorption and PRL response to mCPP. Plasma mCPP levels in the men were twice that of the women for a given dose, but subsequent PRL responses were greater in the women despite the lower drug concentrations (Kahn et al., 1991).

Investigation of dieting-induced receptor supersensitivity requires the use of mCPP at a dose capable of producing reliable, but submaximal PRL responses (Kahn and Wetzler, 1991). A submaximal response before dieting would enable an enhanced post-dieting response (due to the development of receptor supersensitivity) to be seen as a further elevation in plasma PRL. In contrast, a dose that was too high may lead to a ceiling effect, obscuring the effects of receptor supersensitivity, while a dose that was too low would fail
to produce any consistent PRL elevation. The gender differences described above suggest that a smaller dose than those outlined in the literature may produce a satisfactory submaximal PRL response in women. A pilot study was, therefore, carried out to determine whether or not an intravenous dose of 0.04mg/kg mCPP would prove to be suitable for use in women enabling the detection of supersensitivity effects.

Subjects, Methods and Statistics

Fifteen healthy female volunteers were recruited as described under the Selection and Assessment section in Chapter Two. All subjects were of normal weight as determined by a body mass index between twenty and twenty-eight (mean body mass index ± sem=23.4 ± 0.44; range 20.8-26.3). The women were aged between twenty-one and thirty seven years (mean age ± sem=25.6years ± 1.12), and all had given written, informed consent to participating in the study which had been approved by the local ethics committee.

The study was of a double-blind placebo controlled design. Subjects underwent neuroendocrine testing on two occasions approximately one week apart during the early to mid-follicular phase of a single menstrual cycle (determined by plasma oestradiol levels; see section 3.3.1.1). On one occasion the women received intravenous placebo (20mls of 0.9% NaCl administered by infusion pump [Sage instruments] over ninety seconds), and on the other occasion they received intravenous mCPP 0.04mg/kg (Mandeville Medicines Ltd; Aylesbury: UK) made up to 20mls with 0.9% NaCl and administered by infusion pump over ninety seconds. Subjects received either mCPP or placebo at time 0. Blood sampling for PRL estimation was carried out at fifteen minute intervals between -30 minutes and +120 minutes, and at fifteen minute intervals between time 0 and +120 minutes for drug level estimation. Other neuroendocrine procedure details were identical to those outlined in section 3.2.3. Similarly, assay procedure and statistical analysis was also carried out in the manner described in sections 3.2.2 and 3.2.4 respectively.

Results

Three females were determined by plasma oestradiol concentrations to have undergone neuroendocrine testing outside the early to mid-follicular phase of the menstrual cycle and were, therefore, excluded from further analysis. Following mCPP administration, detectable concentrations of mCPP were present in plasma from +15 minutes. Peak mean ± sem plasma mCPP concentrations of 181.3ng/ml ± 35.8 were reached at +15 minutes (Figure 3.1a).
Figure 3.1. (a) mCPP mean ± sem plasma drug levels following intravenous mCPP 0.04mg/kg administration to twelve female volunteers. (b) Effect of intravenous mCPP administration (0.04mg/kg) on PRL response in twelve female volunteers. The PRL response to mCPP was significantly increased following mCPP administration compared to placebo at the time points indicated. Fisher's test: ** p < 0.01.

Intravenous administration of mCPP was associated with rapid onset of action of the drug. This led to an increase in plasma PRL levels without the usual initial fall observed following oral drug administration, in keeping with the circadian fall in PRL release across the morning. Basal PRL concentrations were not significantly different at placebo and mCPP challenges (mean ± sem PRL concentration at time 0: placebo=198.1mIU/l ± 21.6; mCPP=163.1mIU/l ± 16.3; p=0.12), but a significantly enhanced PRL response was seen following mCPP administration compared to placebo (Figure 3.1b). When analysed with two way repeated measures analysis of variance (ANOVA), the PRL data (change from baseline) revealed a significant main effect of occasion [i.e. effect of mCPP versus placebo] (F=18.97; d.f.=1,7; p=0.001), no significant main effect of time [i.e. variation in PRL over time] (F=1.63; d.f.=7,77; p=0.14), but a significant occasion by time interaction [i.e. effect of mCPP on variation of PRL over time] (F=4.60; d.f.=7,77; p=0.0002). Similarly, when analysed as area under the curve (AUC-B), PRL responses were significantly increased following mCPP administration compared to placebo (mean ± sem PRL: placebo=-5362mIU/l.min⁻¹ ± 1051; mCPP=2818mIU/l.min⁻¹ ± 1770; p=0.0009).

Conclusions
Intravenous administration of mCPP at a dose of 0.04mg/kg led to detectable concentrations of the drug being present in plasma from +15minutes. Plasma drug
concentrations were marginally lower than those reported in the literature with the higher intravenous dose of 0.1mg/kg (Pigott et al, 1993), but led to a significant increase in PRL compared to placebo. Additionally, the PRL levels achieved in response to 0.04mg/kg intravenous mCPP were less than those reported in the literature (Charney et al, 1988; Murphy et al, 1989), suggesting the dose would be suitable in determining receptor supersensitivity in dieting women.

3.2.1.1 *d*-Fenfluramine

In contrast to data on *m*CPP, a substantial body of evidence exists in the literature demonstrating significant increases in PRL release following administration of *d*-fenfluramine in humans (Quattrone et al, 1978; Quattrone et al, 1983; Lewis and Sherman, 1985; Lerer et al, 1988; O'Keane and Dinan, 1991). Furthermore, the dose required to produce reliable and consistent PRL responses has been well established, with *d*-fenfluramine appearing to produce a dose-dependent release of PRL (Quattrone et al, 1978; Quattrone et al, 1983). In a study by Silverstone and Feeney (1989), a significant but submaximal increase in PRL was seen following administration of 30mg oral *d*-fenfluramine to healthy volunteers. The authors concluded that this dose produced consistent PRL responses while causing a minimum of side effects. The dieting study would also require the use of *d*-fenfluramine at a dose that produced a reliable, but submaximal PRL response, enabling the detection of dieting-induced receptor supersensitivity through further enhancement of the PRL response (see above section on *m*CPP). Minimal side effects would ensure a stress reaction did not occur, making interpretation of any enhanced PRL response difficult. For these reasons, the dose of 30mg *d*-fenfluramine was chosen for use in the dieting study.

3.2.2 SUBJECTS AND DIET INSTRUCTIONS

3.2.2.1 Repeat *d*-Fenfluramine Administration Study

Ten healthy female volunteers were recruited as described under the Selection and Assessment of Subjects in Chapter Two (section 2.4.2). To ensure comparability with female dieters, all subjects were of average weight or no more than one and one-half stone overweight, as determined by a body mass index between twenty and twenty eight. Mean body mass index was 22.9 and ranged from 21.0-24.9. Subjects had a mean age of 24.4 years and ranged between twenty and twenty-eight. All gave written, informed consent to participating in the study which was been approved by the local ethics committee.

Subjects underwent neuroendocrine testing on two occasions one month apart. Testing was timed to mirror those of the dieting study i.e. challenges were carried out in the early
to mid-follicular phase of two consecutive menstrual cycles with menstrual cycle phase confirmed by plasma oestradiol levels. Subjects were requested to eat normally throughout the study and not to diet.

3.2.2.2 Effects of Dieting on d-Fenfluramine-induced PRL Response

Twenty-six healthy volunteers (fourteen women and twelve men) were recruited as described under Selection and Assessment in Chapter Two. All subjects were of normal weight, or no more than one and half stone over weight as measured by a body mass index of between twenty-one and twenty-eight for women (mean body mass index=24.0; range 21.6-27) and twenty-one to twenty-eight for all but one of the men (mean body mass index=25.2; range 20.9-32). One male had a body mass index above twenty-eight but as this was judged to be largely muscle bulk rather than fat he was included in the study. The women were aged between twenty and thirty-seven (mean age=26.1 years) and the men between twenty and forty-two years of age (mean age=30.8 years). Subjects gave written, informed consent to participating in the study which was passed by the local ethics committee.

Subjects underwent neuroendocrine testing before, and following a three week calorie controlled diet of 1000 kcal for women and 1200 kcal for men. The onset of dieting was delayed by one week following the initial challenge. This ensured that both challenges in the women were carried out at the same phase of two consecutive menstrual cycles. Neuroendocrine tests were timed so that they took place in the early to mid follicular phase of the menstrual cycle and confirmed by plasma oestradiol concentration. To ensure comparability with the women, men also delayed the onset of their diet by one week following the initial challenge.

To replicate the work of Anderson et al (1990a), an identical diet was used which provided equal absolute carbohydrate intake in males and females, because of the potential effect carbohydrate intake has on plasma TRP. The diet was provided by the Oxford University Department of Nutrition and Dietetics and was designed to provide a set number of calories in specific macronutrient proportions. Women received 1000 kcal per day as 31% protein, 44% carbohydrate and 25% fat. Men received 1200 kcal per day as 30% protein, 36% carbohydrate and 34% fat. Subjects were provided with a daily eating plan which they were instructed to follow. The eating plan provided flexibility and variety through a fixed number of carbohydrate and protein "exchanges", coupled with a set amount of fat (as low-fat spread) and a list of unlimited "free foods" which were low in both calories and protein (eg. salad vegetables, low calorie soft drinks). The daily eating plan is outlined in Appendix Twelve. Subjects were asked to keep a food diary which was
checked weekly to ensure compliance. Weekly weights were also recorded to monitor progress and weekly fasting plasma TRP levels were measured.

3.2.2.3 Effect of Dieting on mCPP-induced PRL Response

Twelve healthy female volunteers were recruited as described under Selection and Assessment of Subjects in Chapter Two. All subjects were of normal weight, or no more than one and half stone over weight as measured by a body mass index of between twenty-one and twenty-eight (mean body mass index=25.0; range 22.3-28). The women were aged between twenty and thirty-eight (mean age=29.2 years). Subjects gave written, informed consent to participating in the study which was approved by the local ethics committee.

Subjects underwent neuroendocrine testing before, and following a three week calorie controlled diet of 1000 kcal (see section 3.2.1.2 for diet details). The onset of dieting was delayed by one week following the initial challenge. This ensured both challenges were carried out at the same phase of two consecutive menstrual cycles. Neuroendocrine tests were timed so that they took place in the early to mid follicular phase of two consecutive menstrual cycles and menstrual cycle phase confirmed by plasma oestradiol concentration.

3.2.3 NEUROENDOCRINE CHALLENGE PROCEDURE

Neuroendocrine testing was carried out following an overnight fast with subjects requested not to eat or drink from midnight. Subjects were brought to the Research Unit at approximately 08.30 hours and an indwelling venous cannula inserted into the arm. During testing, subjects rested semi-supine on a bed and were not allowed to eat, drink, smoke or sleep.

3.2.3.1 d-Fenfluramine Challenges

After insertion of the venous cannula, a blood sample for baseline plasma PRL and fasting TRP estimation was taken. Subjects were then left to rest quietly on the bed for thirty minutes before administration of the test drug between 09.00 hours and 09.30 hours (time 0). Dexfenfluramine hydrochloride, 30mg (Servier Laboratories Ltd; Slough; U.K.), was administered orally in two 15mg capsules. Blood sampling for plasma PRL estimation was carried out every thirty minutes from time 0 until +240 minutes. At hourly intervals from time 0 until +240 minutes, blood was removed for drug (d-fenfluramine and d-norfenfluramine) concentration analysis.
3.2.3.2 mCPP Challenge

Following venous cannula insertion, blood samples for baseline plasma PRL and fasting TRP estimation were taken and subjects then left to rest quietly on the bed for sixty minutes. Administration of the test drug took place between 09.30 hours and 10.00 hours (time 0). mCPP (0.04mg/kg) for intravenous use (Mandeville Medicines Ltd; Aylesbury; UK) was made up to 20ml with 0.9% NaCl and administered by infusion pump (Sage instruments) over ninety seconds at time 0. Blood samples for PRL estimation and drug concentration were taken every fifteen minutes from -60 minutes to +120 minutes after drug administration. An extra 10ml blood sample was taken at -30 minutes for fasting TRP levels.

All assays were processed using the methods described in Chapter Two with analysis of all samples from each individual volunteer carried out in the same batch.

3.2.4 SUBJECTIVE RATINGS

3.2.4.1 d-Fenfluramine Challenges

d-Fenfluramine is generally well tolerated by humans but some side effects have been reported to occur, especially at higher doses. Although the dose of 30mg utilised in these experiments could be expected to be largely side-effect free, VA scales for drowsiness, depression, nausea, happiness, light-headedness, hunger and dizziness were presented to subjects throughout testing (Appendix One). The VA rating scales were presented in the standardised manner outlined in section 2.3.1 at hourly intervals from time 0 until +240 minutes. Blood pressure and pulse readings were monitored automatically by machine every thirty minutes from -30 minutes until +240 minutes.

3.2.4.2 mCPP Challenge

mCPP is also well tolerated by humans, but transient side effects have been reported to occur, especially at higher doses. Although the intravenous dose of 0.04mg/kg utilised in the present experiment could be expected to produce minimal side-effects, VA scales for happiness, anxiety, hunger, light-headedness, nausea, mellowness, sweatiness and a sense of unreality were presented to subjects throughout testing (Appendix Two). The VA rating scales were presented in the standardised manner outlined in section 2.3.1 at -40 min, then at fifteen minute intervals from time 0 to +60 minutes, and thereafter at thirty minute intervals until +120 minutes. Blood pressure and pulse readings were monitored automatically by machine at -40 minutes, every fifteen minutes between time 0 and +60 minutes, and thereafter at thirty minute intervals until +120 minutes.
3.2.5 STATISTICAL ANALYSIS

Data approximated satisfactorily to a normal distribution. PRL data were plotted against time (as change from baseline) and then analysed with two way repeated measures analysis of variance (ANOVA) with Fisher's test of least significant difference as a post hoc range test. PRL data were also analysed as area under the curve with subtraction of baseline secretion (AUC-B) extrapolated from time 0 using the trapezoid method. Drug concentration data were measured as area under the curve (AUC) from time 0. VA data were plotted against time as change from baseline (at time 0), and then analysed with ANOVA.

3.3 RESULTS

3.3.1 REPEAT d-FENFLURAMINE ADMINISTRATION STUDY

3.3.1.1 Oestriadiol Levels and Plasma TRP

Three females were found to have undergone neuroendocrine testing at different phases of the menstrual cycle (as determined by plasma oestriadiol concentration differences of greater than 50pg/ml), and were therefore, excluded. Plasma oestriadiol concentrations at first and second challenge tests in the excluded subjects were 46 and 170pg/ml, 100 and 380pg/ml, and 80 and 280pg/ml respectively.

![Graph showing plasma total and free TRP levels](a)

![Graph showing plasma d-fenfluramine and d-norfenfluramine drug concentrations](b)

Figure 3.2. (a): Mean ± sem plasma total and free TRP and (b): plasma d-fenfluramine and d-norfenfluramine drug concentrations (mean AUC ± sem; ng/ml⁻¹.min⁻¹) in seven female subjects at repeat d-fenfluramine challenges one month apart.
Plasma total and free TRP concentrations were not significantly different at baseline sampling at repeat d-fenfluramine challenges (Figure 3.2a). Mean ± sem plasma total TRP concentrations at first and second challenges were 13.39µg/ml ± 1.31 and 12.33µg/ml ± 0.93 respectively \((p=0.55)\), and mean ± sem plasma free TRP concentrations were 0.68µg/ml ± 0.13 and 0.78µg/ml ± 0.06 \((p=0.34)\) respectively.

3.3.1.2 Plasma Drug Levels: d-Fenfluramine and d-Norfenfluramine

Concentrations of d-fenfluramine and d-norfenfluramine were detected in the plasma following d-fenfluramine administration from +60 minutes and no differences in drug levels were seen between first and second challenge (Figure 3.2b). Peak d-fenfluramine concentrations of 34.7ng/ml and 31.4ng/ml were reached at +180 minutes during the first and second challenges respectively. ANOVA revealed no significant main effect of occasion [i.e. effect of repeat challenge] \((F=1.07; d.f.=1,3; p=0.34)\), a significant main effect of time [i.e. variation in plasma drug levels over time] \((F=19.76; d.f.=3,18; p=0.0001)\) but no significant main occasion by time interaction [i.e. effect of repeat challenge on drug level variation over time] \((F=0.28; d.f.=3,18; p=0.84)\). When analysed as AUC, d-fenfluramine levels were not altered between challenges (Fig 3.2b). Mean ± sem plasma d-fenfluramine responses were 6191ng/ml⁻¹.min⁻¹ ± 1505 and 4986ng/ml⁻¹.min⁻¹ ± 755 respectively \((p=0.36)\). Peak d-norfenfluramine levels of 8.2ng/ml and 7.2ng/ml were reached at +240 minutes during the first and second challenge test. The ANOVA again revealed no significant main effect of occasion \((F=0.56; d.f.=1,3; p=0.48)\), a significant main effect of time \((F=41.76; d.f.=3,18; p=0.0001)\) but no significant main occasion by time interaction \((F=1.99; d.f.=3,18; p=0.15)\). Similarly, when analysed as AUC, mean ± sem d-norfenfluramine plasma responses were not significantly different between challenges at 980ng/ml⁻¹.min⁻¹ ± 142 and 786ng/ml⁻¹.min⁻¹ ± 15 (Figure 3.2b; \(p=0.50\)).

3.3.1.3 PRL Response to Repeat d-Fenfluramine Administration

Plasma PRL levels showed an initial fall, in keeping with the natural circadian fall in PRL release seen across the morning (see section 2.1.3), then increased in response to d-fenfluramine administration at both challenges (Figure 3.3). Basal PRL concentrations were not significantly different at either challenge \(\text{mean} ± \text{sem PRL at time } 0: 1\text{st challenge}=263\text{mIU/l} ± 59; 2\text{nd challenge}=267\text{mIU/l} ± 37; p=0.09\), and PRL responses to d-fenfluramine were not significantly different between challenges.
Figure 3.3. Mean ± sem plasma PRL concentration (change from baseline) following two d-fenfluramine challenges one month apart in seven female volunteers. PRL responses were not significantly different between challenges.

The ANOVA on change from baseline data revealed no significant main effect of occasion [i.e. the effect of a repeat challenge] (F=0.20; d.f.=1,7; p=0.67), a significant main effect of time [i.e. the variation in PRL response over time] (F=5.81; d.f.=7,42; p=0.0001) but no significant occasion by time interaction [i.e. effect of a repeat challenge on the variation in PRL response over time] (F=1.42; d.f.=7,42; p=0.22). When analysed as AUC-B no significant effect of repeat d-fenfluramine challenge was revealed on PRL response. Mean ± sem plasma PRL at first and second challenge were -13194mIU/l-1.min-1 ± 5735 and -15574mIU/l-1.min-1 ± 4858 respectively (p=0.75).

3.3.1.4 Subjective Ratings

Some of the women reported an increase in drowsiness following d-fenfluramine administration at first and second challenges. Baseline drowsiness ratings at first and second challenges were not significantly different (p=0.41) and no significant effect of repeat d-fenfluramine administration on drowsiness was found. The ANOVA on change from baseline data revealed no significant main effect of occasion [i.e. effect of repeat challenge] (F=0.34; d.f.=1,3; p=0.58), no significant main effect of time [i.e. variation in
drowsiness over time] (F=2.74; d.f.=3,18; p=0.07) and no significant occasion by time interaction [i.e. effect of repeat challenge on variation in drowsiness over time] (F=0.64; d.f.=3,18; p=0.60).

Two women reported increases in nausea following d-fenfluramine administration at the first challenge, but none reported increases in nausea at the second challenge. No baseline differences between challenges were seen, however (p=0.36), and no significant effect of repeat d-fenfluramine administration on nausea found. The ANOVA on change from baseline data revealed no significant main effect of occasion (F=18; d.f.=1,3; p=0.69), no significant main effect of time (F=1.65; d.f.=3,18; p=0.21) and no significant occasion by time interaction (F=1.65; d.f.=3,18; p=0.21).

Similarly, with VA ratings of light-headedness and dizziness, four women experienced increases following d-fenfluramine administration at first challenge and three women reported increases at the second challenge test. Baseline ratings in light-headedness and dizziness were not significantly different between tests (p=0.30 and p=0.36 respectively). The ANOVAs again revealed no significant main effect of occasion (light-headedness: F=1.50; d.f.=1,3; p=0.27; dizziness: F=1.0; d.f.=1,3; p=0.36), no significant main effect of time (light-headedness: F=1.43; d.f.=3,18; p=0.27; dizziness: F=2.11; d.f.=3,18; p=0.13) and no significant occasion by time interaction (light-headedness: F=1.87; d.f.=3,18; p=0.17; dizziness: F=1.80; d.f.=3,18; p=0.18).

No significant effects in terms of baseline changes or effects of repeat d-fenfluramine administration were seen on hunger (baseline difference: p=0.96; ANOVA occasion by time interaction: F=2.64; d.f.=3,18; p=0.08), depression (baseline difference: p=0.20; ANOVA occasion by time interaction: F=1.33; d.f.=3,18; p=0.30) or happiness (baseline difference: p=0.92; ANOVA occasion by time interaction: F=0.56; d.f.=3,18; p=0.65).

### 3.3.1.5 Correlations

No significant correlation was found between plasma d-fenfluramine or d-norfenfluramine concentration and PRL response at the first (d-fenfluramine: r=0.27; p=0.56; d-norfenfluramine: r=0.19; p=0.68) or second (d-fenfluramine: r=0.25; p=0.59; d-norfenfluramine: r=0.46; p=0.30) challenge. When basal PRL levels were examined, no significant correlation was found between basal PRL and PRL response at first (r=0.75; p=0.05) or second (r=0.57; p=0.18) challenge. Plasma total and free TRP levels did not correlate with PRL response. At first challenge no significant correlation was found between PRL response and plasma total or free TRP (total TRP: r=-0.19; p=0.69; free TRP: r=-0.24; p=0.61). Similarly, at second challenge, no significant correlations were found between PRL response and plasma total TRP (r=-0.37; p=0.41) or plasma free TRP.
Furthermore, no significant correlation was found between plasma oestradiol levels and PRL response at first (r=-0.23; p=0.63) or second challenge (r=-0.59; p=0.17).

A significant, positive correlation was found between plasma d-fenfluramine and d-norfenfluramine concentrations at first challenge (r=0.89; p=0.008; Figure 3.4a) but not at second challenge (r=0.07; p=0.89). When analysed as change in drug concentration across challenges, a highly significant positive correlation was found between plasma d-fenfluramine and d-norfenfluramine levels (r=0.93; p=0.002; Figure 3.4b).

![Graphs showing drug level correlations in women undergoing repeat d-fenfluramine challenges.](image)

(a) Correlation between AUC plasma d-fenfluramine and plasma d-norfenfluramine drug levels (ng.ml⁻¹.min⁻¹) at the first challenge.

(b) Correlation between change in AUC plasma d-fenfluramine and d-norfenfluramine drug levels (ng/ml⁻¹.min⁻¹) between first and second challenge.

### 3.3.2 EFFECTS OF DIETING ON d-FENFLURAMINE-INDUCED PRL RESPONSE

#### 3.3.2.1 Weight Loss, Oestradiol Levels and Plasma TRP

All dieters lost weight. Male dieters lost more weight on average than female dieters but the difference in weight loss did not reach statistical significance (mean ± sem: 3.77kg ± 0.30 vs 3.38kg ± 0.19; p=0.29). When calculated as percentage body weight loss, weight
loss by the women was greater than the men but again, the difference did not reach statistical significant (mean ± sem; 5.27% ± 0.38 vs 4.75% ± 0.37; p=0.33).

Two female subjects were found to have undergone neuroendocrine testing at different phases of the menstrual cycle (50pg/ml or greater difference in plasma oestradiol concentration) and were, therefore, excluded from further analysis. The excluded subjects’ plasma oestradiols at the time of neuroendocrine testing were 44 and 115pg/ml, and 180 and 20pg/ml respectively.

Dieting significantly decreased total and free plasma TRP in the women but not in the men (Figure 3.5). Following dieting, mean ± sem total plasma TRP in the women fell from 12.05µg/ml ± 0.76 to 9.27µg/ml ± 0.40 (p=0.002), while mean ± sem free plasma TRP was reduced from 0.90µg/ml ± 0.07 to 0.72µg/ml ± 0.05 (p=0.006). In the men, mean ± sem total plasma TRP concentrations before and after dieting were 12.70µg/ml ± 0.60 and 11.82µg/ml ± 0.74 respectively (p=0.24), and mean ± sem plasma free TRP concentrations were 0.83µg/ml ± 0.13 and 0.84µg/ml ± 0.13 respectively (p=0.96).

3.3.2.2 Plasma Drug Levels: d-Fenfluramine and d-Norfenfluramine

Following d-fenfluramine administration, detectable concentrations of d-fenfluramine and d-norfenfluramine were present in plasma from +30 minutes. Dieting did not
significantly alter plasma concentrations of either \(d\)-fenfluramine or \(d\)-norfenfluramine in male or females (Figure 3.6).

![Graph A](image1)

**Figure 3.6.** Plasma \(d\)-fenfluramine (a) and \(d\)-norfenfluramine (b) drug concentrations (shown as mean AUC \(\pm\) sem; \(\text{ng/ml} \cdot \text{min}^{-1}\)) in twelve male and female volunteers before and after dieting.

In the men, peak plasma concentrations of \(d\)-fenfluramine (mean \(\pm\) sem: pre-diet=18.6 \(\text{ng/ml}\) \(\pm\) 1.53; post-diet=17.8 \(\pm\) 1.49) were detected before and after dieting at +240 min and +210 minutes respectively. The ANOVA revealed no significant main effect of occasion [i.e. an effect of dieting] \((F=0.04; \text{d.f.}=1.8; p=0.85)\), a significant main effect of time [i.e. a variation in drug level over time] \((F=105.4; \text{d.f.}=8.88; p=0.0001)\) but no significant occasion by time interaction [i.e. effect of dieting on variation in drug level over time] \((F=0.47; \text{d.f.}=8.88; p=0.88)\). Similarly, when analysed as AUC, dieting had no significant effect on \(d\)-fenfluramine (mean \(\pm\) sem: pre-diet=2598 \(\text{ng/ml} \cdot \text{min}^{-1}\) \(\pm\) 230; post-diet=2551 \(\text{ng/ml} \cdot \text{min}^{-1}\) \(\pm\) 244; \(p=0.89\); Figure 3.6a). Peak \(d\)-norfenfluramine plasma levels were seen at +240 minutes before dieting (mean \(\pm\) sem=8.1 \(\text{ng/ml}\) \(\pm\) 1.02) and +210 minutes after dieting (mean \(\pm\) sem=9.0 \(\text{ng/ml}\) \(\pm\) 1.05). The ANOVA again revealed no significant main effect of occasion \((F=0.23; \text{d.f.}=1.8; p=0.64)\), a significant main effect of time \((F=57.70; \text{d.f.}=8.88; p=0.0001)\) but no significant occasion by time interaction \((F=0.63; \text{d.f.}=8.88; p=0.75)\). When analysed as AUC plasma \(d\)-norfenfluramine in the males were also unaltered by dieting (Fig 3.6b). Mean \(\pm\) sem plasma \(d\)-norfenfluramine: pre-diet=878 \(\text{ng/ml} \cdot \text{min}^{-1}\) \(\pm\) 96; post-diet=1009 \(\text{ng/ml} \cdot \text{min}^{-1}\) \(\pm\) 183; \(p=0.42\).

In women dieters, peak mean \(\pm\) sem plasma \(d\)-fenfluramine concentrations of 25.3 \(\text{ng/ml}\) \(\pm\) 2.64 were seen at +210 minutes before dieting, and 25.30 \(\text{ng/ml}\) \(\pm\) 2.62 at +240 minutes after dieting. The ANOVA revealed no significant main effect of occasion
(F=0.70; d.f.=1,8; p=0.42), a significant main effect of time (F=43.3; d.f.=8,88; p=0.0001) but no significant occasion by time interaction (F=1.38; d.f.=8,88; p=0.22). When d-fenfluramine data were analysed as AUC, no significant effect of dieting was found (Figure 3.6a). Mean ± sem plasma d-fenfluramine before and after dieting were 3832ng/ml-1.min-1 ± 580 and 3569ng/ml-1.min-1 ± 456 (p=0.35). Mean ± sem peak d-norfenfluramine levels before and after dieting were 6.5ng/ml ± 2.32 and 8.8ng/ml ± 2.48 respectively and were reached at +240 minutes. The ANOVA again revealed no significant main effect of occasion (F=4.68; d.f.=1,8; p=0.05), a significant main effect of time (F=8.99; d.f.=8,88; p=0.0001) but no significant occasion by time interaction (F=1.06; d.f.=8,88; p=0.40). Similarly, when analysed as AUC, mean ± sem d-norfenfluramine was not significantly different following dieting (Fig 3.6b): pre-diet=758ng/ml-1.min-1 ± 327; post-diet=911ng/ml-1.min-1 ± 343; p=0.07.

3.3.2.3 PRL Response to d-Fenfluramine

In women dieters, plasma PRL levels initially fell, in keeping with the circadian fall in PRL release seen across the morning, and then rose in response to d-fenfluramine administration both before and after dieting (Fig 3.7).

![Figure 3.7](image-url)

Figure 3.7. Mean ± sem plasma PRL concentration (change from baseline) following d-fenfluramine administration in twelve female volunteers before and after dieting. The PRL response was significantly enhanced after dieting at the time points indicated when compared to the pre-diet challenge (ANOVA). Fisher's test: ** p < 0.01.
Basal PRL concentrations were significantly lower following dieting (mean ± sem PRL concentration at time 0: pre-diet=293mIU/l ± 29; post-diet=254mIU/l ± 32; p=0.03). However, the PRL response to d-fenfluramine was significantly enhanced in women after dieting when compared to the pre-diet challenge. The ANOVA on change from baseline data revealed a significant main effect of occasion [i.e. an effect of dieting] (F=5.76; d.f.=1,7; p=0.04), a significant main effect of time [i.e. a variation in PRL response over time] (F=17.21; d.f.=7,77; p=0.0001) and a significant occasion by time interaction [i.e. an effect of dieting on the variation in PRL response over time] (F=2.72; d.f.=7,77; p=0.01). The post hoc range testing revealed plasma PRL levels to be significantly elevated at +90, +120, +180, +210 and +240 minutes after dieting (p<0.01). Similarly, when analysed as AUC-B, PRL levels were significantly elevated after dieting. Mean ± sem PRL before and following dieting was -9723mIU/l.min⁻¹ ± 5043 and 4716mIU/l.min⁻¹ ± 6075 respectively (p=0.03).

Plasma PRL levels in male dieters also increased (after the initial fall seen reflecting circadian release patterns) following d-fenfluramine administration both before, and after dieting (Figure 3.8).

Figure 3.8. Mean ± sem plasma PRL concentration (change from baseline) following d-fenfluramine administration in twelve male volunteers before and after dieting. PRL responses were not significantly different after dieting.
Basal PRL concentrations in the men were not significantly different before and after dieting (mean ± sem PRL concentrations at time 0: pre-diet=199mIU/l ± 42; post-diet=170mIU/l ± 34; p=0.12), and the PRL response to d-fenfluramine was unaltered by dieting (Figure 3.8). The ANOVA on change from baseline data revealed no significant main effect of occasion (F=0.37; d.f.=1,7; p=0.55), a significant main effect of time (F=15.0; d.f.=7,77; p=0.0001) but no significant occasion by time interaction (F=0.68; d.f.=7,77; p=0.70). When analysed as AUC-B, the PRL response to d-fenfluramine in men was unchanged by dieting. Mean ± sem plasma PRL: pre-diet=-5803mIU/l⁻¹.min⁻¹ ± 5570; post-diet=-3906mIU/l⁻¹.min⁻¹ ± 5200; p=0.31).

3.3.2.4 Subjective Measures

Data from two male subjects for drowsiness, nausea, dizziness and hunger VA ratings, and from one male subject for light-headedness, depression and happiness VA ratings were not collected. Data from one female subject on all VA were not collected. Data from ten males for drowsiness, nausea, dizziness and hunger and from eleven males for light-headedness, depression and happiness is presented below, along with the VA data from eleven female dieters.

An increase in drowsiness following d-fenfluramine administration was experienced by some males before and after dieting. Baseline drowsiness in the men was not significantly higher following dieting (p=0.36) and no significant effect of dieting on drowsiness was found. The ANOVA on change from baseline data revealed no significant main effect of occasion [i.e. effect of dieting] (F=0.02; d.f.=1,3; p=0.90), no significant main effect of time [i.e. variation in drowsiness over time] (F=1.82; d.f.=3,27; p=0.17) and no significant occasion by time interaction [effect of dieting on variation in drowsiness over time] (F=0.50; d.f.=3,27; p=0.68). All but three of the women dieters experienced an increase in drowsiness following d-fenfluramine administration before dieting, and all but two after dieting. However, baseline drowsiness in the women before and after dieting was not significantly different (p=0.70) and no significant effect of dieting on drowsiness was found. The ANOVA on change from baseline data revealed no significant main effect of occasion (F=2.77; d.f.=1,3; p=0.13), a significant main effect of time (F=3.36; d.f.=3,30; p=0.03) but no significant occasion by time interaction (F=0.41; d.f.=3,30; p=0.75).

No men reported increases in nausea in response to d-fenfluramine before dieting and only three reported an increase during the post-dieting challenge. Baseline nausea was not significantly elevated after dieting (p=0.17), and no significant effect of dieting on nausea was seen. The ANOVA on change from baseline data revealed no significant main effect of occasion (F=0.50; d.f.=1,3; p=0.50), no significant main effect of time (F=1.0; d.f.=3,27; p=0.41) and no significant occasion by time interaction (F=1.0; d.f.=3,27;
Three women reported increases in nausea following *d*-fenfluramine administration before dieting and three after dieting. Baseline nausea in the women was not significantly altered by dieting (*p*=0.34), and again no significant effect of dieting on nausea was seen when analysed with ANOVA on change from baseline data.

An increase in dizziness in response to *d*-fenfluramine was reported by one male before, and by three males after dieting. Baseline dizziness was not significantly elevated after dieting (*p*=0.62) and no significant effect of dieting on dizziness was found. The ANOVA on change from baseline data in the male dieters revealed no significant main effect of occasion (*F*=2.25; d.f.=1,3; *p*=0.17), no significant main effect of time (*F*=0.47; d.f.=3,27; *p*=0.70) and no occasion by time interaction (*F*=1.14; d.f.=3,27; *p*=0.35). Amongst the female dieters, increased dizziness was reported by three subjects in response to *d*-fenfluramine administration before, and by four subjects following dieting. Basal VA ratings before and after dieting were not significantly different (*p*=0.18) and no significant effect of dieting on dizziness was found. The ANOVA on change from baseline data in the women revealed no significant main effect of occasion (*F*=1.95; d.f.=1,3; *p*=0.19), no significant main effect of time (*F*=1.65; d.f.=3,30; *p*=0.20) and no significant occasion by time interaction (*F*=0.24; d.f.=3,30; *p*=0.87).

Similar result were seen with the VA ratings for light-headedness. Three men reported increases in light-headed following *d*-fenfluramine administration before dieting, and four men reported increases in light-headedness after dieting. However, basal light-headedness ratings were not significantly different before and after dieting (*p*=0.17), and again no significant effect of dieting was seen The ANOVA on change from baseline data revealed no significant main effect of occasion (*F*=0.73; d.f.=1,3; *p*=0.41), no significant main effect of time (*F*=0.24; d.f.=3,30; *p*=0.87) and no significant occasion by time interaction (*F*=0.23; d.f.=3,30; *p*=0.88). Amongst the female dieters, three reported increases in light-headedness following administration of *d*-fenfluramine before dieting and five after dieting, although baseline light-headedness ratings were not significantly different before and after dieting (*p*=0.05). The ANOVA on change from baseline data revealed no significant main effect of occasion (*F*=1.97; d.f.=1,3; *p*=0.19), a significant main effect of time (*F*=3.42; d.f.=3,30; *p*=0.03) but no significant occasion by time interaction (*F*=0.82; d.f.=3,30; *p*=0.50).

Two male dieters reported increases in feelings of depression in response to *d*-fenfluramine administration before, and three after, dieting. Basal depression VA ratings were not significant lower following dieting (*p*=0.22), and no significant effect of dieting on depression was found. The ANOVA on change from baseline data revealed no significant main effect of occasion (*F*=0.0006; d.f.=1,3; *p*=0.98), no significant main effect of time (*F*=1.45; d.f.=3,30; *p*=0.25) and no significant occasion by time interaction
None of the female dieters reported feelings of depression throughout testing either before or after dieting.

All but two of the male dieters reported increases in hunger following d-fenfluramine administration before dieting and all but one after dieting (p=0.93) and no significant effect of dieting on hunger was seen. The ANOVA on change from baseline data revealed no significant main effect of occasion (F=0.31; d.f.=1,3; p=0.59), a significant main effect of time (F=7.33; d.f.=3,27; p=0.001) but no significant occasion by time interaction (F=2.33; d.f.=3,27; p=0.10). An increase in hunger ratings was also seen in five of the women following d-fenfluramine administration before, and four of the women after dieting. Baseline hunger ratings in the female dieters were not significantly different (p=0.65), and no significant effect of dieting on hunger was seen. The ANOVA on change from baseline data revealed no significant main effect of occasion (F=0.64; d.f.=1,3; p=0.44), no significant main effect of time (F=0.33; d.f.=3,30; p=0.80) and no significant occasion by time interaction (F=1.66; d.f.=3,30; p=0.20).

A significant effect of dieting on ratings of happiness was seen in the male dieters although basal happiness ratings were not significantly different before and after dieting (p=0.79). The ANOVA on change from baseline data revealed no significant main effect of occasion (F=1.90; d.f.=3,30; p=0.20), no significant main effect of time (F=1.76; d.f.=3,30; p=0.18) but a significant occasion by time interaction (F=3.13; d.f.=3,30; p=0.04). In the women dieters, baseline happiness ratings were not significantly different before and after dieting (p=0.28). The ANOVA on change from baseline data revealed no significant main effect of occasion (F=2.34; d.f.=3,30; p=0.16), a significant main effect of time (F=4.71; d.f.=3,30; p=0.008), but no significant occasion by time interaction (F=2.24; d.f.=3,30; p=0.10).

3.3.2.5 Correlations

Following d-fenfluramine administration, no significant correlations were found between PRL response and plasma d-fenfluramine or d-norfenfluramine drug levels. In the men, correlations between plasma d-fenfluramine (measured as AUC) and plasma PRL (measured as AUC-B) before and after dieting were r=0.11; p=0.74 and r=-0.33; p=0.30 respectively, while correlations between plasma d-norfenfluramine and plasma PRL before and after dieting were r=-0.52; p=0.08 and r=-0.19; p=0.56. In the women dieters, correlations between d-fenfluramine and plasma PRL responses before and after dieting were r=-0.37; p=0.24 and r=-0.23; p=0.48 respectively, while correlations between d-norfenfluramine and plasma PRL responses before and after dieting also failed to reach significance (r=-0.07; p=0.84 and r=0.32; p=0.30).
No significant correlation was found between basal plasma PRL concentration and PRL response in female dieters either before (r=-0.39; p=0.22), or following (r=-0.34; p=0.28) dieting, but a significant negative correlation was found in male dieters before (r=-0.66; p=0.02), and after (r=-0.83; p=0.0008) dieting such that the lower the PRL level at baseline, the greater the PRL response (Figure 3.9).

![Graph](a)  ![Graph](b)  

Figure 3.9. Correlations between basal plasma PRL levels and PRL response (mIU/l.min⁻¹) in the males before (a), and after (b) dieting. A significant negative correlation was found between basal plasma PRL levels (time 0) and subsequent PRL response to the d-fenfluramine challenge both before and after dieting.

When PRL response and weight loss were compared, no significant correlation between the PRL response after dieting and absolute weight loss (men: r=0.17; p=0.59; women: r=0.36; p=0.25) or percentage body weight loss (men: r=0.37; p=0.24; women: r=0.17; p=0.60) was found.

Plasma total and free TRP levels did not correlate significantly with either PRL response or weight loss. In the men, no significant correlation was found between plasma total TRP and PRL response before (r=0.31; p=0.32), or after (r=0.11; p=0.73; Figure 3.10a) dieting. Similarly, no significant correlation was found between plasma free TRP levels and the PRL response before (r=-0.27; p=0.40) and after (r=0.17; p=0.60; Figure 3.10b) dieting. There was no significant correlation between weight loss and change in plasma TRP levels in male dieters in terms of either absolute weight loss (total TRP: r=0.14; p= 0.66; free TRP: r=0.35; p= 0.26) or percentage body weight loss (total TRP: r=0.11; p=0.74; free TRP: r=0.02; p=0.95).
Figure 3.10. Correlations in men after dieting between plasma total TRP and AUC-B PRL response (mIU/l-1.min-1) (a), and between plasma free TRP and AUC-B PRL response (mIU/l-1.min-1) (b).

In the women, neither plasma total or free TRP levels were found to be significantly correlated with PRL response before (total TRP: r=0.24; p=0.46; free TRP: r=-0.18; p=0.57) or following dieting (total TRP: r=-0.30; p=0.34; free TRP: r=0.05; p=0.87: Figure 3.11).

Figure 3.11. Correlations between plasma total TRP and AUC-B PRL response (mIU/l-1.min-1) (a) and plasma free TRP levels and AUC-B PRL response (mIU/l-1.min-1) in twelve women after dieting (b).
No significant correlation was found between weight loss in the women and change in plasma total or free TRP levels when assessed either as absolute weight loss versus total TRP ($r=0.10; p=0.75$) or free TRP ($r=0.39; p=0.21$), or as percentage body weight loss (total TRP: $r=-0.05; p=0.87$; free TRP: $r=0.28; p=0.38$).

A positive and significant correlation between plasma oestradiol concentration and PRL response was found in women before dieting ($r=0.61; p=0.03$; Figure 3.12) but failed to reach significance after dieting ($r=0.38; p=0.23$).

![Figure 3.12. Correlation between plasma oestradiol levels and AUC-B PRL response (mIU/l⁻¹.min⁻¹) in the women dieters before dieting. A significant and positive correlation was found before, but not after, dieting.](image)

In male dieters, no significant correlation was found between plasma $d$-fenfluramine and $d$-norfenfluramine concentrations before dieting ($r=0.49; p=0.10$), but a significant positive correlation between the two drugs was found after dieting ($r=0.84; p=0.0006$; Figure 3.13a). Furthermore, a positive and significant correlation was found between the changes in $d$-fenfluramine and $d$-norfenfluramine drug concentrations between the two challenge tests ($r=0.63; p=0.03$; Figure 3.13b). In female dieters, no significant correlations were found between $d$-fenfluramine and $d$-norfenfluramine drug concentrations either before dieting ($r=0.27; p=0.39$), after dieting ($r=0.35; p=0.26$) or when measured as change in drug level ($r=0.46; p=0.13$).
3.3.3 EFFECTS OF DIETING ON $m$CPP-INDUCED PRL RESPONSE

3.3.3.1 Weight Loss, Oestradiol Levels and Plasma TRP

All dieters lost weight with a mean ± sem weight loss of 3.31kg ± 0.27 and a percentage body weight loss of 4.84% ± 0.41. No dieters were determined, by disparate plasma oestradiol levels (> 50pg/ml difference in plasma oestradiol between tests), to have been tested at different phases of the menstrual cycle and all were, therefore, included in the final analysis.

Dieting did not significantly alter total or free plasma TRP (Figure 3.14). Mean ± sem total plasma TRP concentrations before and after dieting were 10.67µg/ml ± 0.57 and 10.07µg/ml ± 0.42 respectively ($p=0.31$). There was a trend towards a decrease in plasma free TRP concentrations after dieting, but the decrease failed to reach statistical significance. Mean ± SEM plasma free TRP before and after dieting were 1.05µg/ml ± 0.11 and 0.88µg/ml ± 0.06 respectively ($p=0.06$).
Figure 3.14. Mean ± sem plasma total TRP and free TRP in twelve females before and after dieting. No significant differences in plasma total and free TRP levels were found after dieting.

3.3.3.2 Plasma Drug Levels: mCPP

Following mCPP administration, detectable concentrations of mCPP were present in plasma from +15 minutes. Dieting did not significantly alter plasma concentrations of mCPP (Figure 3.15).

Figure 3.15. Plasma mCPP drug concentrations (shown as mean AUC ± sem; ng/ml⁻¹.min⁻¹) in twelve female dieters before and after dieting. Dieting did not significantly alter plasma mCPP drug concentrations.
Peak plasma concentrations of mCPP were detected before (mean ± sem=652ng/ml ± 211) and after dieting (mean ± sem=698 ± 244) at +15 minutes. The ANOVA revealed no significant main effect of occasion [i.e. an effect of dieting] (F=0.03; d.f.=1,8; p=0.85), a significant main effect of time [i.e. a variation in mCPP drug levels over time] (F=8.84; d.f.=8.88; p=0.0001) but no significant occasion by time interaction [i.e. an effect of dieting on the variation of mCPP drug levels over time] (F=0.15; d.f.=8.88; p=0.70). Similarly, when analysed as area under the curve (AUC), dieting was found to have had no significant effect on mCPP drug concentrations (Figure 3.15). Mean ± sem mCPP response in the female dieters before and after dieting was 13819ng/ml⁻¹.min⁻¹ ± 3539 and 14201ng/ml⁻¹.min⁻¹ ± 4320 respectively (p= 0.84).

3.3.3.3 PRL Response to mCPP

Intravenous administration of mCPP was associated with rapid onset of action of the drug. This led to an increase in plasma PRL levels, without the initial fall observed following d-fenfluramine administration, in the female dieters both before and after dieting (Fig 3.16).

Figure 3.16. Mean ± sem plasma PRL concentration (change from baseline) following mCPP administration in twelve female volunteers before and after dieting. The PRL response was not significantly altered after dieting.
Basal PRL concentrations were not significantly different before and after dieting (mean ± sem PRL concentration at time 0: pre-dieting=224mIU/l ± 24; post-diet=234mIU/l ± 22; p=0.54). Similarly, the PRL response to mCPP was not significantly altered in the women after dieting when compared to the pre-diet challenge (Figure 3.16). The ANOVA on change from baseline data revealed no significant main effect of occasion [i.e. an effect of dieting] (F=0.02; d.f.=1,7; p=0.89), a significant main effect of time [i.e. the variation in PRL response over time] (F=4.26; d.f.=7,77; p=0.005) but no significant occasion by time interaction [i.e. an effect of dieting on the variation in PRL response over time] (F=0.24; d.f.=7,77; p=0.98. When analysed as AUC-B, PRL responses were not significantly elevated after dieting. Mean ± sem PRL responses before and following dieting were 2796mIU/l-1.min-1 ± 1770 and 2938mIU/l-1.min-1 ± 2014 respectively (p=0.94).

3.3.3.4 Subjective Ratings

All but four of the women reported an increase in VA ratings of nausea following mCPP administration before dieting and all but three women reported an increase after dieting (p=0.80) and no effect of dieting was found on nausea. The ANOVA on change from baseline data revealed no significant main effect of occasion [i.e. effect of dieting] (F=0.09; d.f.=1,6; p=0.77), a significant main effect of time [i.e. variation in nausea ratings over time] (F=8.10; d.f.=6,66; p=0.0001), but no significant occasion by time interaction [i.e. effect of dieting on variation in nausea ratings over time] (F=2.04; d.f.=6,66; p=0.07).

Following mCPP administration, all women except one reported an increase in light-headedness before dieting, and all but three women reported an increase after dieting. VA ratings of basal light-headedness were not significantly different before and after dieting (p=0.97), and no significant effect of dieting on light-headedness was found. The ANOVA on change from baseline data revealed no significant main effect of occasion (F=0.02; d.f.=1,6; p=0.90), a significant main effect of time (F=12.47; d.f.=6,66; p=0.0001), but no significant occasion by time interaction (F=0.87; d.f.=6,66; p=0.53).

Similar effects were seen on anxiety ratings. Half the women reported an increase in anxiety following mCPP administration before dieting, and five after dieting. Basal ratings of anxiety, however, were not significantly different before and after dieting (p=0.10), and again, no significant effect of dieting on anxiety ratings were seen. The ANOVA on change from baseline data revealed no significant main effect of occasion (F=3.08; d.f.=1,6; p=0.11), no significant main effect of time (F=2.06; d.f.=6,66; p=0.07) and no significant occasion by time interaction (F=2.06; d.f.=6,66; p=0.55).
Increases in VA ratings of unreality were found in all but one of the women following mCPP administration before dieting, and in all the women after dieting. Basal ratings of unreality were not significantly different before and after dieting ($p=0.52$), and no significant effect of dieting on unreality ratings were found. The ANOVA on change from baseline data revealed no significant main effect of occasion ($F=2.17$; d.f.=1,6; $p=0.17$), a significant main effect of time ($F=10.49$; d.f.=6,66; $p=0.0001$) and no significant occasion by time interaction ($F=0.68$; d.f.=6,66; $p=0.67$).

VA ratings of sweatiness were measured and no significant effect of dieting found. While all but five women reported an increase in ratings of sweatiness following mCPP administration before dieting, only five reported increases after dieting ($p=0.28$), and no significant effect of dieting on sweatiness was found. The ANOVA on change from baseline data revealed no significant main effect of occasion ($F=0.61$; d.f.=1,6; $p=0.45$), no significant main effect of time ($F=0.28$; d.f.=6,66; $p=0.94$) and no significant occasion by time interaction ($F=0.97$; d.f.=6,66; $p=0.45$).

All but one of the women reported increased ratings of hunger following mCPP administration before dieting, and all but three after dieting. Basal hunger ratings were not significantly different before and after dieting ($p=0.27$) and no significant effect of dieting on hunger was seen. The ANOVA on change from baseline data revealed no significant main effect of occasion ($F=1.14$; d.f.=1,6; $p=0.31$), no significant main effect of time ($F=0.93$; d.f.=6,66; $p=0.48$) and no significant occasion by time interaction ($F=1.37$; d.f.=6,66; $p=0.24$).

Increased ratings of happiness were reported following mCPP administration in all but two women, both before and after dieting. Basal happiness ratings were not significantly different before and after dieting ($p=0.73$) and no significant effect of dieting was seen on happiness. The ANOVA on change from baseline data revealed no significant main effect of occasion ($F=2.88$; d.f.=1,6; $p=0.12$), a significant main effect of time ($F=2.26$; d.f.=6,66; $p=0.05$) but no significant occasion by time interaction ($F=0.89$; d.f.=6,66; $p=0.51$).

Finally, VA ratings of mellowness also failed to show any significant differences before and after dieting. Increased ratings of feeling mellow were reported following mCPP administration in all women but three before and in all but two women after dieting respectively. Basal ratings were not significantly different before and after dieting ($p=0.33$) and no significant effect of dieting on mellowness was seen. The ANOVA on change from baseline data revealed no significant main effect of occasion ($F=2.15$; d.f.=1,6; $p=0.17$), no
significant main effect of time ($F=0.90$; d.f.$=6,66$; $p=0.50$) and no significant occasion by time interaction ($F=0.99$; d.f.$=6,66$; $p=0.44$).

### 3.3.3.5 Correlations

Following $m$CPP administration, no significant correlation was found between PRL response and plasma $m$CPP drug levels. Correlations between plasma $m$CPP and plasma PRL before and after dieting were $r=0.40$; $p=0.20$ and $r=0.02$; $p=0.96$ respectively.

No significant difference in basal PRL levels were found before and after dieting (see section 3.3.3.3), but a significant negative correlation was found between basal plasma PRL concentration and PRL response before dieting ($r=-0.70$; $p=0.01$; Figure 3.17a), although the correlation failed to reach significance following dieting ($r=0.19$; $p=0.56$). When PRL response and weight loss were compared, no significant correlation between the PRL response after dieting and absolute weight loss ($r=-0.12$; $p=0.72$) or percentage body weight loss ($r=-0.15$; $p=0.65$) was found.

No significant correlation was found between plasma total TRP and PRL response before ($r=-0.22$; $p=0.50$), and after ($r=0.13$; $p=0.69$) dieting. Similarly, no significant correlation was found between plasma free TRP levels and the PRL response before ($r=0.39$; $p=0.21$), and after ($r=-0.15$; $p=0.64$) dieting. There was no significant correlation between weight loss and change in plasma TRP levels in terms of either absolute weight loss (total TRP:
r=0.32; p=0.31; free TRP: r=-0.29; p=0.36) or percentage body weight loss (total TRP: r=0.32; p=0.30; free TRP: r=-0.23; p=0.46). A positive and significant correlation between plasma oestradiol concentration and PRL response was found in the women, however, after dieting (r=0.72; p=0.008; Figure 3.17b) but failed to reach significance before dieting (r=-0.02; p=0.94).

3.3.3.6 Comparisons Between d-Fenfluramine and mCPP dieters

When the females who participated in the d-fenfluramine and mCPP dieting studies were compared, no significant differences in age (p=0.20), body mass index (p=0.20), starting weight (p=0.27), plasma oestradiol concentration (p=0.60) or initial plasma total (p=0.16) and free (p=0.28) TRP levels were found. Similarly, weight loss between the two groups was not significantly different in terms of either absolute weight loss (p=0.82) or percentage body weight loss (p=0.37).

3.4 DISCUSSION

3.4.1 DIETING AND REPEAT ADMINISTRATION STUDIES WITH d-FENFLURAMINE

Two women dieters and three women in the repeat d-fenfluramine administration study were determined by disparate plasma oestradiol levels to have been tested at different phases of their menstrual cycle and were, therefore, excluded from further analysis. Studies by O’Keane et al (1991) and Hrboticky et al (1989) have demonstrated changes in PRL responses to d-fenfluramine and in TRP metabolism respectively, across the menstrual cycle. Exclusion of the above females was supported by the finding of a positive and significant correlation between plasma oestradiol level and PRL response before dieting, and is in keeping with work by O’Keane et al (1991) suggesting that variations in d-fenfluramine-induced PRL responses across the menstrual cycle are caused by fluctuations in oestrogen levels. No significant correlation was found, however, between oestradiol levels and PRL response after dieting and in the repeat d-fenfluramine administration study.

A key finding of this study was an enhanced PRL response in women to d-fenfluramine following a three week period of moderate calorie restriction and subsequent weight loss. Although male dieters lost an equivalent amount of weight in both absolute and percentage body weight terms, no increase in PRL response was seen in the men after dieting. Females who did not diet (i.e. repeat d-fenfluramine administration study) also showed no alteration in PRL response to repeat d-fenfluramine challenges one month apart. Thus, the enhanced PRL response to d-fenfluramine seen in the female dieters could
not be explained by an "order" effect of \( d \)-fenfluramine testing. Indeed, PRL responses in women undergoing the second challenge in the repeat \( d \)-fenfluramine administration study, were slightly lower when compared to the first challenge, although neither baseline PRL changes nor PRL response to \( d \)-fenfluramine reached statistical significance when the second challenge was compared to the first. Furthermore, dieting did not alter plasma levels of \( d \)-fenfluramine or its active metabolite, \( d \)-norfenfluramine, suggesting that changes in \( d \)-fenfluramine disposition could not account for the increase in PRL release after dieting. An enhanced PRL response may be seen secondary to a "stress" reaction (see section 2.1.5), but this is unlikely to explain the enhanced response seen in the female dieters. VA ratings revealed no significant effects of dieting on \( d \)-fenfluramine-induced subjective changes (e.g. nausea, light-headedness and dizziness) that normally accompany such a reaction.

On the basis of earlier studies with TRP, it was hypothesised that moderate weight loss through dieting decreases plasma TRP sufficiently in women to reduce brain 5-HT synthesis, as synthesis of brain 5-HT has been shown to be dependent upon the availability of TRP from plasma (see section 1.2.1 for a full explanation). A subsequent reduction in 5-HT neurotransmission across the synapse results, which in turn produces a supersensitivity of post-synaptic 5-HT receptors, as evidenced by an enhanced PRL response to a TRP challenge after dieting. Animal studies in which rats fed a low TRP diet demonstrate enhanced PRL responses to 5-HT precursors such as 5-hydroxytryptophan support this hypothesis (Clemens \textit{et al}, 1980). Further support comes from the human study by Delgado \textit{et al} (1989) in which healthy volunteers maintained on a low TRP, non calorie-reducing diet for ten days also demonstrated enhanced PRL responses to a subsequent TRP infusion. The precise synaptic mechanism of the enhanced 5-HT mediated neuroendocrine response is, however, unknown although compensatory up-regulation of post-synaptic 5-HT receptors secondary to decreased 5-HT release appears the most likely explanation.

Consistent with this hypothesis, dieting in the present study led to a significant decrease in both plasma total and free TRP in women but no significant fall in plasma TRP was seen in the men. The findings contrast with those of earlier studies in two ways. Anderson \textit{et al} (1990a) reported significant decreases in total TRP for both men and women dieters (with falls in plasma total TRP greater in the women compared to men), but no significant change in plasma free TRP was found for either sex. One possible explanation for the finding that dieting lowered plasma total and free TRP in women alone is that plasma TRP levels in women are, in general, more vulnerable to dysregulation. Support for this possible explanation comes from a study of depressed patients who had lost weight, where it was found that significant decreases in plasma TRP were confined to female patients (Anderson \textit{et al}, 1990b). Another possible explanation for the failure of the men studied to lower their plasma TRP would be a smaller weight loss compared to the
women dieters. This was not borne out, however, in terms of absolute weight loss or percentage body weight loss which were not significantly different between the sexes. Moreover, no significant correlation was found between weight loss and plasma total or free TRP levels for either male or female dieters. The finding that plasma total and free TRP levels did not fall in women who did not diet, further suggests that no systematic change over time is likely to explain the significant fall in plasma TRP amongst female dieters.

An alternative explanation to the one outlined above for the enhanced PRL response seen in female dieters would be that dieting induces non-specific alterations in the PRL response to a 5-HT challenge drug. For example, a dieting-induced increase in the pituitary reserve of PRL would lead to an enhanced PRL response. Earlier work carried out by Anderson et al (1989), however, demonstrated that the enhanced PRL response in women after dieting was not secondary to direct stimulatory effects upon the pituitary lactotrophs as dieting had no effect upon TRH-induced PRL release. It was also shown that dieting did not alter dopaminergic neurotransmission as no effect of dieting was seen on PRL release by the dopamine antagonist metaclopramide, nor was there any effect on the physiological release (sleep-induced) of PRL (Anderson et al, 1989). These findings, coupled with the results from the present study with the selective 5-HT drug d-fenfluramine, argue against a non-specific effect, suggesting instead that dieting selectively alters 5-HT-mediated PRL release.

The selective 5-HT drug d-fenfluramine, is thought to increase PRL through indirect enhancement of serotonergic neurotransmission secondary to pre-synaptic release and re-uptake inhibition (Fuller et al, 1988; Campbell, 1991). It could be argued, therefore, that partial interruption of pre-synaptic 5-HT function could lead to an enhanced d-fenfluramine-induced PRL response (similar to that seen after dieting with TRP). Work by Meltzer et al (1982), in which rats were pretreated with the tryptophan hydroxylase inhibitor para-chloro-phenylalanine supports this theory. Pre-treatment with para-chlorophenylalanine, which leads to pre-synaptic depletion of 5-HT, has been shown to result in an enhanced PRL response to a subsequent dl-fenfluramine challenge when compared to saline pre-treatment. Dieting may produce a partial interruption of pre-synaptic 5-HT function secondary to chronic plasma TRP depletion leading to reduced 5-HT synthesis. Based on this hypothesis, the failure of the male dieters to lower their plasma TRP could explain their subsequent lack of an enhanced PRL response to d-fenfluramine. However, it is of interest that in the earlier dieting study by Anderson et al (1990a), significant falls in plasma total TRP in male dieters was still not accompanied by enhanced PRL responses to TRP. It appears therefore, that even when dieting in men does lead to significant falls in plasma TRP they are still less likely to demonstrate enhanced PRL responses, suggesting that women may be more vulnerable than men to diet-induced changes in brain 5-HT
function. An increased vulnerability in women with respect to alterations in brain 5-HT function is further suggested the study by Delgado et al. (1989) in which healthy human volunteers maintained on a low TRP diet for ten days demonstrated enhanced PRL responses to TRP. TRP depletion in the female subjects was found to produce a more robust increase in PRL response compared to the men. Interestingly, the fall in plasma TRP was also significantly greater in women compared to men.

It is possible that plasma TRP concentrations do not closely mirror brain 5-HT function (see section 1.2.1.1 for full discussion), and that this is reflected in the variable results in plasma total TRP seen across all dieting studies to date. If this is the case, it could also explain why a significant fall in free TRP occurred in the present study but not in earlier work, despite the utilisation of an identical diet and dieting regime and the achievement of similar amounts of weight loss. Furthermore, such an explanation is consistent with the contrasting correlations found between plasma TRP and PRL across studies. In the present study, no correlation was found between absolute or change in plasma free and total TRP and subsequent PRL response amongst dieters, while Anderson et al. (1990a) reported a finding of a trend towards a significant but positive correlation between change in plasma total TRP and change in PRL response, such that the greater the fall in plasma total TRP the higher the subsequent PRL rise. While this finding was seen for the dieting group as a whole, it was not seen when both sexes were considered separately. The presence of a complex relationship between plasma TRP and brain 5-HT levels in humans is further suggested by a study which utilised CSF TRP as an indirect index of brain TRP levels and in which the authors concluded that the relationship between plasma free TRP and brain TRP was not clear cut (Perez-Cruet et al., 1974). Moreover, the presence of such a complex and variable relationship argues against the earlier proposed hypothesis which suggests a direct relationship between plasma TRP depletion, decreased brain 5-HT function and subsequent enhanced PRL response.

The present finding of an enhanced PRL response to d-fenfluramine is also consistent with the hypothesis that dieting increases brain 5-HT function as proposed by Curzon (1988). In his work on rats, Curzon et al. (1972) demonstrated that plasma total TRP levels were unaltered in response to fasting yet brain 5-HT levels rose, apparently as a consequence of body fat store mobilisation increasing plasma unesterified fatty acids which directly compete with TRP for albumin binding. This results in increased concentrations of plasma free TRP available for transport into the brain, increasing 5-HT synthesis (see section 1.3.3.). Increased brain 5-HT synthesis in turn leads to enhanced synaptic 5-HT neurotransmission and to increased PRL responses to d-fenfluramine. What is not clear, however, is how the effects of total fasting compare to moderate calorie restriction in humans. While the finding of a significant fall in plasma TRP in the female dieters is against this latter hypothesis, the possibility remains that plasma TRP changes
are transient and/or do not directly relate to brain TRP levels. If this is indeed the case, it may explain the finding of variable plasma TRP levels in male dieters despite consistent findings of non enhancement of PRL responses to 5-HT challenge drugs after dieting.

### 3.4.2 EFFECTS OF DIETING ON mCPP-INDUCED PRL RESPONSE

A three week period of moderate calorie restriction and subsequent weight loss was found to have no significant effect on mCPP-induced PRL responses in women. Thus, in contrast to the female dieters in the d-fenfluramine study, the mCPP-induced PRL response was not enhanced after dieting. Furthermore, a three week period of moderate calorie restriction did not lead to a significant reduction in either plasma total or free TRP levels.

The finding of normal plasma TRP levels along with unaltered mCPP-induced PRL responses in women after dieting raises two possible explanations. Based on the hypothesis originally proposed, that dieting-induced changes in PRL response to a 5-HT challenge drug is dependent on plasma TRP depletion, failure to achieve such depletion in the present study appears the most likely explanation for the subsequent unaltered PRL response. What is not clear, however, is why a fall in plasma TRP did not occur. The diet utilised along with the dieting regime was identical in both studies, and mCPP female dieters lost as much weight as the d-fenfluramine female dieters in both absolute and percentage body weight terms. Moreover, starting weights and body mass indexes were similar and no significant correlation was found amongst mCPP dieters between change in plasma TRP concentration and weight loss, in either absolute or percentage body weight loss terms.

As outlined above, PRL responses to some 5-HT challenge drugs (O'Keane et al, 1991) are known to vary across the menstrual cycle. While no studies looking at the effects of menstrual cycle phase on mCPP-induced PRL responses have been published, the present finding of a significant positive correlation between plasma oestradiol levels and PRL response after dieting suggests that similar effects occur with mCPP and confirms the need to control for menstrual cycle phase. This finding is, however, unlikely to explain the disparate PRL results seen between the two groups of female dieters, as all women were challenged in the early to mid-follicular phase as confirmed by plasma oestradiol concentration. No significant differences in plasma oestradiol levels were found between the two groups of females at the pre- and post dieting challenge.

Dieting-induced alterations in mCPP absorption leading to lower plasma levels are unable to explain the failure to find an enhanced PRL response in the women after dieting. Plasma mCPP drug levels were not significantly different between challenges. Similarly, the use of mCPP at a dose unable to detect the presence of receptor supersensitivity is also
an unlikely explanation, as the pilot study demonstrated that an intravenous dose of 0.04mg/kg mCPP produces a significant but submaximal increase in PRL release in women when compared to placebo. Moreover, plasma mCPP concentrations measured in the study were comparable to those reported in the literature to produce reliable PRL responses (Kahn and Wetzler, 1991), and no significant correlation was found between mCPP drug levels and PRL response, either before or after dieting.

A significant, negative correlation was found between basal PRL concentration and PRL response before, but not after dieting, suggesting that the lower the baseline PRL concentration, the greater the subsequent PRL response. Basal PRL levels, however, were not significantly different before and after dieting. It is, therefore, unlikely that disparate basal PRL levels can account for the unaltered PRL results.

Based on the findings of the present study, the most likely explanation for unaltered plasma TRP levels amongst women mCPP dieters is the possibility that, in humans, plasma TRP levels do not closely reflect brain 5-HT function and that previously noted falls in plasma TRP are either transient and/or do not directly relate to the diet-induced changes seen in brain 5-HT function. Furthermore, the present finding that PRL responses to mCPP are unaltered by dieting suggests that post-synaptic 5-HT$_{2C}$ receptors are not supersensitive.

While the findings argue against the originally proposed hypothesis that dieting leads to decreased brain 5-HT function with the consequent development of post-synaptic receptor supersensitivity, they are consistent with the hypothesis that dieting leads to increased brain 5-HT neurotransmission. As outlined earlier in the discussion, Curzon (1988) suggested that while fasting has no systematic effect on plasma TRP, it does lead to increased brain 5-HT function. Thus if we assume that plasma TRP levels are variable and do not relate directly to brain 5-HT function, then the failure to find a significant decrease in plasma TRP in the mCPP dieters would be in keeping with this hypothesis. Furthermore, from this point of view, pre-synaptic enhancement of 5-HT neurotransmission secondary to increased 5-HT synthesis, is consistent with both an enhanced PRL response to d-fenfluramine (a pre-synaptic challenge) and a lack of dieting-induced changes following mCPP (a post-synaptic challenge). Enhanced pre-synaptic brain 5-HT neurotransmission is thought to lead to an adaptive downregulation of post-synaptic receptors in which the receptors become sub-sensitive. Such an adaptive response would result in either a reduced, or possibly an unchanged, PRL response to a direct post-synaptic receptor agonist such as mCPP. For example, chronic electroconvulsive therapy and antidepressant administration have been shown to lead to an attenuation of 5-HT$_{1A}$ receptor-mediated responses (8-OH-DPAT-induced hypothermia and serotonin syndrome in the rat), secondary to down-regulation of 5-HT$_{1A}$ receptors as an adaptive response to increased
availability of pre-synaptic 5-HT (Goodwin et al, 1987a). More recently a similar adaptive response has been shown to occur in humans. Lesch et al (1990) demonstrated attenuation of 5-HT_{1A}-mediated hypothermia (ipsapirone-induced) in a group of depressed patients following chronic amitriptyline administration.

One final alternative explanation is suggested by the mCPP results. It is possible that dieting-induced changes in brain 5-HT function are not mediated through the post synaptic receptors with which mCPP interacts. If this were the case, it would explain the lack of an enhanced PRL response to mCPP. It would also suggest that an enhanced response may follow administration of agonist drugs more specific for other 5-HT receptor subtypes, for example 5-HT_{1A} receptors.

3.5 CONCLUSIONS

Findings from the present study provide further evidence in support of the hypothesis that dieting-induced alterations in PRL release are 5-HT mediated, and add to the data indicating that even moderate dieting can alter brain 5-HT function in women. In addition, the findings suggest that the enhanced PRL responses seen in women after dieting probably reflect pre-synaptic changes in brain 5-HT function, perhaps as a consequence of alterations in the synthesis and release of 5-HT. The present findings do not support the proposed hypothesis that dieting-induced enhanced PRL responses occur secondary to the development of post-synaptic 5-HT receptor supersensitivity.
Chapter 4
EFFECTS OF \textit{m}CPP ON FOOD INTAKE AND PLASMA PROLACTIN IN WOMEN

4.1 INTRODUCTION

Substantial evidence now exists in support of a central role by serotonergic pathways in the control of appetite in both animals and humans. As outlined in the literature review in Chapter One, one of the most striking and consistent findings in the area of appetite research has been the suppression of food intake by experimental conditions that either directly, or indirectly, enhance brain 5-HT neurotransmission (Samanin \textit{et al}, 1980; Gottfries, 1981; Silverstone and Goodall, 1986; Leibowitz, 1990). The converse also appears to hold true, with an increase in food consumption accompanying reduced brain 5-HT function although the finding appears more sensitive to experimental design (Lavenstein \textit{et al}, 1962; Breisch \textit{et al}, 1976; Silverstone and Goodall, 1986). More recent experiments have suggested that 5-HT may also be important in the control of circadian feeding patterns, and in determining macronutrient choice through satiety mechanisms that involve the termination of feeding behaviour (Silverstone and Goodall, 1986; Leibowitz, 1990). Serotonin appears to be primarily concerned with the control of carbohydrate and protein intake, with serotonergic stimulation leading to a reduction in the proportion of carbohydrate consumed in the diet. For example, the administration of the 5-HT releasing drug \textit{d}-fenfluramine to healthy male volunteers was found to exert an anorectic effect in terms of reduced total calorie consumption and a significant reduction in carbohydrate consumption while sparing protein intake (Goodall \textit{et al}, 1991). In contrast, acute depletion of plasma TRP by a low TRP drink was found to have no effect on total calorie or carbohydrate consumption in a group of healthy male subjects, but did lead to a significant reduction in protein intake (Young \textit{et al}, 1988).

Interest in the role that different 5-HT receptor subtypes play in mediating human feeding behaviour has been stimulated by the recent increased availability of selective 5-HT drugs suitable for human research. Animal data highlighted the role of 5-HT1\textsubscript{B}/2C
receptors in feeding behaviour and appetite control in the rat. 5-HT$_{1B}$ receptors however are absent from the human brain. In a study by Goodall _et al_ (1984), _d_-fenfluramine-induced anorexia was blocked in three out of four human subjects by pre-treatment with the non-selective 5-HT$_{1/2}$ antagonist melperone. _d_-Fenfluramine-induced anorexia in another group of twelve healthy male volunteers was attenuated following pre-treatment with the 5-HT$_{2A/2C}$ antagonist ritanserin (Goodall _et al_., 1993), suggesting a role of 5-HT$_2$ receptors in the control of food intake in humans.

The identification of _m_CPP as a highly selective probe of serotonergic function provided the opportunity to investigate further the role of 5-HT pathways in the control of human feeding behaviour. As outlined in section 2.1.4.2, _m_CPP appears to act primarily as a 5-HT agonist and is most potent at 5-HT$_2C$ receptors. A recent paper by Baumann _et al_., (1993), however, has raised the possibility that _m_CPP may also act pre-synaptically to enhance release of 5-HT in a similar way to _d_-fenfluramine. Animal studies have shown that, in keeping with other 5-HT agonist drugs (leading to enhanced serotonergic neurotransmission), both peripheral and central administration of _m_CPP leads to hypophagia in rats (Samanin _et al_., 1979; Kennett and Curzon, 1988b). Furthermore, hypophagia induced by centrally administered _m_CPP has been blocked by pre-treatment with the potent 5-HT$_2C$ antagonist mianserin (Kennett and Curzon, 1988b). The effect of _m_CPP on appetite in humans has not been explored. Animal work, however, suggests that _m_CPP administration to healthy female subjects should lead to a reduction in food intake, along with a selective decrease in carbohydrate consumption, secondary to enhancement of 5-HT neurotransmission. The finding of a reduction in food intake would provide data in support of a role by serotonergic pathways in the control of human feeding behaviour and suggest 5-HT$_2C$ receptor involvement in the mediation of these effects. A reduction in carbohydrate intake would further suggest a significant role by serotonergic pathways in determining human macronutrient choice.

Administration of oral _m_CPP, as opposed to intravenous _m_CPP, to female subjects also provided an opportunity to examine its effects on PRL release, as to date, few studies utilising oral _m_CPP as a 5-HT challenge drug in women have been published.

In the present study a combined neuroendocrine and test meal approach was utilised to examine the effects of oral _m_CPP in healthy women. The aims of the present study were four fold. Firstly, a pilot study was carried out to determine a suitable oral dose of _m_CPP for use in neuroendocrine and appetite studies in women. Secondly, the dose determined by the pilot study was used to examine the effects of _m_CPP on food intake in healthy female volunteers. More specifically, the effects of oral _m_CPP on two aspects of food consumption were studied i.e. total food consumption (as measured by calorie intake) and macronutrient selection. In addition, the effects of oral _m_CPP on PRL response in
women were determined. The use of \textit{m}CPP, as an agonist drug with highest affinity for the 5-HT\textsubscript{2C} receptor, also enabled a closer examination of the receptor subtypes involved in mediating serotonergic control of food intake in humans.

\section*{4.2 METHODS}

\subsection*{4.2.1 DETERMINATION OF DRUG DOSAGE (ORAL \textit{m}CPP PILOT STUDY)}

\subsubsection*{4.2.1.1 Introduction}

While the data presented in section 2.1.4.2 demonstrates the reliability of \textit{m}CPP-induced PRL release in humans, few published studies have utilised oral \textit{m}CPP as a 5-HT challenge in women. This fact, coupled with the absence of a clear dose-response curve for the drug, and the presence of gender differences in absorption of \textit{m}CPP, make it difficult to generalise findings between the sexes (Kahn and Wetzler, 1991). Earlier studies in the literature suggested that oral doses of at least 0.25mg/kg were required to produce consistent elevations in plasma PRL (Kahn \textit{et al}, 1990; Kahn and Wetzler, 1991). More recent work, however, demonstrated a significant decrease in slow wave sleep following the administration of oral \textit{m}CPP at 0.125mg/kg to healthy volunteers, an effect probably mediated through activation of 5-HT\textsubscript{2C} receptors (Katsuda \textit{et al}, 1993; Sharpley \textit{et al}, 1994). Furthermore, personal experience with \textit{m}CPP in dieters suggested that smaller intravenous doses of \textit{m}CPP than those previously described in the literature are also sufficient to produce significant elevations in PRL increases in women (see Chapter Three).

In choosing a dose to examine the effects of \textit{m}CPP on food intake, the drug's propensity to produce side effects such as nausea and light-headedness, which might in themselves affect appetite, must be considered. While these effects are largely confined to higher doses, especially following intravenous administration, the use of \textit{m}CPP at a dose as low as possible would be important in order to minimise potential side effects that may make interpretation of appetite and neuroendocrine effects difficult. Thus, the dose chosen would need to be high enough to produce sufficient activation of 5-HT receptors (thereby enhancing brain 5-HT neurotransmission), but low enough to ensure a minimum of unwanted side effects.

Based upon data outlined above suggesting that low \textit{m}CPP doses in women produce reliable PRL release, coupled with the need to avoid side effects capable of independently affecting food intake, a placebo controlled pilot study was designed to
examine the effects of oral mCPP at doses of 7.5mg and 15mg (approximately 0.13mg/kg and 0.25mg/kg mCPP) on food intake and PRL release in women.

4.2.1.2 Subjects and Method

Twelve healthy female volunteers were recruited as described under the Selection and Assessment of Subjects section in Chapter Two. Subjects were aged between twenty-two and thirty-seven years (mean age=29 years ± 1.5), and all were of average weight as determined by a body mass index of between nineteen and twenty-eight (mean ± sem body mass index=22.4 ± 0.56; range 19.5-25.5). All gave written, informed consent before participating in the study which had been approved by the local ethics committee.

The study was a double-blind, placebo controlled, cross-over design with a balanced order of drug administration. Subjects were tested on three occasions within the early to mid-follicular phase of the menstrual cycle with menstrual cycle phase confirmed by plasma oestradiol levels. As challenge days were separated by at least five days, the study extended over two consecutive menstrual cycles. Each subject received placebo, 7.5mg mCPP and 15mg mCPP, given in the form of a single opaque capsule. Subjects received either placebo or mCPP at time 0, and blood sampling for PRL and mCPP drug levels was carried out at thirty minute intervals from -30 minutes until +180 minutes. Full details of the neuroendocrine procedure will be outlined below in section 4.2.3.

The test meal (procedure outlined in section 4.2.4) was presented three and a half hours after the ingestion of the test drug. Subjects were allowed to take as long as they wished over their meal and food intake analysed in the standard manner as outlined in section 2.3.3.1. Plasma collection and analysis was carried out using the standard method outlined in Chapter Two.

4.2.1.3 Statistics

The PRL results were analysed with a two-way repeated measures ANOVA, with post hoc t-tests where the interaction term was significant. Data from the test meal were analysed with a one way repeated measures ANOVA. The PRL data were also measured as area under the curve with subtraction of baseline secretion (AUC-B) extrapolated from time 0 using the trapezoid method. Plasma drug concentrations of mCPP were measured as area under the curve (AUC) from time 0.

4.2.1.4 Results

mCPP Drug Levels
Oral administration of 7.5mg and 15mg mCPP led to detectable concentrations of mCPP in plasma from +60 and +30 minutes respectively. No detectable concentrations of plasma mCPP were seen after placebo administration. Peak plasma concentrations following 7.5mg mCPP (mean ± sem = 7.07ng/ml ± 3.27) and 15mg mCPP (mean ± sem = 12.38ng/ml ± 5.21) were seen at +120 and +180 minutes respectively. (Figure 4.1a)

![Figure 4.1. (a): Mean ± sem plasma mCPP levels in twelve female volunteers following oral administration of 7.5mg and 15mg mCPP. (b): Effect of oral mCPP (7.5mg and 15mg) on plasma PRL (shown as mean ± sem change from baseline) in twelve female volunteers. Plasma PRL was significantly elevated following mCPP, compared to placebo, at the time points indicated. Paired-t test: * p < 0.05; ** p < 0.01.

Plasma drug concentrations were not significantly different following the two doses (Figure 4.1a). The ANOVA revealed no significant main effect of occasion [i.e. effect of 7.5mg versus 15mg mCPP] (F=0.81; d.f.=1,6; p=0.39), a significant main effect of time [i.e. variation in mCPP levels over time] (F=5.60; d.f.=6,66; p<0.0001), but no significant occasion by time interaction [i.e. effect of 15mg versus 7.5mg mCPP on variation in mCPP levels over time] (F=1.79; d.f.=6,66; p=0.11). Similarly, when analysed as AUC, plasma mCPP was not significant different following either dose (mean plasma mCPP ± sem: 7.5mg=851.6ng/ml⁻¹.min⁻¹ ± 403.5; 15mg=988.2ng/ml⁻¹.min⁻¹ ± 273.8; p=0.59).

**PRL Response to mCPP**

Following placebo administration, plasma PRL levels fell, in keeping with the circadian fall in PRL release seen across the morning, reaching a plateau at approximately...
+120 minutes. In contrast when compared to placebo, mCPP administration significantly increased plasma PRL concentrations after an initial fall reflecting the circadian decline (Figure 4.1b). The ANOVA on change from baseline data revealed a significant main effect of occasion [i.e. effect of mCPP versus placebo] (F=7.45; d.f.=10,110; p<0.001), a significant main effect of time [i.e. variation in PRL over time] (F=2.77; d.f.=5,55; p=0.03), and a significant occasion by time interaction [i.e. effect of mCPP on variation in PRL over time] (F=7.45; d.f.=10,110; p<0.001). Post-hoc testing revealed that PRL levels were significantly higher following 7.5mg and 15mg mCPP, when compared to placebo, at +150 and +180 minutes and +90, +120, +150, and +180 minutes respectively. Plasma PRL levels were not significantly different between the two mCPP doses. When analysed as AUC-B, plasma PRL response was significantly higher following mCPP compared to placebo (mean ± sem: placebo=-13304mIU/l⁻¹.min⁻¹ ± 1780; 7.5mg=-6631mIU/l⁻¹.min⁻¹ ± 5176; 15mg=-3060mIU/l⁻¹.min⁻¹ ± 3158; p=0.002), but the PRL response was not significantly different between the 7.5mg and 15mg mCPP doses (p=0.39).

**Effect of Oral mCPP on Food Intake**

Food intake during the buffet-style lunch was not significantly altered by mCPP (Figure 4.2), in terms of either total intake (p=0.24) or macronutrient choice (all p values >0.1).

![Figure 4.2. Food intake in twelve healthy female volunteers following placebo, 7.5mg and 15mg mCPP. Food intake following mCPP administration was not significantly different when compared to placebo.](image-url)
VA Ratings

No significant effects of oral mCPP was found on neuroendocrine VA ratings (nausea, anxiety, light-headedness, happiness and depression), or upon VA ratings of appetite (hunger and wish to eat).

4.2.1.4 Conclusions

Oral administration of 7.5mg and 15mg doses of mCPP led to detectable levels of mCPP in plasma from +60 and +30 minutes respectively. Peak plasma drug levels following the 15mg mCPP dose (approximately 0.25mg/kg mCPP) were slightly lower than those reported in the literature in male subjects receiving the same oral dose, but are in keeping with the gender differences in plasma levels noted for the drug (Kahn et al, 1990; Kahn et al, 1991). Oral mCPP at doses of 7.5mg and 15mg led to significant increases in PRL release compared to placebo. No effect on food intake, in terms of total consumption or macronutrient selection was seen.

The results suggest that while smaller doses of mCPP than those reported in the literature do appear sufficient to consistently increase plasma PRL, higher oral doses may be required before the anorectic effects widely reported in animal studies are seen in human females. An oral dose of 0.4mg/kg was, therefore, chosen for use in the main study designed to examine the effect of mCPP on food intake and PRL response in women. It was felt that this dose would provide sufficient stimulation of 5-HT receptors, while minimising side effects.

4.2.2 SUBJECTS

Twelve healthy female volunteers were recruited as described under the Selection and Assessment of Subjects section in Chapter Two. All subjects were of average weight as determined by a body mass index of between nineteen and twenty-eight (mean ± sem body mass index=22.5 ± 0.47; range 19.1-24), and were aged between nineteen and thirty-four years of age (mean ± sem age=26.4years ± 1.18). All gave written, informed consent to participating in the study which had been approved by the local ethics committee.

Subjects underwent neuroendocrine testing with placebo and mCPP on separate occasions approximately one week apart. Test meals were carried out at the completion of blood sampling. Challenges took place in double-blind, randomised order in the early to mid-follicular phase of a single menstrual cycle for all but one of the subjects. One female was forced to delay her second challenge test due to illness, so this was carried out at the
corresponding time of her following menstrual cycle. Menstrual cycle phase was confirmed by plasma oestradiol concentration.

4.2.2 NEUROEDOCRINE CHALLENGE PROCEDURE

Neuroendocrine testing was carried out following an overnight fast with subjects requested not to eat or drink from midnight. Subjects were brought to the Research Unit at approximately 08.30 hours and an indwelling venous cannula inserted into the arm. During testing, subjects rested semi-supine on a bed and were not allowed to eat, drink, smoke or sleep.

After insertion of the venous cannula, a blood sample for baseline plasma PRL estimation was taken. Subjects were then left to rest quietly on the bed for thirty minutes before administration of the test drug between 09.00 and 09.30 hours (time 0). mCPP, 0.4mg/kg or placebo was administered orally in a single unmarked opaque capsule (Littlemore Hospital Pharmacy; Oxford; UK). Blood samples for plasma PRL and mCPP drug levels were taken every thirty minutes from time 0 until +150 minutes.

4.2.3 TEST MEAL PROCEDURE

Fifteen minutes after the completion of neuroendocrine testing, subjects were placed at a table in a quiet room on their own. Meal instructions were given by the author in the standardised manner outlined in section 2.3.3.1. The food was then presented on trays in the standardised format and placed on the table in front of the subject. Subjects were left alone to eat and asked to come out of the room when they had finished their meal. Following completion of the meal, the trays were removed without comment. Food not consumed was weighed and calorie consumption calculated as outlined in section 2.3.3.1.

4.2.4 SUBJECTIVE RATINGS

mCPP is generally well tolerated by human subjects but side effects have been reported, especially at high doses. While our previous experience with the drug suggested that an oral dose of 0.4mg/kg would be well tolerated by women, it was also likely that transient side effects would occur. VA rating scales for happiness, anxiety, hunger, light-headedness, nausea, mellowness, sweatiness and a sense of reality were, therefore, presented to subjects throughout testing. The VA rating scales were presented in the standardised manner outlined in section 2.3.1 at thirty minute intervals between -30 and +150 minutes.
4.2.5 STATISTICAL ANALYSIS

4.2.5.1 Neuroendocrine Challenge Data Analysis

PRL data were plotted against time (as change from baseline) and then analysed with two way repeated measures ANOVA with Fisher's test of least significance difference as a post hoc range test. PRL data were also analysed as area under the curve with subtraction of baseline secretion (AUC-B) extrapolated from time 0 using the trapezoid method. Drug concentration data were measured as area under the curve (AUC) from time 0. VA data were plotted against time and then analysed with ANOVA, with Fisher's test as a post hoc range test.

4.2.5.2 Test Meal Analysis

All food items were weighed to the nearest 0.1gm before and after the meal. Calculation of final calorie consumption was carried out as described in section 2.3.3.1. Data from the test meals were analysed using paired t testing.

4.3 RESULTS

4.3.1 PLASMA DRUG LEVELS: \( m \)CPP

Figure 4.3. Mean ± sem plasma \( m \)CPP concentrations in twelve female volunteers following oral administration of 0.4mg/kg \( m \)CPP.
Following \(m\)CPP administration, detectable concentrations of \(m\)CPP were present in plasma from +30 minutes, while no detectable concentrations of plasma \(m\)CPP were found following placebo administration (Figure 4.3). Peak plasma concentrations of \(m\)CPP (mean ± sem=13.47ng/ml ± 2.48) were detected at +120 minutes. When analysed as AUC, mean ± sem plasma \(m\)CPP following 0.4mg/kg \(m\)CPP administration was 1131.5ng/ml\(^{-1}\).min\(^{-1}\) ± 211.2.

### 4.3.2 PRL RESPONSE TO \(m\)CPP

Following an initial fall, in keeping with the natural circadian fall in PRL release seen across the morning, plasma PRL levels increased following \(m\)CPP administration. In contrast, after placebo administration PRL levels continued to fall (Figure 4.4).

![Figure 4.4](Image)

Figure 4.4 Mean ± sem plasma PRL concentration (change from baseline) following placebo and 0.4mg/kg \(m\)CPP challenges in twelve female volunteers. PRL responses were significantly increased following \(m\)CPP at the time points indicated. Fisher's test: * p < 0.05, ** p < 0.01

Basal PRL concentrations at placebo and \(m\)CPP challenges were not significantly different (mean ± sem PRL at time 0: placebo=236mIU/l ± 41.7; \(m\)CPP=242mIU/l ± 55.3; \(p=0.84\)) but the PRL response following \(m\)CPP administration was significantly different
compared to placebo. The ANOVA on change from baseline data revealed no significant main effect of occasion [i.e. effect of mCPP] (F=2.80; d.f.=1,4; p=0.12), no significant main effect of time [i.e. variation in PRL over time] (F=0.86; d.f.=4,44; p=0.50), but a significant occasion by time interaction [i.e. effect of mCPP on variation in PRL over time] (F=3.50; d.f.=4,44; p=0.01). However, when analysed as AUC-B, PRL responses following mCPP administration were not significantly different compared to placebo. Mean ± sem PRL response following mCPP and placebo were -1687mIU/l⁻¹.min⁻¹ ± 7335 and -10663mIU/l⁻¹.min⁻¹ ± 3091 respectively (p=0.15).

**4.3.3 EFFECT OF mCPP ON FOOD INTAKE**

mCPP administration was found to have a significant effect on food intake in women (Figure 4.5).

![Figure 4.5. Effect of mCPP on food intake in twelve female volunteers, shown as mean ± sem kJs consumed. Oral mCPP (0.4mg/kg) had a significant effect on food intake in terms of both total energy and macronutrient consumption. Paired t test: **p < 0.01.](image)

Total calorie consumption in the women was reduced following mCPP administration when compared to placebo (mean ± sem: mCPP=2659.5kJ ± 286.1;
Macronutrient intake was similarly reduced following mCPP administration when compared to placebo. Reductions in protein intake (mean ± sem: mCPP=367.2kJ ± 49.7; placebo=572.4kJ ± 47.0; p=0.0007), fat intake (mean ± sem: mCPP=1051.8kJ ± 159.3; placebo=1578.3kJ ± 160.4; p=0.01) and carbohydrate intake (mean ± sem: mCPP=1240.3kJ ±119.1; placebo=1715.0kJ ± 128.5; p=0.007) were found.

### 4.3.4 SUBJECTIVE RATINGS

#### 4.3.4.1 Neuroendocrine VA

An increase in nausea was reported by two women following placebo administration, and by all but three women following mCPP administration. Baseline nausea ratings in the women were not significantly different between challenges (p=0.15), but a significant effect of mCPP on nausea was seen compared to placebo (Figure 4.6a).

![Figure 4.6](image-url)

**Figure 4.6.** Effect of oral administration of 0.4mg/kg mCPP on VA ratings of nausea (a) and anxiety (b), shown as mean ± sem. Fisher's test: **p < 0.01.

The ANOVA revealed a significant main effect of occasion [i.e. effect of mCPP vs placebo] (F=7.63; d.f.=1,5; p=0.02), a significant main effect of time [i.e. variation in nausea over time] (F=4.77; d.f.=5,55; p=0.001), and a significant occasion by time interaction [i.e. effect of mCPP on variation in nausea over time] (F=3.68; d.f.=5,55; p=0.006). At +150 minutes, however, nausea ratings following mCPP administration had fallen close to baseline levels and no significant differences between placebo and mCPP ratings were seen (mean ± sem nausea at +150 minutes: placebo=2.42 ± 2.01; mCPP=5.58
When measured as AUC-B, differences in nausea ratings for placebo and mCPP challenges just failed to reach significance (mean AUC-B ± sem nausea: placebo=157.5 ± 134.1; mCPP=1315.4 ± 521.4; p=0.06).

Four women reported increased anxiety following placebo administration and all but three women reported increases after mCPP administration (Figure 4.6b). Basal anxiety ratings were not significantly different between placebo and mCPP challenges (p=0.30). The ANOVA revealed no significant main effect of occasion [i.e. effect of mCPP vs placebo] (F=2.90; d.f.=1,5; p=0.12), a significant main effect of time [i.e. variation in anxiety over time] (F=2.47; d.f.=5,55; p=0.04), but no significant occasion by time interaction [i.e. effect of mCPP on variation in anxiety over time] (F=2.12; d.f.=5,55; p=0.08).

Seven women reported increased light-headedness after administration of placebo and nine women reported increases after mCPP. Baseline ratings of light-headedness were not significantly different between placebo and mCPP challenges (p=0.94), but a significant effect of mCPP on light-headedness was seen. The ANOVA revealed a significant main effect of occasion [i.e. effect of mCPP vs placebo] (F=8.97; d.f.=1,5; p=0.01), a significant main effect of time [i.e. variation in light-headedness over time] (F=3.72; d.f.=5,55; p=0.006), and a significant occasion by time interaction [i.e. effect of mCPP on variation in light-headedness over time] (F=2.96; d.f.=5,55; p=0.02; Figure 4.7a).

![Figure 4.7. Effect of mCPP on VA ratings of light-headedness (a) and hunger (b). Oral administration of 0.4mg/kg mCPP significantly increased ratings of light-headedness (a), and significantly decreased ratings of hunger (b). Fisher's test: * p < 0.05; ** p < 0.01.](image-url)
By +150 minutes, light-headedness ratings were no longer significantly different and had fallen to near baseline levels (mean ± sem light-headedness at +150 minutes: placebo=11.75 ± 3.67; mCPP=18.25 ± 6.74; p=0.34)

Following the administration of placebo, all but one woman reported increased ratings of hunger and following mCPP administration seven out of the twelve women reported an increase. Basal hunger ratings were not significantly different between the two challenges (p=0.26), but a significant decrease in hunger followed mCPP administration (Figure 4.7b). The ANOVA revealed no significant main effect of occasion [i.e. effect of mCPP vs placebo] (F=2.80; d.f.=1,5; p=0.12), a significant main effect of time [i.e. variation in hunger over time] (F=3.42; d.f.=5,55; p=0.009), and a significant occasion by time interaction [i.e. effect of mCPP on variation in hunger over time] (F=4.35; d.f.=5,55; p=0.002). Similarly, when analysed as AUC-B, mCPP administration was followed by a significant decrease in hunger ratings (mean ± sem hunger: placebo=1531 ± 593; mCPP=-733 ± 750; p=0.02). Hunger ratings at the end of neuroendocrine testing remained significantly different between the two challenges. Mean ± sem hunger at +150 minutes for placebo and mCPP were 60.58 ± 8.52 and 44.08 ± 7.55 respectively (p=0.04).

In contrast, no significant effects were seen on desire to eat. Seven women experienced an increase in desire to eat ratings following placebo administration and three women reported a similar increase after mCPP. Baseline desire to eat ratings were not significantly different between placebo and mCPP challenges (p=0.10), and no significant effect of mCPP on desire to eat was found. The ANOVA revealed no significant main effect of occasion [i.e. effect of mCPP vs placebo] (F=0.94; d.f.=1,5; p=0.35), no significant main effect of time [i.e. variation in desire to eat over time] (F=2.30; d.f.=5,55; p=0.06), and no significant occasion by time interaction [i.e. effect of mCPP on variation in desire to eat over time] (F=1.82; d.f.=5,55; p=0.12).

Similarly, baseline happiness ratings at placebo and mCPP challenges were not significantly different (p=0.16). Happiness following mCPP administration tended to be lower than after placebo. The ANOVA revealed a main effect of occasion that just reached significance [i.e. effect of mCPP versus placebo] (F=4.63; d.f.=1,5; p=0.05), but no significant main effect of time [i.e. variation in happiness over time] (F=1.47; d.f.=5,55; p=0.21) and no significant occasion by time interaction [i.e. effect of mCPP on variation in happiness over time] (F=1.81; d.f.=5,55; p=0.12).

Nine out of the twelve women experienced an increase in depression following placebo administration while six experienced an increase following mCPP administration. Basal depression ratings between the two challenges were not significantly different
(p=0.14), but mCPP administration was followed by a significant reduction in the rise in ratings of depression across the morning when compared to placebo (Figure 4.8).

![Figure 4.8](image)

**Figure 4.8.** Effect of mCPP on VA ratings of depression (shown as mean ± sem) in twelve female volunteers. Ratings of depression were significantly reduced following mCPP administration when compared to placebo. Fisher's test: * p < 0.05; ** p < 0.01

The ANOVA revealed no significant main effect of occasion [i.e. effect of mCPP versus placebo] (F=2.51; d.f.=1.5; p=0.14), a significant main effect of time [i.e. variation in depression over time] (F=2.57; d.f.=5.55; p=0.04), and a significant occasion by time interaction [i.e. effect of mCPP on variation in depression over time (F=4.59; d.f.=5.55; p=0.001).

### 4.3.5 CORRELATIONS

Following mCPP administration, no significant correlation was found between PRL response and plasma mCPP drug levels (r=0.39; p=0.20). No significant correlation was found between nausea ratings (measured as AUC-B) and PRL response for either the placebo (r=0.32; p=0.32) or mCPP challenge (r=-0.09; p=0.78). Similarly, no significant correlations were found between PRL response and food intake for either placebo or mCPP challenges. Correlations between PRL response and food intake following placebo were r=0.07; p=0.83 (PRL response versus protein intake); r=0.14; p=0.67 (PRL response versus fat intake); r=-0.34; p=0.29 (PRL response versus carbohydrate intake) and r=-0.07; p=0.84 (PRL response versus total calorie intake). Following mCPP, correlations between PRL response and food intake were not significant at r=0.08; p=0.82 (PRL response versus protein intake), r=0.24; p=0.45 (PRL response versus fat intake), r=0.09; p=0.78 (PRL...
response versus carbohydrate intake) and $r=0.18; p=0.57$ (PRL response versus total calorie intake).

Nausea ratings were analysed as AUC-B and at +150 minutes. Correlations between nausea ratings and food intake were not significant following placebo administration for protein (AUC-B nausea: $r=-0.13; p=0.68; +150$ minutes nausea: $r=0.11; p=0.74$), fat (AUC-B nausea: $r=-0.29; p=0.35; +150$ minutes nausea: $r=-0.40; p=0.20$), or total calorie intake (AUC-B nausea: $r=-0.50; p=0.09; +150$ minutes nausea: $r=-0.50; p=0.10$). A significant, negative correlation was found between nausea ratings and carbohydrate intake following placebo administration (AUC-B nausea: $r=-0.65; p=0.02; +150$ minutes nausea: $r=-0.60; p=0.04$), but examination of the data revealed the correlation to be due to a single outlying data point and not therefore a true finding. At mCPP challenge, no significant correlations were found between nausea and food intake in terms of protein (AUC-B nausea: $r=0.06; p=0.85; +150$ minutes nausea: $r=0.03; p=0.92$), fat (AUC-B nausea: $r=0.18; p=0.59; +150$ minutes nausea: $r=-0.19; p=0.56$), carbohydrate (AUC-B nausea: $r=0.01; p=0.97; +150$ minutes nausea: $r=-0.09; p=0.79$) or total calorie consumption (AUC-B nausea: $r=0.11; p=0.73; +150$ minutes nausea: $r=-0.13; p=0.68$; Figure 4.9).

![Figure 4.9. Correlations between VA ratings of nausea and food intake. No significant correlation was seen following the mCPP challenge between nausea rating at +150 minutes and food intake [total food intake only shown].](image-url)
Hunger ratings were also analysed as AUC-B and at +150 minutes, and correlated with food intake. When analysed as AUC-B, no significant correlations were found between hunger ratings and food intake following placebo administration in terms of protein (r=0.17; p=0.60), fat (r=0.02; p=0.95), carbohydrate (r=0.25; p=0.44) or total calorie intake (r=0.16; p=0.62). Similarly, no significant correlations following mCPP administration were found between AUC-B hunger ratings and food intake for protein (r=0.19; p=0.56), fat (r=0.25; p=0.43), carbohydrate (r=0.17; p=0.59) or total calorie intake (r=0.25; p=0.44). When hunger ratings at +150 minutes were correlated with food intake however, significant correlations were found. Following placebo administration, a significant correlation was found between hunger and protein intake (r=0.62; p=0.03) and between hunger and total calorie intake (r=0.63; p=0.03), but not between hunger and fat (r=0.43; p=0.16) or carbohydrate intake (r=0.56; p=0.06). Following mCPP administration, hunger ratings at +150 minutes were significantly and positively correlated with both fat (r=0.63; p=0.03; Figure 4.10b) and total calorie consumption (r=0.60; p=0.04; Figure 4.10a), but no significant correlations were seen between hunger at +150 minutes and protein (r=0.38; p=0.23) or carbohydrate intake (r=0.44; p=0.15).

Figure 4.10. Correlations between ratings of hunger and food intake following mCPP administration. A significant positive correlation was found between VA ratings of hunger at +150 minutes and both fat (b) and total food intake (b).

VA ratings of light-headedness were analysed as AUC-B and at 150 minutes. Following placebo challenge, no significant correlation was found between light-headedness and protein (AUC-B light-headedness: r=0.08; p=0.80; light-headedness +150
minutes: $r=0.53; \ p=0.08$), fat (AUC-B light-headedness: $r=-0.31; \ p=0.33$; light-headedness +150 minutes: $r=0.14; \ p=0.66$) or total food consumption (AUC-B light-headedness: $r=-0.45; \ p=0.14$; light-headedness +150 minutes: $r=0.13; \ p=0.69$). A negative correlation, just reaching significance, was found after placebo administration between light-headedness (measured as AUC-B) and carbohydrate intake ($r=-0.60; \ p=0.04$; Figure 4.11a), but failed to reach significance when light-headedness was measured at +150 minutes ($r=-0.10; \ p=0.76$). Following mCPP administration, a significant positive correlation was found between light-headedness at +150 minutes and fat intake ($r=0.65; \ p=0.03$; Figure 4.11b) but not between AUC-B light-headedness and fat intake ($r=0.25; \ p=0.43$). No significant correlation was seen following mCPP administration between ratings of light-headedness and protein (AUC-B light-headedness: $r=0.07; \ p=0.83$; light-headedness +150 minutes: $r=0.003; \ p=0.99$), carbohydrate (AUC-B light-headedness: $r=0.19; \ p=0.55$; light-headedness at +150 minutes: $r=0.33; \ p=0.29$) or total food intake (AUC-B light-headedness: $r=0.23; \ p=0.47$; light-headedness at +150 minutes: $r=0.50; \ p=0.10$).

Depression ratings were similarly analysed as AUC-B and at +150 minutes. Following placebo administration, no significant correlation was found between ratings of AUC-B depression and food intake (protein: $r=-0.01; \ p=0.96$; fat: $r=0.33; \ p=0.29$; carbohydrate: $r=0.33; \ p=0.30$; total intake: $r=0.35; \ p=0.27$). When depression was

![Figure 4.11. Significant correlations between VA ratings of light-headedness and food intake in twelve female volunteers. A significant, negative correlation was found following placebo administration between ratings of light-headedness (measured as AUC-B) and carbohydrate intake (a). A significant, positive correlation was found between light-headedness and fat intake in women following mCPP administration (b).](image-url)
measured at +150 minutes, a significant positive correlation was found between ratings of depression and protein intake after placebo (r=0.62; p=0.03), but not for other measures of food intake (fat: r=0.34; p=0.27; carbohydrate: r=0.54; p=0.07), although a trend towards a positive correlation was seen between depression at +150 minutes and total food intake (r=0.56; p=0.06). Following mCPP administration, no significant correlation was found between AUC-B depression and food intake (protein: r=-0.18; p=0.57; fat: r=0.20; p=0.53; carbohydrate: r=0.08; p=0.80; total intake: r=0.12; p=0.72), but when depression ratings were analysed at +150 minutes, a significant positive correlation was seen between ratings of depression and fat intake and between depression and total food consumption (fat: r=0.63; p=0.03; total intake: r=0.61; p=0.03). No significant correlation was seen between depression ratings at +150 minutes and protein (r=0.44; p=0.16) or carbohydrate intake (r=0.45; p=0.14).

mCPP drug levels did not significantly correlate with food intake, in terms of protein (r=-0.11; p=0.74), carbohydrate (r=-0.14; p=0.66), and fat intake (r=0.04; p=0.91) or in terms of total energy consumption (r=-0.06; p=0.86). No significant correlation was found between mCPP drug levels and nausea ratings (nausea (AUC-B): r=0.48; p=0.11; nausea +150 minutes: r=0.17; p=0.61), or between mCPP drug levels and ratings of light-headedness (light-headedness (AUC-B): r=0.58; p=0.05; light-headedness +150 minutes: r=0.02; p=0.95). Similarly, no significant correlation was found between mCPP plasma levels and ratings of hunger when analysed as AUC-B hunger (r=-0.48; p=0.12) or hunger at +150 minutes (r=-0.26; p=0.41), or between mCPP plasma levels and ratings of depression (depression at +150 minutes: r=-0.34; p=0.29; depression AUC-B: r=-0.34; p=0.27).

4.4 DISCUSSION

Administration of oral mCPP at a dose of 0.4mg/kg was found to significantly increase plasma PRL in women, a finding in keeping with both animal and human data demonstrating enhanced PRL responses to mCPP (Aloi et al, 1984; Mueller et al, 1985; Bagdy et al, 1989b). The results add further support to recent data suggesting that smaller doses than those originally described in the literature appear capable of stimulating PRL release in women. Earlier human studies had suggested that consistent mCPP-induced PRL responses followed administration of oral doses between 0.5 and 0.75mg/kg, with only marginal effects seen at 0.25mg/kg (Mueller et al, 1985; Murphy et al, 1989; Kahn et al, 1990). The present results with 0.4mg/kg however, coupled with the findings of the pilot study (approximately 0.13 and 0.24mg/kg oral) and the mCPP diet study in women (0.04mg/kg intravenous), suggest that consistent and reliable PRL release is seen in females after substantially lower oral and intravenous doses than those previously reported.
The results are also consistent with data suggesting gender differences in mCPP-induced PRL release in humans. Two previous studies have found lower mCPP plasma concentrations per given dose in women compared to men, although the lower plasma levels in women were followed by greater PRL release (Charney et al., 1987; Kahn et al., 1991). It has been suggested that circulating oestrogens in women may be responsible for potentiating mCPP-induced PRL release, as similar PRL potentiation has been reported with other 5-HT challenge drugs (Charney et al., 1987; Kahn et al., 1991; O'Keane et al., 1991). Indeed, reports of oestradiol potentiation of PRL led to the decision to exclude results of any women found to have been tested out of phase in terms of menstrual cycle, although in this study no exclusions were necessary.

The significant increase in PRL response following mCPP administration cannot be explained by baseline differences in PRL, as basal PRL levels were not significantly different at placebo and mCPP challenge. Increased PRL release may accompany a "stress" reaction, but this explanation is also unlikely to account for the current findings. Significant increases in ratings of nausea and light-headedness were seen following mCPP administration compared to placebo, but the increases were transient and in keeping with ratings seen following other 5-HT challenge drugs (for further discussion, see below). Furthermore, no significant correlations were found between ratings of nausea and light-headedness (when assessed as either AUC-B and absolute rating at +150 minutes) and subsequent PRL response.

mCPP administration to women led to a significant reduction in food intake. Food intake was decreased in terms of total calorie consumption and for all the macronutrients measured i.e. protein, fat and carbohydrate. The finding of a decrease in total calorie consumption is in keeping with other studies using drugs that enhance brain 5-HT neurotransmission. To date, most studies looking at the effects of serotonin on food intake in humans have used the 5-HT releasing drug d-fenfluramine. Administration of d-fenfluramine to both lean and obese subjects has been shown to reduce total food intake by 11% to 40%, depending upon experimental design (Blundell and Hill, 1991). Reported effects on macronutrient intake, however, have been more variable. While a reduction in carbohydrate consumption is the most consistent finding following d-fenfluramine administration, decreases in both fat and protein intake have also been reported. A study by Wurtman et al. (1985), demonstrated a reduction in high fat/carbohydrate foods amongst obese subjects after d-fenfluramine administration, while both total calorie intake and fat consumption were reduced in healthy males following d-fenfluramine (Goodall et al., 1993). In a study by Hill and Blundell (1986), a significant reduction in all three macronutrients was found, while again in contrast, no effect on fat consumption followed d-fenfluramine administration to male subjects in another study by Goodall et al. (1993). Thus the current finding of a reduction in all three macronutrients in women following oral
mCPP at a dose of 0.4mg/kg is consistent with some reports in the literature with d-fenfluramine. While significant reductions in total calorie, carbohydrate and fat intake are more frequently reported, reductions in protein consumption have been found. Indeed a recent paper by Baumann et al (1993), suggested a common mechanism of action (through pre-synaptic release of 5-HT) for mCPP, the trifluoromethyl analog of mCPP 1-(m-trifluoromethylphenyl)piperazine, and d-fenfluramine in mediating PRL release in the rat. It is possible that the presence of a common mechanism of action on serotonergic pathways is also reflected in similar effects on food intake.

The present finding of a significant effect of 0.4mg/kg mCPP on food intake is in contrast to the findings of the pilot study. However, the most likely explanation for the failure to find an anorectic effect in the pilot study is the low dose of mCPP employed, as both animal and human data utilising 5-HT drugs have suggested dose threshold effects for the induction of anorexia. For example, d-fenfluramine shows a dose-response suppression of food intake in humans (Silverstone et al, 1975) while in rats, although the dose required to produce an anorectic effect is more dependent upon experimental conditions of food restriction, a similar threshold effect is seen with both d-fenfluramine(Campbell, 1991) and mCPP (Leibowitz and Shor-Posner, 1986).

Serotonergic drugs, such as mCPP and d-fenfluramine are thought to exert their effects on food intake through specific satiety mechanisms. For example, administration of d-fenfluramine to obese and lean subjects has been shown to reduce meal size while prolonging satiety, as reflected in a decreased number of between meal snacks (Blundell and Hill, 1991). A recent study by Kennett and Curzon (1988a), suggested that mCPP's ability to reduce food intake in rats occurs by a similar mechanism. Administration of mCPP to rats was followed by a significant reduction in food intake and an increase in the behavioural satiety sequence, a behaviour normally displayed by free feeding rats on reaching satiety. The authors concluded that their results supported the hypothesis that mCPP-induced hypophagia does occur specifically through satiety mechanisms, rather than through general behavioural effects such as reduced locomotion, which may in turn decrease food intake.

Subjective aspects of eating behaviour including measures of satiety have attracted less research. In the present study, VA ratings were utilised to assess some subjective aspects of eating behaviour. VA ratings of hunger, but not desire to eat, were significantly reduced following mCPP administration when compared to placebo. While the interpretation of these subjective experiences in humans is complex, Silverstone and Goodall (1986), suggests that hunger is a measure of the psychological sensations that accompany food deprivation, while desire to eat is a more accurate measure of "appetite" i.e. the person's desire for a given food at a particular time, regardless of hunger. Based
upon the theory that 5-HT drugs act purely on satiety mechanisms involving the termination of feeding (see section 1.2.3.1), it could be argued that no effect on hunger but some effect on desire to eat should follow mCPP administration, in direct contrast to the finding of the present study. Reports in the literature with other 5-HT drugs, however, do not clearly follow this pattern. Oral d-fenfluramine administration, like mCPP in the present study, has led to significant decreases in hunger ratings, although the changes appear somewhat variable. Goodall and Silverstone (1988a), found significant reductions in hunger motivation amongst normal weight subjects, during the period between dosing with acute d-fenfluramine and being offered a test meal, while Hill and Blundell (1986), also reported decreases in hunger ratings following d-fenfluramine administration amongst human subjects both before, during and after a test meal. In contrast, 60mg of oral d-fenfluramine led to significant reductions in the ratings of desire to eat, but not in ratings of hunger, amongst healthy volunteers in a study by (Silverstone and Goodall, 1986). Thus it would appear that drugs such as d-fenfluramine which exert their anorectic effects via satiety mechanisms also produce significant ratings of subjective hunger. The present findings with mCPP are in keeping with this.

The finding of a reduction in food intake provides further data in support of the theory that serotonergic pathways are involved in the control of human feeding behaviour. More specifically, it suggests a role by 5-HT \( \text{_{2C}} \) receptors in mediating these effects. The findings are in keeping with animal data which has highlighted the role of 5-HT \( \text{_{1B/2C}} \) receptors in appetite control in the rat (see section 1.2.3.1). Moreover, they are consistent with human work in which the 5-HT \( \text{_{2A/2C}} \) receptor antagonist ritanserin was found to block d-fenfluramine-induced anorexia, suggesting a role by 5-HT \( \text{_{2}} \) receptors in the control of food intake in humans (Goodall et al, 1993). While similarities between mCPP and d-fenfluramine on food intake could be explained by a shared mechanism of direct post-synaptic agonist activity by mCPP and d-norfenfluramine at 5-HT \( \text{_{2C}} \) receptors (since d-norfenfluramine exhibits modest affinity for these receptors), the recent paper by Baumann et al (1993), raises a possible alternate shared mechanism of action. Findings suggested that mCPP, like d-fenfluramine, is capable of stimulating pre-synaptic release of 5-HT and that such a mechanism of action may underlie partial control of mCPP-induced PRL release. Pre-synaptic enhancement of 5-HT neurotransmission, possibly acting through 5-HT \( \text{_{2}} \) receptors, may also mediate mCPP and d-fenfluramine effects on food intake.

An alternative explanation for the reduction in food intake must, however, be considered. The finding of significant increases in nausea and light-headedness following mCPP administration raises the possibility that the decrease in food intake occurred secondary to a more general effect on behaviour (i.e. the subjects feeling unwell) and not as a consequence of 5-HT-mediated anorexia. Consideration of the nausea ratings just before the meal (+150 minutes) show that they had returned to near baseline levels and
were not significantly different when placebo and \( m \text{CPP} \) challenges were compared. Furthermore, although a significant, negative correlation was found between carbohydrate intake and nausea ratings at +150 minutes following placebo, indicating that the higher the nausea rating the less the amount of carbohydrate eaten, no significant correlation was found between nausea ratings (either +150 minutes nausea and AUC-B nausea) and food intake following \( m \text{CPP} \) administration.

VA ratings of light-headedness were also significantly increased following \( m \text{CPP} \) when compared to placebo, but the effects were again transient. By +150 minutes, ratings of light-headedness had fallen close to baseline readings and were no longer significantly different from placebo ratings. Moreover, no consistent correlation was found between light-headedness and food intake. Following placebo administration, a negative correlation, just reaching significance, was seen between AUC-B (but not +150 minutes) light-headedness and carbohydrate intake indicating that the greater the light-headedness, the less the consumption of carbohydrate at test meal. In contrast, \( m \text{CPP} \) administration was followed by a significant positive correlation between ratings of light-headedness at +150 minutes (but not AUC-B light-headedness) and fat intake, suggesting that the greater the experience of light-headedness, the greater the consumption of fat.

Other behavioural changes reflected in the VA ratings that might be expected to affect food intake were those of anxiety and depression. While a significant increase in anxiety over time was seen, no specific effect of \( m \text{CPP} \) was seen on the ratings. In contrast, \( m \text{CPP} \) administration did lead to a significant decrease in the rate of rise in depression ratings seen following both challenges across time. While significant correlations were seen between ratings of depression and fat and total food consumption following \( m \text{CPP} \) administration, the correlation was positive such that the greater the rating of depression, the greater the consumption of fat and food overall. It could be argued that such a correlation would work against the main finding of a decrease in food consumption after \( m \text{CPP} \) administration.

The findings outlined above suggest that no consistent significant effect on general behavioural ratings, apart from positive correlations between food intake and depression and hunger ratings were seen following \( m \text{CPP} \) administration. While hunger ratings closely paralleled subsequent food intake and can be seen to reflect specific effects upon satiety, the influence of the correlation between depression and food intake would have been to minimise the overall decrease in food consumption seen after \( m \text{CPP} \) administration. The findings suggest that the \( m \text{CPP} \)-induced hypophagic effect observed in females was unlikely to have occurred secondary to a more general effect upon behaviour. The current finding is in keeping with work by Kitchener and Dourish (1994) who demonstrated that \( m \text{CPP} \)-induced hypophagia in rats is accompanied by specific satiety
behavioural patterns, in contrast to administration of the 5-HT$_2$ agonist DOI and the 5-HT$_{1B}$ agonist RU 24969. Administration of the latter drugs also induces hypophagia but does not produce behavioural profiles that resemble satiety, suggesting that these drugs' effect on food intake occur through general behavioural changes (such as hypoactivity), in contrast to mCPP-induced hypophagia which is mediated primarily through satiety mechanisms. Further support for the idea that mCPP acts through specific satiety mechanisms comes from another study in rats in which mCPP-induced hypophagia was unaffected by pre-treatment with an anti-emetic drug, suggesting that nausea was not the underlying mechanism leading to a decrease in food intake (Silverstone and Feeney, 1989).

While overall the data strongly suggests that the decrease in food intake seen in women following mCPP administration was mediated through effects on satiety as reflected in subjective ratings of hunger, what is not possible to rule out is the situation where an earlier experience of feeling ill continues to render a person unwilling to eat, even after subjective feelings of nausea and light-headedness have returned to normal. It could be argued that the finding of a overall reduction in food consumption supports this hypothesis, as a purely serotonergic drug would be expected to reduce total calorie intake and to have a more selective effect upon macronutrient intake, although the literature outlined above does suggest that 5-HT drug effects on macronutrient intake can be somewhat variable.

4.5 CONCLUSIONS

An oral mCPP dose of 0.4mg/kg was found to significantly increase plasma PRL in women, adding to the data that the 5-HT agonist drug mCPP consistently enhances PRL release in humans. The finding of a reliable PRL response to 0.4mg/kg mCPP, supports earlier findings that gender differences exist for the drug. Lower doses than those originally reported appear sufficient in producing reliable PRL responses in women.

A decrease in food intake also followed the administration of 0.4mg/kg mCPP in women. The reduction in food consumption was characterised by a decrease in total calories and a decrease in all macronutrients measured i.e. protein, fat and carbohydrate, a finding in keeping with some reports in the literature following administration of the 5-HT releasing drug, d-fenfluramine. Decreases in food intake were accompanied by decreases in VA ratings of hunger suggesting specific effects on satiety. While no consistent effects upon general behaviour were seen that might in itself affect food intake, it is not possible to rule out the possibility that an earlier experience of increased nausea and light-headedness continued to influence food intake, leading to a reduction in food consumption.
The finding that mCPP administration induces anorexia, provides further evidence in support of the theory that 5-HT pathways are involved in the control of food intake in humans. More specifically, it suggests a role by 5-HT$_{2C}$ receptors in mediating these effects, either through direct agonist activity at post-synaptic receptor level or possibly through increased pre-synaptic release of 5-HT.
Chapter 5

EFFECT OF ACUTE TRP DEPLETION ON MOOD AND FOOD INTAKE IN HEALTHY AND RECOVERED BULIMIC FEMALE SUBJECTS

5.1 INTRODUCTION

The data reviewed in Chapter One provide strong evidence in support of the hypothesis that serotonergic dysregulation occurs during clinical episodes of the eating disorder bulimia nervosa (see section 1.3.2 for a full review). For example, decreased levels of the major 5-HT metabolite, 5-HIAA, have been reported in the CSF of bulimic patients compared to controls (Jimerson et al, 1988). Increased platelet 5-HT reuptake compared to controls has also been reported, suggesting decreased availability of 5-HT at post-synaptic receptors (Goldbloom et al, 1990). Neuroendocrine studies have demonstrated blunted PRL responses to the 5-HT agonist mCPP and to the 5-HT releasing drugs dl- and d-fenfluramine in both acutely ill and recently recovered bulimic patients, again suggesting reduced brain 5-HT function (McBride et al, 1991; Brewerton et al, 1992).

It can also be argued that hyposerotonergic activity is consistent with the development of many of the behavioural disturbances that accompany bulimia nervosa. Low CSF 5-HIAA levels have been linked to a greater frequency of bingeing and purging behaviour (Jimerson et al, 1988), while a study by Kaye et al (1988), suggested a direct relationship between the development of satiety (defined as the cessation of bingeing and vomiting) and the ratio of plasma TRP:LNAA amongst a group of nine actively bingeing bulimic patients. The author suggested that when bingeing behaviour led to an increase in the ratio of plasma TRP:LNAA, patients experienced satiety and no longer continued to binge. In those patients where no increase in TRP:LNAA ratio was demonstrated, satiety was not reported and patients continued to binge and purge. The underlying mechanism leading to the development of satiety was thought to be an increase in brain 5-HT neurotransmission as evidenced by a greater PRL response, secondary to an increased plasma TRP:LNAA ratio in patients reporting satiety compared to those patients who did not increase their
The observation that many eating disordered patients choose to binge on high carbohydrate foods further suggests that bingeing may function as a mechanism enabling patients to raise their brain 5-HT levels. Carbohydrate consumption is known to cause insulin secretion which in turn promotes the uptake of branch chain amino acids into muscle. This process leads to an increase in the ratio of plasma TRP:LNAA, thus increasing transportation of TRP across the blood-brain barrier and enhancing brain 5-HT synthesis (see section 1.2).

Further support for a role of hyposerotonergic activity in the development of the eating disorders comes from the area of appetite research involving the pharmacological manipulation of brain 5-HT function. While one of the most consistent findings in this field of research has been a decrease in food consumption following experimental procedures that either directly or indirectly increase brain 5-HT function, the converse i.e. an increase in food consumption following brain 5-HT depletion, also holds true (Silverstone and Goodall, 1986; Fuller and Wong, 1989; Leibowitz, 1990). Moreover, clinical studies utilising selective 5-HT reuptake inhibitor antidepressant drugs in the treatment of bulimia nervosa have demonstrated consistent and significant improvements in both low mood and bingeing/purging behaviour, suggesting benefit from the drugs' ability to enhance brain 5-HT neurotransmission (Fluoxetine Bulimia Nervosa Collaborative Study Group, 1992; Linnoila et al, 1992).

Blunted neuroendocrine responses to 5-HT challenge drugs, suggesting decreased 5-HT neurotransmission, have consistently been reported in affective illness, especially depression (Deakin, 1991; Deakin and Graeff, 1991; Power and Cowen, 1992), and symptoms of low mood along with an increased lifetime risk of major depression are well recognised features of bulimia nervosa (Fairburn and Cooper, 1984; Fairburn and Beglin, 1990). For a subgroup of patients with bulimia nervosa, disorders of impulsivity such as shop-lifting, self harm, sexual promiscuity and alcohol abuse are commonplace. Such behaviours have been linked to low brain 5-HT function as increasing evidence suggests that serotonin exerts a restraining role on behaviour in general (Shaw et al, 1967; Linnoila et al, 1992).

The data reviewed in Chapter One suggests that many cases of clinical eating disorders, including bulimia nervosa, develop from "normal" periods of dieting in which control has been lost (see section 1.1.4). Furthermore, in Chapter Three it was demonstrated that periods of moderate calorie restriction are often associated with falls in plasma TRP, and one hypothesis put forward suggests that a reduction in plasma TRP
leads to a decrease in brain 5-HT function in female dieters (see section 3.4 for a full discussion). Thus dieting-induced falls in plasma TRP and the resultant decrease in brain 5-HT function may represent a key neurobiological mechanism that contributes to the development of eating disorders in vulnerable women (Cowen et al., 1992).

Animal studies have demonstrated that dietary manipulations which acutely lower plasma TRP produce decreases in brain 5-HT turnover in both rodents and non-human primates (Young et al., 1989; Gartside et al., 1992). In a study by Delgado et al. (1989), acute depletion of plasma TRP in humans by a low TRP diet was shown to produce an alteration in the PRL response to a TRP infusion, suggesting a similar decrease in brain 5-HT function occurs in humans. Acute TRP depletion has also been achieved by the use of amino acid mixtures deficient in TRP. The use of such mixtures has been shown to produce a fall in plasma TRP and thus a decrease in the plasma TRP:LNAA, which in turn leads to a reduced uptake of TRP into the brain (see section 2.2). Studies utilising the amino acid mixture have demonstrated an effective and similar magnitude of reduction in free and total plasma TRP following 100g and 52g amino acid drinks (Young et al., 1985; Delgado et al., 1990; Oldman et al., 1994), and have shown that acute TRP depletion is associated with a lowering of mood in both healthy (Young et al., 1985) and newly recovered depressed patients maintained on antidepressants (Delgado et al., 1990), although these findings have not been universally consistent (Oldman et al., 1994). The effect of acute TRP depletion on appetite and food intake in humans, however, has not been widely examined. In one study by Young et al. (1988), the effects of a 100g amino acid drink deficient in TRP on food intake were examined in healthy young males. In contrast to what would be expected from the animal data, acute TRP depletion was found to have no effect on total food intake or carbohydrate consumption, but did lead to a significant reduction in protein selection at a subsequent buffet style meal. No published studies to date have examined the effects of acute TRP depletion on mood and appetite in healthy women.

The data from studies utilising amino acid drinks in depressed patients suggest that TRP depletion is associated with a rapid return of previous depressive symptomatology. Acute TRP depletion in women recovered from bulimia nervosa, therefore, could also be expected to lead to a return of the symptoms associated with the disorder. More specifically, it could be hypothesised that TRP depletion would lead to a lowering of mood and alterations in the subjective experience of satiety, which in turn would be reflected in an increased consumption of food at test meal.
The aim of the present study was, therefore, to examine the effects of acute TRP depletion on mood and food intake in healthy female subjects and in women who had recovered from a previous episode of bulimia nervosa. The study utilised a 52g amino acid drink mixture deficient in TRP, as this mixture had previously been shown to produce a fall in plasma TRP of similar magnitude to the 100g drink (see section 2.2.2), while being far better tolerated by female subjects and causing a much reduced rate of nausea and vomiting (Oldman et al., 1994).

5.2 METHODS

5.2.1 SUBJECTS AND EXPERIMENTAL DESIGN

5.2.1.1 Healthy Female Volunteers

Twelve healthy female volunteers were recruited as described under the Selection and Assessment of Subjects section in Chapter Two. In addition, subjects were carefully screened to ensure normal dietary habits. Strict vegetarians were also excluded. Volunteers were aged between nineteen and twenty-seven years (mean ± sem.=22.3 years ± 0.8), and were all of normal weight for height as determined by a body mass index (mean ± sem. body mass index=23.0 ± 0.98; range=17.4-29). All subject gave written, informed consent to participating in the study which had been approved by the local ethics committee.

Using a within-subject [i.e. examining the effects of TRP depletion on individual groups of control and recovered bulimic subjects] and between-subject [i.e. comparing the effects of TRP depletion on recovered bulimic subjects with the effects of TRP depletion on control subjects] comparison design, the study involved examining the effects of a TRP depletion condition (T-), a balanced amino acid condition (B) and a placebo control condition (P) on mood changes across the morning and on appetite and food intake, as measured by a test meal procedure. The experimental conditions were achieved by the use of amino acid drinks (see below), with subjects undergoing testing on three separate occasions approximately one week apart. In order to avoid the late luteal phase of the menstrual cycle, all test sessions were carried out between day three and day twenty-one. As for most subjects this meant running the study over two consecutive menstrual cycles, randomisation of the different drinks in terms of menstrual cycle phase was also carried out to ensure no systematic bias occurred.

The highly distinctive consistency and taste of the two amino acid drink mixtures meant it was always possible for subjects to distinguish them from the (P) condition (but not from each other). Because of this, a semi-balanced design was adopted, with the (P)
condition always given first in a single-blind fashion. The final two test sessions were carried out in double-blind cross-over design (Table 5.1).

Table 5.1. Design of TRP depletion study in healthy volunteers

<table>
<thead>
<tr>
<th>Group Size</th>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Placebo</td>
<td>(B) Mixture</td>
<td>(T-) Mixture</td>
</tr>
<tr>
<td>6</td>
<td>Placebo</td>
<td>(T-) Mixture</td>
<td>(B) Mixture</td>
</tr>
</tbody>
</table>

5.2.1.2 Subjects Recovered From Bulimia Nervosa

Eight female subjects were recruited as described under the Selection and Assessment of Subjects section in Chapter Two. All subjects had previously met criteria for DSM-IIIR Bulimia Nervosa but had now recovered. Subjects had met recovery criteria (as defined in section 2.4.2.2 in Chapter Two) for a mean period of six years (range=6months-27years), and none had received psychotropic medication within the previous twelve months. Six of the women had previously met criteria for DSM-IIIR affective disorder, while three had a family history of affective illness. Subjects were aged between twenty-four and fifty-two years (mean ± sem age=32.4years ± 3.4), and were all of normal weight for height as determined by body mass index (mean ± sem body mass index=24.8 ± 1.13; range=21.1-29.1). Six of the eight recovered bulimic patients had normal menstrual cycles, one had previously undergone hysterectomy but was still able to recognise a regular monthly pattern of mood and physical changes suggestive of a menstrual cycle. The remaining female subject was post-menopausal and on hormone replacement treatment. All subjects gave written, informed consent to participating in the study which had been approved by the local ethics committee.

The experimental design of the study was identical to that outlined above for the healthy volunteers and is summarised below in Table 5.2.

Table 5.2. Design of TRP depletion study in recovered bulimic patients

<table>
<thead>
<tr>
<th>Group Size</th>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Placebo</td>
<td>(B) Mixture</td>
<td>(T-) Mixture</td>
</tr>
<tr>
<td>4</td>
<td>Placebo</td>
<td>(T-) Mixture</td>
<td>(B) Mixture</td>
</tr>
</tbody>
</table>

5.2.2 ACUTE TRP DEPLETION PROCEDURE

5.2.2.1 Composition of Amino Acid Drink

The procedure for TRP depletion was based on the method described by Young et al (1985), in which subjects ingested the amino acids in the form of flavoured drinks. The
The experimental drink consisted of fifteen amino acids in the same ratio as those found in human milk, but with aspartic acid and glutamic acid omitted because of concerns about their toxicity. The control drink, designed to be nutritionally balanced, consisted of the same composition of amino acids to which had been added 1.15g of TRP. The final weight of the mixture was 51.25g for the experimental drink and 52.40g for the control drink. Thus, apart from being only half the dose, the composition of the amino acid drinks were similar to the 100g drink described by Young et al (1985).

The amino acid powder was prepared for consumption immediately prior to drinking. 300ml of cold water, to which had been added 12.5mg of saccharin and blackcurrant flavouring, was mixed into the powder and the ensuring suspension served in a standard drinking glass with a straw. Subjects were asked to consume the entire drink and then allowed to rinse their mouths with water and if they desired, chew sugar free gum for thirty minutes to remove the taste.

Table 5.3. Amino acid composition of drink.

<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>2.75g</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>2.45g</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>1.35g</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.6g</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>1.6g</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>4.0g</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>6.75g</td>
</tr>
<tr>
<td>L-Lysine monohydrochloride</td>
<td>5.5g</td>
</tr>
<tr>
<td>L-methionine</td>
<td>1.5g</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>2.85g</td>
</tr>
<tr>
<td>L-Proline</td>
<td>6.1g</td>
</tr>
<tr>
<td>L-Serine</td>
<td>3.45g</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>3.45g</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>3.45g</td>
</tr>
<tr>
<td>L-Valine</td>
<td>4.45g</td>
</tr>
<tr>
<td>[L-Tryptophan]</td>
<td>[1.15g]</td>
</tr>
</tbody>
</table>

5.2.2.2 General Procedure

Volunteers were requested to eat normally during the day preceding the test but to fast from midnight, although a small amount of water was allowed prior to the test session. Subjects were brought to the laboratory at 08.30 am and placed in a quiet room on their own. After a settling in period of ten minutes, baseline VA ratings for mood were obtained and a venous blood sample taken for estimation of plasma total and free TRP. Subjects were then given one of the three drinks and returned to their room where they rested quietly for the next 4.5 hours. During this time, VA ratings of mood were presented to all subjects at hourly intervals in the standardised manner outlined in Chapter Two. At +4.5
hours pre-meal VA ratings of appetite were filled out by the subjects and a further blood sample taken for plasma total and free TRP level estimation.

5.2.3 TEST MEAL PROCEDURE

After the second blood sample had been taken, subjects were placed back in the room at a table. The test meal consisted of a buffet lunch which contained a range of foods of different macronutrient content, with each food portion being presented to excess (see section 2.3.3.1 for full details). Meal instructions were given by the author in the standardised manner outlined in section 2.3.3.1. The food was then presented on trays in the standardised format and placed on the table in front of the subject. Subjects were left alone to eat and asked to come out of the room when they had finished their meal. The trays were then removed without comment and post-meal VA ratings of mood and appetite given to the subject for completion. Food not consumed was weighed and calorie consumption calculated as outlined in section 2.3.3.1. The test procedure is summarised below in Table 5.4.

Table 5.4. Summary of test procedure

<table>
<thead>
<tr>
<th>Time</th>
<th>Measure/procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.30</td>
<td>Arrival of subject and settled into test room</td>
</tr>
<tr>
<td>08.40</td>
<td>Baseline VA ratings of mood completed (VAs and POMS)</td>
</tr>
<tr>
<td>08.50</td>
<td>Fasting blood sample for plasma total and free TRP estimation</td>
</tr>
<tr>
<td>09.00</td>
<td>Administration of drink</td>
</tr>
<tr>
<td>09.30-13.30</td>
<td>VA ratings of mood completed at hourly intervals (VAs and POMS)</td>
</tr>
<tr>
<td>13.30</td>
<td>Pre-meal VA ratings of appetite completed</td>
</tr>
<tr>
<td>13.35</td>
<td>Blood sample for +4.5hour plasma total and free TRP estimation</td>
</tr>
<tr>
<td>13.40</td>
<td>Test meal followed by post-meal VA ratings of appetite</td>
</tr>
<tr>
<td>14.15</td>
<td>End of session. Subject returned home by taxi</td>
</tr>
</tbody>
</table>

5.2.4 SUBJECTIVE MEASURES

5.2.4.1 Measures of Mood

Mood was measured using a combination of VA ratings (Appendix 5.1) and the Profile of Mood State Questionnaire (POMS) which had been amended to examine current mood (Appendix 5.2). The VA rating forms of mood (despondent/sad; anxious; irritable and
tense), were presented in the standard manner as outlined in section 2.3.4, at baseline (08.40 hours) and then at hourly intervals across the morning until 13.30 hours. The POMS was presented at baseline (08.30 hours) and again at 13.30 hours.

5.2.4.2 Measures of Appetite

VA ratings of hunger were presented hourly across the morning from baseline to 13.30. Pre- and post-meal VA rating scales (wish to eat, hunger, feel full and find/found meal satisfying) were used to measure satiety before and after completion of the test meal respectively (Appendix 5.3). VA rating scales of appetite were presented to subjects in the standardised manner outlined in section 2.3.3.

5.2.5 BIOCHEMICAL MEASURES

10ml venous blood samples were collected into heparin tubes and immediately separated by centrifugation. Plasma samples were stored at -30° C until assay. Plasma total and free TRP levels were determined by fluorometric detection according to the method of Bloxam and Warren (1974) and Bloxam et al (1977) as outlined in section 2.3.2.6.

5.2.6 STATISTICAL ANALYSIS

The data were analysed with a two or three way repeated measures analysis of variance using both within and between subject comparisons, with Greenhouse-Geisser (Greenhouse and Geisser, 1959) correction for repeated measures. The factors analysed were group (bulimic patients versus controls), condition [(P), (B) and (T-) drinks] and time. Significant F values were followed up with post-hoc t-tests.

5.3 RESULTS

5.3.1 AMINO ACID DRINK ADMINISTRATION IN HEALTHY FEMALE SUBJECTS

5.3.1.1 Effect of Placebo and Amino Acid Drinks on Plasma TRP Levels

Plasma concentrations of total and free TRP were analysed by three way repeated measures analysis of variance. Baseline plasma total TRP levels were not significantly different for the three conditions (mean ± sem baseline total TRP: (T-)=13.26µg/ml ± 0.75; (B)=13.08µg/ml ± 0.72; (P)=12.98µg/ml ± 0.63; p>0.05). The ANOVA on total TRP following (P) and amino acid drink administration revealed a significant main effect of condition [i.e. effect of (T-) versus (B) versus (P) drinks] (F=33.77; d.f.=2,22; p<0.001), a
significant main effect of time [i.e. variation of plasma total TRP over time] (F=45.13; d.f.=2,22; p<0.001) and a significant condition by time interaction [i.e. effect of (T-), (B) and (P) drinks on variation in plasma total TRP over time] (F=45.13; d.f.=2,22; p<0.001). Post-hoc testing revealed that while no significant difference between baseline and +4.5 hour plasma total TRP was found following the (B) drink condition (mean ± sem total TRP: baseline=13.08µg/ml ± 0.72; +4.5 hours=15.42µg/ml ± 1.48; p>0.05; Figure 5.1a), a significant decrease in plasma total TRP concentration between baseline and +4.5 hours followed administration of the (T-) drink (mean ± sem total TRP: baseline=13.26µg/ml ± 0.75; +4.5 hours=2.89µg/ml ± 0.72; p<0.0005; Figure 5.1a) and the (P) drink condition (mean ± sem total TRP: baseline=12.98µg/ml ± 0.63; +4.5 hours=9.93µg/ml ± 0.63; p<0.0005; Figure 5.1a). Following the (T-) condition, a 78% decrease in plasma total TRP was seen across the 4.5 hours, while a 23% decrease followed administration of the (P) drink condition.

Basal plasma free TRP concentrations were not significantly different between the three conditions (mean ± sem baseline plasma free TRP: (T-)=0.91µg/ml ± 0.09; (B)=0.88µg/ml ± 0.09; (P)=0.84µg/ml ± 0.08; p>0.05), but the ANOVA following (P) and amino acid administration revealed a significant main effect of condition [i.e. effect of (T-) versus (B) versus (P) drinks] (F=11.14; d.f.=2,22; p<0.001), a significant main effect of time [i.e. variation in plasma free TRP over time] (F=30.32; d.f.=1,11; p<0.001) and a significant condition by time interaction [i.e. effect of (T-), (B) and (P) drinks on variation in plasma free TRP over time] (F=33.27; d.f.=2,22; p<0.001). Post-hoc testing revealed

![Figure 5.1. Effect of (P) and amino acid drinks on plasma total (a) and free (b) TRP shown as mean ± sem at time 0 and time +4.5 hours. A significant fall in plasma total and free TRP followed administration of the (T-) drink condition. Paired t test: ** p < 0.0005; * p < 0.001.](image)
significant decreases in plasma free TRP levels at +4.5 hours compared to baseline following the (T-) condition (mean ± sem free TRP: baseline=0.91µg/ml ± 0.09; +4.5 hours=0.31µg/ml ± 0.03; p<0.0005; Figure 5.1b) and following the (P) condition (mean ± sem free TRP: baseline=0.84µg/ml ± 0.08; +4.5 hours=0.64 µg/ml ± 0.05; p<0.001; Figure 5.1b), but plasma free TRP levels were not significantly different at baseline and +4.5 hours after administration of the (B) drink (mean ± sem free TRP: baseline=0.88µg/ml ± 0.09; +4.5 hours=0.97µg/ml ± 0.08; p>0.05; Figure 5.1b). Following the (T-) drink condition, a 67% decrease in plasma free TRP was seen across the 4.5 hours, while a 24% decrease followed administration of the (P) drink condition.

5.3.1.2 Subjective Measures

Measures of Mood

VA ratings of mood were examined to see whether or not the (T-) condition had any additional effect on changes in subjective ratings of mood over the (B) and (P) conditions. While significant decreases in the ratings of sad/despondency were seen across the morning following administration of all three drink conditions (Figure 5.2a), the effect of the (T-) condition on ratings of sad/despondency was not significantly different.

![Change in ratings of sadness](a)

![Change in ratings of irritability](b)

Figure 5.2. Changes in VA ratings of (a) sad/despondency and (b) irritability following administration of (P) and amino acid drinks in twelve healthy female volunteers. Data are shown as mean ± sem ratings at time 0 and time +4.5 hours. Significant falls in ratings of sad/despondency were seen following administration of all three drink conditions [ANOVA] (a). Ratings of irritability were significantly higher in the initial (P) condition session compared to the (B) and (T-) conditions [ANOVA] (b).
The ANOVA following (P) and amino acid drink administration revealed no significant main effect of condition [i.e. effect of (P) vs (B) vs (T-) on ratings of sad/despondency] (F=0.38; d.f.=2.20; p>0.05), a significant main effect of time [i.e. variation on ratings of sad/despondency over time] (F=4.58; d.f.=6.66; p<0.05; Figure 5.2a) but no significant condition by time interaction [i.e. effect of (T-) condition on variation in sad/despondency ratings over time] (F=0.87; d.f.=12,120; p>0.05).

Mean VA ratings of irritability decreased between baseline and +4.5 hours following administration of all three conditions but the decrease did not reach statistical significance. The ANOVA following administration of (P) and amino acid drinks revealed a significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) on ratings of irritability] (F=4.95; d.f.=2.20; p<0.05), no significant main effect of time [i.e. variation in irritability over time] (F=2.54; d.f.=6.60; p>0.05) and no significant condition by time interaction [i.e. effect of (P) vs (B) vs (T-) drink on variation in irritability over time] (F=0.92; d.f.=12,120; p>0.05) was found. Examination of the data revealed that the significant main effect of condition reflected increased scores in the initial (P) condition session and that amino acid loading per se had no effect on ratings of irritability (Figure 5.2b).

Figure 5.3. Changes in VA ratings of tension (a) and anxiety (b) following administration of (P) and amino acid drink conditions in twelve healthy female volunteers. Data are shown as mean ± sem ratings at time 0 and +4.5 hours. Significant falls in ratings of tension (a) and anxiety (b) followed administration of all three drink conditions [ANOVA], but no additional effects of (T-) administration on ratings of tension and anxiety were seen.
The ANOVA on VA ratings of tension and anxiety (Figure 5.3) following administration of (P) and amino acid drinks revealed no significant main effects of condition [i.e. effect of (P) vs (T-) vs (B) drink on ratings of tension and anxiety] (Tension: \(F=4.31; \text{d.f.}=2,20; p=0.06\); Anxiety: \(F=4.03; \text{d.f.}=2,20; p=0.07\)), significant main effects of time [i.e. variation in ratings of tension and anxiety over time (Tension: \(F=5.13; \text{d.f.}=6,60; p<0.01\); Anxiety: \(F=4.15; \text{d.f.}=6,60; p<0.05\)] and significant condition by time interactions [i.e. effect of (P) vs (T-) vs (B) drinks on variation in ratings of tension and anxiety over time] (Tension: \(F=3.43; \text{d.f.}=12,120; p<0.05\); Anxiety: \(F=3.44; \text{d.f.}=12,120; p<0.05\)). Closer examination of the data, however, revealed that all of these effects were a reflection of the high scores seen in the first session i.e. (P) condition, suggesting that the amino acid loading conditions had no real impact on the VA scales.

POMS ratings of depression demonstrated a significant fall across the morning following administration of the (T-) drink, but no significant effect of TRP depletion was seen on the ratings of depression (Figure 5.4a). The ANOVA revealed no significant main effect of condition [i.e. effect of (T-) drink] (\(F=2.21; \text{d.f.}=2,20; p>0.05\)), a highly significant main effect of time [i.e. variation in ratings of depression over time] (\(F=41.25; \text{d.f.}=2,20; p<0.00001\)) but no significant condition by time interaction [i.e. effect of (T-) drink on variation in ratings of depression over time] (\(F=1.68; \text{d.f.}=4,40; p>0.05\)).

![Figure 5.4](image-url)
Similar findings were seen with the POMS rating of anxiety/tension (Figure 5.4b). Again, a significant fall in the VA ratings was seen across the morning following administration of all three drink conditions, but no additional effect of (T-) drink administration over (P) and (B) drink administration on changes in ratings of anxiety/tension were seen. The ANOVA following administration of (P) and amino acid drinks revealed no significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] (F=0.19; d.f.=2,20; p>0.05), a highly significant main effect of time [i.e. variation in ratings of anxiety/tension over time] (F=17.91; d.f.=2,20; p<0.0005), but no significant condition by time interaction [i.e. effect of (P) vs (T-) vs (B) drink on variation in ratings of anxiety/tension over time] (F=1.07; d.f.=4,40; p>0.05).

Measures of Appetite

VA ratings of appetite were examined to see whether or not the (T-) condition had any additional effects on subjective ratings of appetite over and above those seen with the (P) and (B) conditions. A significant increase in hunger occurred across the morning following administration of all three conditions (Figure 5.5a).

![Figure 5.5](image)

(a) Change in VA ratings of hunger (a) and satiety VA measures of wish to eat (b) following administration of (P) and amino acid drinks in twelve healthy female volunteers. Data are shown as mean ± sem ratings at time 0 and +4.5 hours, and pre-and post-meal respectively. A significant increase in rating of hunger followed administration of all three conditions [ANOVA] (a), and a significant decrease in rating of wish to eat was seen post-meal following administration of all three drinks when compared to the pre-meal ratings [ANOVA] (b).
The ANOVA following administration of (P) and amino acid drinks revealed a significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] (F=8.52; d.f.=2,20; p<0.005), a highly significant main effect of time [i.e. variation in ratings of hunger across time] (F=37.7; d.f.=6,60; p<0.00001) but no significant condition by time interaction [i.e. effect of (P) vs (T-) vs (B) drink on variation in ratings of hunger across time] (F=2.16; d.f.=12,120; p=0.09). While the ANOVA suggested a significant condition effect, examination of the means demonstrated that baseline hunger ratings for the (B) drink condition was considerably lower than the other two conditions, and that the condition effect suggested was merely a contamination effect of this difference throughout the test session (Figure 5.5a).

Pre- and post-meal VAs of satiety i.e. wish to eat, feel full and find/found the meal satisfying were also examined to see if the (T-) drink condition had any additional effect on subjective ratings of satiety compared to administration of (P) and (B) conditions (Figure 5.5b and 5.6).

![Figure 5.6](image_url)

Figure 5.6. Changes in VA ratings of feel full (a) and find meal satisfying (b) following administration of (P) and amino acid drinks in twelve healthy female volunteers. Data are shown as mean ± sem VA ratings before and after test meal. Significant increases in ratings of feel full and meal satisfaction were found over time following administration of all three drinks (ANOVA).

The ANOVAs following administration of (P) and amino acid drinks revealed significant main effects of time [i.e. variations in ratings over time i.e. variations after compared to before the meal] (wish to eat: F=132.3; d.f.=1,10; p<0.00001; feel full:
F=220.4; d.f.=1,10; p<0.0001; find meal satisfying: F=8.69; d.f.=1,10; p<0.01), but no significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] (wish to eat: F=0.82; d.f.=2,20; p>0.05; feel full: F=1.68; d.f.=2,20; p>0.05; find meal satisfying: F=0.66; d.f.=2,20; p>0.05) and no significant condition by time interaction [i.e. effect of (P) vs (T-) vs (B) drink on variation in ratings seen before and after the meal] (wish to eat: F=3.26; d.f.=2,20; p=0.06; feel full: F=1.55; d.f.=2,20; p>0.05; find meal satisfying: F=1.83; d.f.=2,20; p>0.05).

5.3.1.3 Effect of Amino Acid Drinks on Food Intake

No significant effect on food intake either in terms of total calorie consumption, or in terms of macronutrient selection was seen following administration of all three drink conditions. The ANOVA following administration of (P) and amino acid drinks revealed no significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] for carbohydrate intake (F=0.35; d.f.=2,20; p>0.05), protein intake (F=0.6; d.f.=2,20; p>0.05), fat intake (F=2.37; d.f.=2,20; p>0.05) or total food consumption (F=1.67; d.f.=2,20; p>0.05). Food intake at test meal for all three conditions is summarised below in Table 5.5.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (mean ± sem)</th>
<th>(T-) Drink (mean ± sem)</th>
<th>(B) Drink (mean ± sem)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (kJ)</td>
<td>1839 (± 167)</td>
<td>1907 (± 187)</td>
<td>1848 (± 178)</td>
</tr>
<tr>
<td>Protein (kJ)</td>
<td>648 (± 64)</td>
<td>688 (± 48)</td>
<td>649 (± 56)</td>
</tr>
<tr>
<td>Fat (kJ)</td>
<td>1824 (± 177)</td>
<td>2075 (± 170)</td>
<td>1899 (± 185)</td>
</tr>
<tr>
<td>Total Food Intake (kJ)</td>
<td>4445 (± 307)</td>
<td>4663 (± 377)</td>
<td>4395 (± 388)</td>
</tr>
</tbody>
</table>

5.3.1.4 Correlations

No significant correlations were found between changes in free or total plasma TRP, mood and appetite ratings or food intake.

5.3.2 EFFECT OF AMINO ACID DRINK ADMINISTRATION IN RECOVERED BULIMIC PATIENTS

5.3.2.1 Effect of Amino Acid Drink Administration on Plasma TRP Levels

Plasma total and free TRP levels were compared for both within (i.e. effects within the recovered bulimic group) and between (i.e. comparison of control versus recovered bulimic subjects) group effects across the three drink conditions.
The ANOVA on plasma total TRP data following administration of (P) and amino acid drink conditions revealed a significant main effect of group [i.e. recovered bulimic subjects versus control subjects] (F=11.32; d.f.=1,16; p<0.005). Post hoc testing demonstrated that basal plasma total TRP levels (time 0) were significantly lower in the recovered bulimic subjects compared to controls for all three test conditions (Figure 5.7; (T-) and (B) conditions only shown). In addition, recovered bulimic subjects' total plasma TRP levels were significantly lower compared to controls at +4.5 hours, following both (T-) and (B) drinks but not following (P). A highly significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] (F=60.28; d.f.=2,32; p<0.00001), and a significant condition by time interaction [i.e. effect of (T-) drink on variation in total TRP over time] (F=64.33; d.f.=2,32; p<0.00001) was found. Examination of the data demonstrated that these significant effects reflected the ability of the (T-) drink to lower plasma total TRP at +4.5 hours. No significant interaction of group [i.e. recovered bulimic subjects versus control subjects] with time or of group by time by condition was found (all p values > 0.1).

![Figure 5.7](image)

(a) Controls Rec Bulimics

(b) Controls Rec Bulimics

Figure 5.7. Change in plasma total TRP levels following administration of (a) (T-) and (b) (B) drinks conditions in twelve healthy controls and eight recovered bulimic subjects. Data shown as mean ± sem at times 0 and +4.5 hours. Baseline and +4.5 hour plasma total TRP levels were significantly lower in the recovered bulimic group compared to controls. In addition, (T-) administration led to a significant fall in plasma total TRP in both control and bulimic subjects at +4.5 hours. Paired t test: ** p < 0.01.

For plasma free TRP, no significant group effect [i.e. effect of recovered bulimic subjects versus control subjects] following administration of (P) and amino acid drinks
was seen. Administration of the (T-) drink did, however, lead to a significant lowering of plasma free TRP by +4.5 hours (Figure 5.8; (T-) and (B) conditions only shown). The ANOVA revealed a significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drinks] (F=19.97; d.f.=2,32; p<0.00001), and a significant condition by time interaction [i.e. effect of (P) vs (T-) vs (B) drink on variation in plasma free TRP levels over time] (F=69.15; d.f.=2,32; p<0.00001). Again, no significant group by time or group by time by condition interactions were seen (all p values >0.1).

![Figure 5.8](image)

Figure 5.8. Change in plasma free TRP levels in twelve healthy and eight recovered bulimic subjects following administration of [a] (T-) and [b] (B) drink conditions. Data shown as mean ± sem at times 0 and +4.5 hours. Baseline and +4.5 hour concentrations were not significantly different between control and bulimic groups, but administration of (T-) led to a significant fall in plasma free TRP at +4.5 hours [a]. Paired t test: **p < 0.01.

**Measures of Mood**

VA ratings of mood were examined to see whether or not the (T-) condition had any additional effect on subjective ratings of mood compared to those seen following administration of (P) and (B) drinks, and to determine any between group differences (i.e. recovered bulimic versus controls) in effects of (T-) drink administration. Following administration of (P) and amino acid drinks, a significant main effect of group was seen on all VA ratings of mood [i.e. effect of recovered bulimic versus control subjects] (sad/despondency: F=5.85; d.f.=1,16; p<0.05; tense: F=4.53; d.f.=1,16; p<0.05; anxiety: F=7.26; d.f.=1,16; p<0.05; irritable: F=7.37; d.f.=1,16; p<0.05), and examination of the
data demonstrated that these effects came from an overall higher rating of negative mood in the recovered bulimic subjects compared to controls. No significant main effect of condition was seen following administration of the drinks [i.e. effect of (T-) drink versus (B) and (P) drinks] (sad/despondency: $F=0.20$; d.f.=2,32; $p>0.05$; tense: $F=2.32$; d.f.=2,32; $p>0.05$; anxiety: $F=1.71$; d.f.=2,32; $p>0.05$; irritable: $F=0.16$; d.f.=2,32; $p>0.05$), and no significant group by condition interaction [i.e. effect of bulimic versus control subjects on (T-) drink] (sad/despondency: $F=1.01$; d.f.=2,32; $p>0.05$; tense: $F=2.32$; d.f.=2,32; $p>0.05$; anxiety: $F=1.71$; d.f.=2,32; $p>0.05$; irritable: $F=2.42$; d.f.=2,32; $p>0.05$) was seen. Furthermore, no significant effect of time was seen on ratings of mood and no significant interactions between time, group and condition were seen (all $p$ values > 0.1), indicating that the (T-) drink did not increase negative ratings of mood in either the recovered bulimic or control subject groups.

Figure 5.9. Changes in POMS ratings of depression following (P) and amino acid drink administration. (a): Mean ± sem POMS dysphoria ratings following (P) and amino acid drink administration at +4.5 hours in eight recovered bulimic women and twelve healthy female controls. POMS ratings for all measures except depression were significantly higher in bulimics compared to controls [ANOVA]. (b): Mean ± sem POMS ratings of depression at +4.5 hours following administration of (P) and (T-) drinks. Ratings were significantly higher for both control and bulimic subjects during the (P) condition compared to the (T-) and (B) conditions (ANOVA).

Following administration of (P) and amino acid drinks, POMS ratings of depression demonstrated a significant main effect of group [i.e. effect of recovered bulimic
versus control subjects] (F=8.62; d.f.=1,13; p=0.01; Figure 5.9a), again reflecting the higher ratings of negative mood in the recovered bulimic subjects, and a significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] (F=4.34; d.f.=2,26; p<0.05; Figure 5.9b) with ratings of depression for both subject groups higher during the initial (P) drink day. No significant group by condition interaction [i.e. effect of recovered bulimic versus control subjects on (P) vs (T-) (B) drinks] (F=2.35; d.f.=2,24; p>0.05) and no significant effect of time was seen on POMS ratings of depression. Furthermore, no significant interactions between time, group and condition was seen, indicating that the (T-) drink did not increase negative ratings of mood in either group of subjects.

For POMS rating of anxiety/tension, no significant main effect of group [i.e. effect of recovered bulimic versus control subjects] (F=5.78; d.f.=1,13; p>0.05), no significant main effect of condition [i.e. effect of (P) vs (T-) vs drinks] (F=2.33; d.f.=2,26; p>0.05) and no significant group by condition interaction [i.e. effect of bulimic versus control subjects on (P) vs (T-) vs (B) drinks effects] (F=0.19; d.f.=2,26; p>0.05) was seen. Similarly, no significant effect of time was seen on ratings of anxiety/tension and no interactions between time, group and condition were found (all p values > 0.1), again indicating that the (T-) drink did not increase negative ratings of mood in either the recovered bulimic or control subjects.

Measures of Appetite

Appetite ratings were examined to determine the effects of the (T-) drink within the groups of recovered bulimic subjects, and to determine if differences between the groups resulting from (T-) drink administration existed.

Following administration of (P) and amino acid drinks, VA ratings of hunger increased in both subject groups across the morning, as reflected in a highly significant main effect of time [i.e. variation in ratings of hunger across time] being demonstrated on ANOVA (F=43.59; d.f.=6,96; p<0.00001; Figure 5.10a: (T-) condition only shown). A significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drinks] (F=9.74; d.f.=2,32; p=0.0005) but no significant condition by time interaction [i.e. effect of (P) vs (T-) vs (B) drink on variation in ratings of hunger over time] (F=2.92; d.f.=12,192; p>0.050) was seen. Examination of the data revealed that the effect of condition appeared to result from a significant suppression of hunger following administration of both (T-) and (B) drinks, and that no additional effect following the (T-) drink was present. No significant main effect of group [i.e. effect of recovered bulimic versus control group] (F=1.10; d.f.=1,16; p>0.05), and no significant group by condition interaction [i.e. effect of recovered bulimic versus control subjects on (P) vs (T-) vs (B) drinks] (F=2.57; d.f.=2,32; p=0.09) was seen.
Figure 5.10. Change in VA ratings of hunger following administration of (P) and amino acid drinks in eight recovered bulimic women and twelve healthy controls. Data are shown as mean ± sem. (a): A significant increase in ratings of hunger was seen in both control and bulimic groups over time [(T-) drink condition only shown] (ANOVA). (b): A significantly lower rise in ratings of hunger was seen in both subject groups following administration of (T-) and (B) amino acid drinks compared to placebo (ANOVA; bulimic data only shown).

VA ratings of satiety (i.e. wish to eat; feel full; satisfaction of meal and could eat a large amount) were measured pre- and post-meal. Ratings of wish to eat were decreased following the test meal, and a significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] (F=5.61; d.f.=2,32; p=0.01) was found such that both amino acid drinks significantly suppressed the desire to eat (Figure 5.11a), although the effect was weaker when the control subjects were analysed separately (perhaps because of the smaller group size). Similarly, a significant group by time interaction was found [i.e. effect of recovered bulimic versus control subjects on variation in wish to eat over time] (F=5.26; d.f.=1,16; p<0.05; Figure 5.11b), in which the recovered bulimic subjects expressed less desire to eat prior to the meal, but greater desire after the meal compared to controls.
Figure 5.11. Changes in VA ratings of wish to eat in twelve healthy control and eight recovered bulimic subjects following administration of (P) and amino acid drinks. (a): A significant decrease in wish to eat was seen in both control and recovered bulimic subjects following administration of (T-) and (B) amino acid drinks compared to (P) [ANOVA]. (b): Recovered bulimic subjects rated significantly less wish to eat before, and significantly greater wish to eat after the test meal compared to controls (ANOVA; (T-) condition only shown).

As expected, ratings of fullness were increased significantly after the meal following administration of all three drinks as demonstrated by a significant main effect of time [i.e. variation in fullness from before to after the meal] (F=182.3; d.f.=1,16; p<0.0001), although all other time interactions failed to reach significance (all p values >0.1). Similarly, no significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drink] (F=0.46; d.f.=2,32; p>0.05) and no significant condition by group interaction [i.e. effect of (P) vs (T-) vs (B) drink on effect of recovered bulimic versus control groups] (F=0.76; d.f.=2,32; p>0.05) was seen.

Following administration of (P) and amino acid drinks, a significant main effect of group [i.e. effect of recovered bulimic versus control subjects] (F=22.43; d.f.=1,16; p<0.0005) was seen for VA ratings of meal satisfaction with control subjects rating meal satisfaction significantly higher than recovered bulimic subjects. No significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drinks] (F=1.41; d.f.=2,32; p>0.05) and no
significant group by condition interaction [i.e. effect of recovered bulimic versus control group on (P) vs (T-) vs (B) drink effects] (F=0.59; d.f.=2,32; p>0.05) was found.

Following administration of (P) and amino acid conditions, VA ratings of "could eat a large amount of food" were significantly decreased after the test meal as revealed by a highly significant main effect of time on ANOVA (F=74.9; d.f.=1,16; p<0.0001). A significant group by time interaction [i.e. effect of recovered bulimic versus control subjects on variation in ratings over time] (11.35; d.f.=1,16; p<0.005; Figure 5.12a) and a significant group by time by condition interaction [i.e. effect of recovered bulimic versus control subjects on variation in ratings following (P) vs (T-) vs (B) drink over time] (F=5.23; d.f.=2,32; p<0.05). Post-hoc t-tests revealed that recovered bulimic subjects scored less on the rating of "could eat a large amount of food" prior to the meal following both (P) and (T-) drinks (Figure 5.12b).

Figure 5.12. Changes in VA ratings of "could eat a large amount of food" following administration of (P) and amino acid drinks in eight recovered bulimic women and twelve healthy female controls. Data shown as mean ratings ± sem pre-and post-meal (a); pre-meal (b). Bulimic women scored significantly less desire to eat a large amount of food prior to test meal following (P) and (T-) drinks compared to controls. Unpaired t test: * p < 0.05.

Overall, the data do not indicate any specific effect of the (T-) condition on VA ratings of appetite in either control or recovered bulimic subjects. Administration of both (T-) and (B) drinks, however, did appear to decrease the desire to eat.
5.3.2.3 Effect of Amino Acid Drink Administration on Food Intake

Food intake at test meal was compared within the subject groups, as well as examining differences between the groups. Following administration of (P) and amino acid drinks, total calorie intake at test meal was significantly less in the recovered bulimic group compared to the control subjects (post-hoc t-testing: \( p<0.05 \); Figure 5.13). The ANOVA on total food intake revealed a significant main effect of group [i.e. effect of recovered bulimic versus control subjects] (\( F=4.75; \) d.f.=1,16; \( p<0.05 \)), a significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) vs (P) drinks], in that both subject groups ate less at the initial (P) test (\( F=4.15; \) d.f.=2,32; \( p<0.05 \)), but no significant group by condition interaction [i.e. effect of recovered bulimic versus control subjects on effects of (P) vs (T-) vs (B) drinks] (\( F=3.0; \) d.f.=2,32; \( p=0.08 \)) was found, indicating no specific effect of the (T-) condition on total calorie intake in recovered bulimic women.

![Figure 5.13](image_url)

Figure 5.13. Mean ± sem food intake at test meal in twelve healthy and eight recovered bulimic females following administration of (P) and amino acid drinks. Total calorie consumption in the recovered bulimic subjects was significantly less than in controls. Unpaired t test: * \( p < 0.05 \).

Analysis of macronutrient data following administration of (P) and amino acid drinks demonstrated a significant decrease in consumption of fat by recovered bulimic
subjects compared to controls. The ANOVA revealed a significant main effect of group [i.e. effect of recovered bulimic versus control subjects] on fat intake (F=4.60; d.f.=1,16; p<0.05), a significant main effect of condition (F=4.99; d.f.=2,32; p<0.05) but no significant group by condition interaction (F=2.69; d.f.=2,32; p=0.09). The significant effect of condition was seen following administration of the (P) drink and appeared to result from the decrease in total calories seen during the initial session.

In terms of carbohydrate and protein intake, the ANOVA following administration of (P) and amino acid drinks revealed no significant main effect of group [i.e. effect of recovered bulimic versus controls] (carbohydrate: F=3.91; d.f.=1,16; p=0.07; protein: F=3.06; d.f.=1,16; p>0.05), no significant main effect of condition [i.e. effect of (P) vs (T-) vs (B) drinks] (carbohydrate: F=0.21; d.f.=2,32; p>0.05; protein: F=1.63; d.f.=2,32; p>0.05) and no significant group by condition interaction [i.e. effect of recovered bulimics versus controls on effects of (P) vs (T-) vs (B)] (carbohydrate: F=1.35; d.f.=2,32; p>0.05; protein: F=1.46; d.f.=2,32; p>0.05).

In summary, no additional effect of the (T-) drink over either (P) or (B) drinks on food intake was seen, in terms of total calorie or macronutrient choice in recovered bulimic or control subjects.

5.3.2.4 Correlations

No significant correlations were seen between changes in plasma total or free TRP, mood and appetite ratings or food intake.

5.4 DISCUSSION

5.4.1 EFFECT OF PLASMA TRP LOWERING ON MOOD AND FOOD INTAKE IN HEALTHY FEMALE SUBJECTS

The administration of a 52g amino acid drink deficient in TRP led to a significant reduction in both plasma total and free TRP over 4.5 hours, confirming the efficacy of this smaller dose to lower plasma TRP and in keeping with the findings of Oldman et al (1994). In addition to being well tolerated by the female subjects, the results in terms of TRP depletion were comparable to those obtained by Young et al (1985), using the 100g dose. In the present study, a reduction in total TRP of 78% and in free TRP of 67% was obtained, while Young et al (1985), reported decreases of 76% and 60% respectively. The two studies did, however, contrast in respect to changes in plasma TRP following administration of the (B) drink. Young et al (1985) reported significant increases in plasma free and total TRP following the 100g (B) drink (in the order of 72% and 84%
respectively), while in contrast, no significant change in plasma total and free TRP were seen following administration of the 52g (B) drink. Furthermore, the present study design incorporated a placebo condition following which significant falls in total (23%) and free (24%) plasma TRP across the 4.5 hours were seen. While the fall in plasma TRP seen in the placebo condition was of a much smaller magnitude than that seen following TRP depletion, it is of interest to note such changes which presumably reflect the effects of overnight fasting.

In contrast to the hypothesis proposed in the introduction, TRP depletion was not associated with a lowering of mood in healthy women. This finding differs from those of Young et al (1985) and Smith et al (1987) who both reported small but significant increases in depression subscale scores in healthy male volunteers following TRP depletion with a 100g amino acid drinks. One possible explanation for the failure to find a lowering of mood in the present study is the variation in drinks utilised between the two studies. As outlined in Chapter Two, amino acid loading reduces brain TRP by two mechanisms. In addition to directly lowering plasma TRP levels (due to incorporation of TRP into protein), the (T-) drink also increases the ratio of LNAA:TRP, which in turn, increases competition for the transportation of TRP into the brain across the blood brain barrier (see section 2.2.2). While the 52g dose led to a comparable fall in levels of both total and free plasma TRP to that seen with the 100g drink, it is possible that the smaller amino acid load inherent in the 52g drink would result in less LNAA to TRP competition at the blood brain barrier, such that a greater percentage of TRP would reach the brain. In this situation, brain 5-HT synthesis may not be reduced sufficiently to produce a detectable lowering of mood. It is of interest that in a study by Abbott et al (1992), which also utilised the 50g amino acid dose, TRP depletion was found to block the analgesic effect of morphine on tolerance to cold pressor pain in healthy male subjects but had no effect on mood.

All published studies to date examining the effects of TRP depletion on mood have used male subjects, and it is possible that sex differences in responses to TRP depletion account for the current findings i.e. that women are more resilient to the effects of TRP depletion in terms of mood. This possibility seems unlikely, however, in view of the fact that rates of affective disorder are twice as high in women compared to men, and results from previous dieting research suggests that they are more vulnerable in terms of alterations in brain 5-HT function. As discussed in Chapter Three, considerable evidence exists to suggest that moderate dieting in women, but not men, is associated in alterations in brain 5-HT function and that one possible mechanism underlying these dieting-induced changes is plasma TRP depletion (Anderson et al, 1990a; Cowen et al, 1992). Furthermore, while a diet that restricted TRP intake to 200mg/day was found to lead to alterations in brain 5-HT function in both men and women, a more moderate restriction of
TRP (700mg/day) was found to only affect brain 5-HT function in women (Delgado et al, 1989). Taken together, these data suggest that women should be more vulnerable to the effects of TRP depletion, in contrast to the present finding.

Mood and appetite changes are well documented across the menstrual cycle, especially during the pre-menstrual phase (Goodall et al, 1991; Goodall and Silverstone, 1993). In the current study, all tests were completed outside the pre-menstrual phase and no evidence suggestive of systematic bias in terms of allocation of particular drinks to a specific phase of the cycle was found. It would seem unlikely, therefore, that menstrual cycle effects confounding the effects of TRP depletion could account for the failure to see a mood lowering effect in women.

In keeping with the effects on mood, TRP depletion induced by the (T-) drink was found to have no significant effect on subjective ratings of appetite or on food intake in healthy women. Although few studies have examined the effects of TRP depletion on food intake, the present findings are in contrast to those of Young et al (1988) who found a small but significant reduction in protein intake amongst twenty-two male volunteers following a 50g amino acid load. Sex differences in appetite responses to TRP depletion is a possible explanation for the contrasting findings. In support of this hypothesis, 2g and 3g TRP loading has been found to produce significant reductions in calorie consumption in men but not women (Leiter et al, 1987).

5.4.2 EFFECT OF TRP DEPLETION ON MOOD AND FOOD INTAKE IN SUBJECTS RECOVERED FROM BULIMIA NERVOSA

Examination of the data revealed differences between the group of women who had recovered from bulimia nervosa and the group of healthy controls. The differences were characterised by abnormalities of mood, subjective measures of appetite and food intake. In addition, although no specific effects of TRP depletion were seen, significant differences in basal TRP levels were found.

In keeping with the findings outlined above in healthy female subjects, a 52g amino acid drink deficient in TRP significantly lowered plasma total and free TRP over 4.5 hours in recovered bulimic women. In addition, basal plasma total TRP levels were significantly lower in recovered bulimic women compared to controls. The difference in basal total TRP levels was found for all three drink conditions, and following the (T-) and (B) drinks, persisted at +4.5 hours. In view of the hypothesis suggesting impaired 5-HT function in bulimia nervosa and, more specifically, that hyposerotonergic activity is consistent with bulimic behaviour, this finding is of considerable interest. To date few studies in the literature have addressed this issue, but one study by Lydiard et al (1988)
found no baseline plasma TRP/LNAA ratio differences in normal weight bulimic patients compared to healthy controls. As this study was carried out on acutely ill patients, however, and the literature reviewed in section 1.3.2 clearly demonstrates alterations in measures of brain 5-HT function between phases of acute illness and recovery, the findings are difficult to compare. As far as the author is aware no published studies have looked at TRP levels in long-term recovered subjects but our findings are consistent with the hypothesis of persistent hyposerotonergic activity amongst women recovered from bulimia nervosa.

An alternative explanation for the reduced basal plasma total TRP levels found in recovered bulimic women must be considered. Ongoing dietary restrictions in terms of reduced intake of food could explain the present findings. Data outlined in Chapter Three, coupled with the studies by Anderson et al (1990a) and Anderson et al (1990c) have suggested that moderate calorie restriction leads to reductions in plasma TRP levels, especially amongst women. Furthermore, diets sufficient in calories but moderately deficient in TRP have also been shown to lead to reduced plasma total TRP (Delgado et al, 1989). The women in the present study were carefully screened for persistent abnormal eating habits, including unusual dietary restrictions, and demonstrated normal measures of dietary restraint on the EDE-Q3 when compared to controls. Nevertheless, it is possible that the screening procedures utilised were unable to detect more subtle abnormalities of diet and eating behaviour that persist in clinically recovered bulimic women. Indeed, a high level of dietary restraint would be consistent with the finding of reduced food intake amongst recovered bulimic women seen in the present study at test meal (see below).

Subjective measures of appetite were found to differ in several aspects between control and recovered bulimic subjects. While no significant differences in ratings of hunger were seen between the two groups across the morning, significant difference in pre-meal ratings of satiety were found. The recovered bulimic women rated themselves as having less "wish to eat" and scored significantly lower on ratings of "could eat a large amount of food" prior to the meal. These lower ratings were reflected in actual food intake at test meal, with the recovered bulimic women consuming significantly less calories than controls. Interestingly, post-meal satiety measures also differed between the two groups in that the recovered bulimic women scored significantly lower on ratings of meal satisfaction and significantly higher on ratings of wish to eat. The present findings in subjective appetite ratings combined with the reduced food intake amongst recovered bulimic women can be interpreted in two ways. Firstly, the findings may indicate that recovered bulimic subjects continue to exercise dietary restraint (as reflected in reduced food consumption) in addition to cognitive restraint (as reflected in the lower scores of "wish to eat" and "could eat a large amount of food" prior to the meal). Voluntary dietary restraint would be in keeping with post-meal ratings of significant less meal satisfaction.
and higher scores of "wish to eat", suggesting the subject did not allow herself to eat to complete satiety. Alternatively, the findings may represent an persisting state of satiety dysregulation, perhaps secondary to decreased brain 5-HT function, as indicated by the low plasma TRP levels, although as discussed above, the plasma TRP findings are also consistent with dietary restriction.

Although no effect of TRP depletion was seen on ratings of mood, recovered bulimic subjects scored consistently higher on all ratings of dysphoric mood i.e. depression, tension, anxiety and irritability when compared to controls. To date, there are few long-term studies which have examined residual symptoms amongst patients recovered from bulimia nervosa, and they have tended to focus on aspects of eating behaviour and menstrual functioning as these symptoms have been used as measures of recovery (Swift et al, 1987; Hsu and Sobkiewicz, 1989; Herzog et al, 1991). The women in the present study all met strict criteria for recovery (see section 2.4.2) and many considered themselves to have been well for a number of years, yet despite this, abnormalities of mood appear to persist.

While the ratings of depression found in the recovered bulimic women could not be considered clinical in extent, the results are nevertheless of considerable interest in view of the high incidences of personal and family history of depression amongst patients with bulimia nervosa. Furthermore, the findings raise the issue of the exact nature of the relationship between the eating and affective disorders. Considerable debate exists within the literature as to whether or not the co-existence of eating disorders and depression simply represents co-morbidity in women with a high vulnerability for developing psychiatric illness in general; whether depressed mood occurs as a complication of the eating difficulties, perhaps secondary to the effects of food restriction and weight fluctuations; or whether or not the eating disorders represent a variation in the presentation of an affective illness (Russell, 1979; Hatsukami et al, 1984; Wilson and Lindholm, 1987; Walters et al, 1992). It could be argued that the present finding of persistent depressive symptomatology despite recovery from bulimia nervosa, suggest the presence of a primary, underlying affective disturbance (although it is not possible to rule out a persisting mood disturbance that has developed secondary to the eating disorder), a hypothesis supported by the clinical observation that antidepressant treatment is very effective at improving both mood and disturbed eating behaviours (Pope et al, 1983; Mitchell and Groat, 1984; Fluoxetine Bulimia Nervosa Collaborative Study Group, 1992). Obviously, this is an area that requires further research, perhaps utilising longitudinal studies that would enable the relationship to be untangled.

In contrast to the proposed hypothesis, TRP depletion had no effect on subjective ratings of appetite, objective measures of food intake or ratings of mood. While some
methodological issues and gender differences that may account for these findings have been discussed in general in the section on control subjects, the results are surprising in view of the vulnerability of the patients studied. To date, the amino acid mixture paradigm has been used in several patient groups. For example, TRP depletion in remitted depressed patients on antidepressant drugs is associated with an acute relapse in depressive symptomatology (Delgado et al., 1990), while patients in remission from obsessive-compulsive disorder on selective serotonin reuptake inhibitors also report an exacerbation of depressive but not obsessive compulsive symptoms following acute TRP depletion (Barr et al., 1992).

More recently a study by Weltzin et al. (1994) demonstrated significant increases in ratings of anxiety, indecisiveness, over-reactivity and fear of fatness, along with a trend towards increased ratings in depression and fatigue amongst a group of thirteen bulimic women after TRP depletion. This study contrasted to the present one in that it was carried out on acutely ill patients and utilised a 100g amino acid mixture with or without 2.3g TRP as the (T-) and (B) drink respectively. The authors demonstrated significant reductions in plasma TRP and TRP:LNAA following the (T-) drink, and emphasised the need to obtain this fall in amino acid ratio before adequate brain 5-HT depletion would occur. Although care must be taken in generalising findings from a study on acutely ill bulimic women to women who are in remission, it is possible that the smaller 52g dose of amino acids did not produce sufficient fall in TRP:LNAA (and hence insufficient competition at the blood brain barrier), to decrease brain 5-HT synthesis to a degree enabling changes in mood and appetite to be seen.

Finally, in terms of the failure to find an effect of TRP depletion on appetite ratings and food intake, it is possible that the overnight fast resulted in a ceiling effect in terms of hunger which obscured any effect of TRP depletion on food intake the next day, although Goodall and Silverstone (1988b) were able to demonstrate increased food intake in normal subjects following administration of metergoline utilising a similar experimental design. Bulimic patients may, however, be more sensitive to such effects. Furthermore, the unfamiliarity and unnatural atmosphere of the laboratory setting may also have influenced the test meal results.

5.5 CONCLUSIONS

In conclusion, a 52g amino acid drink deficient in TRP produced a significant reduction in plasma total and free TRP in both healthy and recovered bulimic women over 4.5 hours. In contrast to the proposed hypothesis, however, no effect of TRP depletion, was seen in either control subjects or recovered bulimic patients in terms of subjective ratings of appetite and mood or upon objective measures of food consumption.
Basal plasma total TRP levels were significantly lower in recovered bulimic subjects compared to controls, and recovered bulimic subjects exhibited abnormalities in subjective ratings of appetite and satiety. The findings suggest that either recovered bulimic women continue to exercise dietary restraint, or alternatively, that a persistent state of hyposerotonergic activity exists in women recovered from bulimia nervosa.
Chapter 6
GENERAL DISCUSSION AND CONCLUSIONS

6.1 OVERVIEW

In the introduction to this thesis it was suggested that while clear evidence now exists in support of serotonergic dysregulation occurring during clinical episodes of anorexia and bulimia nervosa, the precise nature of the dysregulation remains unclear. Furthermore, it was discussed that interpretation of research into the eating disorders is confounded by complications of the illnesses themselves, i.e. the effects of low body weight, starvation or chronic food restriction, along with the consequences of behaviours such as bingeing and purging. In addition, understanding of the neurobiological processes involved in the pathogenesis of these illnesses is hampered by our limited knowledge of the serotonergic mechanisms involved in the normal control of feeding behaviour in humans. It was suggested, therefore, that a better understanding of the effects that the confounding factors have on brain 5-HT function, coupled with greater knowledge of the normal mechanisms involved in serotonergic control of feeding behaviour, would lead to increased understanding of the disorders themselves. In particular, such knowledge would enable those factors arising as complications of the illnesses to be disentangled from those neurobiological abnormalities central to the underlying pathogenesis of the disorders.

The present studies utilised a neuroendocrine challenge paradigm to investigate the effects of weight loss on brain 5-HT function in humans. A combined neuroendocrine challenge and test meal approach was then used to examine the effects of the 5-HT agonist mCPP on PRL, food intake and mood in women. In addition, acute TRP depletion in conjunction with a test meal paradigm was utilised to examine the effects of TRP depletion on food intake and mood in healthy females subjects and female patients recovered from bulimia nervosa.

Throughout the thesis, particular attention has been paid to the possibility of gender differences and to the search for female neurobiological vulnerabilities that may lead to the development of the eating disorders. The reason for this emphasis is three-fold. Firstly, anorexia and bulimia nervosa are primarily disorders of young women, with only 10% of
cases occurring in men (Fairburn, 1983). Secondly, earlier work carried out by Goodwin et al (1987b) and Anderson et al (1990a) demonstrated that moderate food restriction and weight loss leads to alterations in brain 5-HT function in women but not men, suggesting that females may have a differing biological response to altered food intake compared to men. Thirdly, recent epidemiological evidence strongly suggests that normal dieting behaviour is in itself a risk factor in the development of eating disorders amongst vulnerable individuals, and in our society, dieting behaviour is more commonly carried out by young women (Jakovovits et al, 1977; Johnson-Sabine et al, 1988; Patton et al, 1990).

The investigations described in this thesis provide further evidence in support of the hypothesis that females are more vulnerable to the effects of food restriction and weight loss than men, by confirming earlier work that suggested moderate weight loss leads to alterations in brain 5-HT function in women alone. In addition, the findings provide evidence to suggest that the mechanism underlying the 5-HT-mediated changes in brain function probably occur pre-synaptically, perhaps as a consequence of alterations in the synthesis or release of 5-HT. No evidence was found to support the hypothesis that dieting leads to the development of post-synaptic 5-HT receptor supersensitivity as proposed by Goodwin et al (1987b) and Anderson et al (1990a). In this light, findings from this thesis are in keeping with the hypothesis that food restriction and weight loss may lead to increased brain 5-HT function secondary to an increase in pre-synaptic synthesis of 5-HT as proposed by Curzon (1988).

Evidence for a central role of serotonergic pathways in the control of food intake in humans was provided by the findings of the mCPP studies in Chapter Four. In keeping with animal data, oral mCPP at a dose of 0.4mg/kg significantly decreased food intake in healthy women in terms of total calorie consumption, a reduction reflected in a general decrease in all three macronutrients measured i.e. protein, fat and carbohydrate. The decrease in food intake was accompanied by a decrease in VA ratings of hunger, suggesting specific effects on satiety, in keeping with serotonergic pathway stimulation. In addition, the finding that mCPP decreased food intake suggested a role of the 5-HT$_2$C receptor subtype in the control of human feeding behaviour.

In view of the data outlined in the introduction suggesting that serotonergic dysregulation occurs during clinical eating disorders, the finding that TRP depletion did not alter mood or food intake in such a vulnerable population as recovered bulimic women was unexpected. It is possible that the neurobiological abnormalities present in bulimia nervosa are not affected by TRP depletion or that methodological issues account for the negative findings and these will be briefly discussed below. Nevertheless, the study did provide interesting and new data to suggest that women who have recovered from bulimia nervosa have low basal plasma total TRP levels and continue to exhibit abnormalities in
ratings of appetite and satiety, along with abnormalities of food intake compared to controls. While the most probable explanation for the present findings is that they reflect dietary restraint on the part of the recovered bulimic women, one alternate and intriguing explanation also in keeping with the data, is that the findings represent evidence of persistent hyposerotonergic activity. For example, it could be argued that if reduced brain 5-HT function is an abnormality intrinsic to the disorder, then women suffering from bulimia nervosa may use dieting as a means of "self medication" to increase their levels of brain 5-HT function. While this intriguing theory would be in keeping with the present dieting results in normal women, it makes interpretation of low plasma TRP levels in apparently asymptomatic and "recovered" bulimic women more difficult to explain.

One possibility is that "recovery" in our bulimia nervosa patients was relative, and that while some women manage to curb the more distressing and problematic behaviours that arise from the disorder, such as bingeing, purging and severe dieting, their persistent vulnerability remains (as reflected in a chronic underlying state of hyposerotonergic activity), but in a modified form through the practice of dietary restraint. Thus low plasma TRP may represent a trait marker for the disorder. This interpretation is in keeping with the findings of Jimmerson et al (1988;1990), who found that bulimia nervosa patients with a binge frequency of two or more per day had lower concentrations of CSF 5-HIAA than less symptomatic patients. As no correlation was found between 5-HIAA levels and either changes in body weight or a history of depression, the authors suggested that the low CSF 5-HIAA levels may reflect persistently reduced central serotonin turnover which in turn may have been reversed by a higher binge frequency. Alternatively, it is also possible that bulimic women practice dietary restraint in order to achieve low plasma TRP levels. Such a practice would suggest an underlying state of hyperserotonergic activity in bulimia nervosa and is in direct contrast to the dieting findings in normal women presented in this thesis.

The association between reduced central serotonin function and the presence or absence of depressive symptomatology has important implications for the current study. A substantive body of research data exists to suggest that abnormalities of serotonergic neurotransmission occur during clinical episodes of depression (Meltzer and Nash, 1988; Deakin and Graeff, 1991; Power and Cowen, 1992). Metabolite studies, although somewhat conflicting, have demonstrated low CSF 5-HIAA levels amongst depressed patients compared to controls, a finding most consistent in a subgroup of depressives prone to impulsive violent suicide (Linnoila et al, 1992). Furthermore, neuroendocrine studies have shown that PRL and GH responses to the 5-HT precursor, TRP, and the 5-HT releasing drug d-fenfluramine are blunted during episodes in depression, again suggesting decreased serotonergic neurotransmission (Power and Cowen, 1992).
The majority of our recovered bulimic patients had previously met criteria for major depression at some stage in their lives, and although none were currently clinically depressed, our subjects scored consistently higher on all ratings of dysphoric mood i.e. depression, tension, anxiety and irritability when compared to controls. It is possible, therefore, that the finding of low plasma TRP amongst our group of recovered bulimic subjects was a reflection of their depressive symptomatology, rather than a trait marker relating specifically to bulimia nervosa. Against this hypothesis is the consistent research finding that changes in serotonergic function amongst depressed patients are state dependent, with abnormalities reverting to normal upon clinical recovery. Furthermore, although both plasma TRP and CSF 5-HIAA concentrations are thought to reflect central 5-HT function, the exact manner in which the two measures relate firstly to each other, and secondly to central 5-HT neurotransmission, is still open to debate.

One of the important themes running throughout this thesis has been that of possible gender differences in vulnerability to altered food intake. In keeping with earlier work (Anderson et al., 1990a), our dieting findings suggested that moderate weight loss leads to alterations in brain 5-HT function in women but not men. Furthermore, amongst recovered bulimic women, the finding of low plasma TRP levels raises the possibility of persistent central hyposerotonergic function. While this latter finding may represent a trait marker, it may equally reflect a persistent hyiserotonergic state consequent upon the disorder itself i.e. as a result of persistent food restriction, bingeing and purging behaviours.

Persistent dysregulation of central serotonergic function as a result of food restriction has been suggested by the work of Keys et al. (1950), in which normal men dieted to lose 26% of their total body weight. Restoration of full body weight along with unrestricted access to adequate food did not immediately reverse typical eating disordered behaviours, including a tendency to binge. Such behaviours persisted for up to six months after full body weight recovery. Furthermore, dieting and weight loss amongst obese subjects who had never previously engaged in bingeing behaviours led to the development of binge eating in the three months following completion of their diet (Telch and Agras, 1993).

In view of the findings from this thesis and earlier dieting research, it is probable that women are particularly vulnerable to such persistent dysregulation. The reasons for such gender differences are unclear, although in terms of brain serotonergic function, data exists to suggest that females have a higher basal rate of turnover of 5-HT compared to males (Young et al., 1980). In evolutionary terms, one might speculate that the consequences of food restriction and subsequent weight loss would be greater for the female sex who carry responsibility for perpetuation of the human species. As normal
menstrual functioning ceases once fat composition of the female body drops below approximately 10%, it is possible that females have greater in-built protective mechanisms against food restriction. For example, it is possible that the threshold for food restriction and weight loss triggering central neurotransmitter dysregulation, and hence compensatory behaviours such as binge eating, is lower in women. Such a suggestion would be in keeping with findings from the dieting work presented in this thesis in which alterations in brain 5-HT function were seen only in women, even though male dieters sustained a greater weight loss, in both absolute and percentage body weight loss terms. Alternatively, the degree of compensatory behavioural change may be greater in women than in men and in view of the research data to date, it is also likely that any central neurotransmitter dysregulation that may result as a consequence of food restriction would also persist longer in women.

Longitudinal studies on bulimic patients and studies using vulnerable populations (e.g. relatives of eating disordered patients, families with affective illness) to examine plasma TRP levels while carefully controlling for dietary habits would help clarify these issues.

In direct contrast to previously reported findings amongst normal males (Young et al, 1985; Young et al, 1988), acute TRP depletion did not lead to a lowering of mood or produce alterations in food intake amongst healthy women. This finding was unexpected in view of the widespread literature published suggesting women are more vulnerable to the mood lowering effects of TRP depletion (Delgado et al, 1989; Anderson et al, 1990a; Cowen et al, 1992). While it is possible that gender differences may account for the findings, in that females are in fact more resilient than men to the effects of TRP depletion (a finding not previously reported), methodological issues make firm conclusions difficult to draw.

6.2 FUTURE RESEARCH

The dieting studies outlined in this thesis suggest that pre-synaptic changes in brain 5-HT function may underlie the dieting-induced changes in PRL responses and provide some evidence against the development of dieting-induced post-synaptic 5-HT receptor supersensitivity. Such conclusions must, however, be considered tentative in that they are based upon a single study and on the assumption that dieting-induced changes are mediated through receptors with which mCPP interacts. Furthermore, in contrast to earlier studies, plasma TRP levels did not fall (Anderson et al, 1990a). Future work should be directed towards further determination of the exact mechanism underlying dieting-induced alterations in brain 5-HT function. This would involve carrying out neuroendocrine challenge tests utilising selective 5-HT agonist and antagonist drugs (as they become
available) that interact with other 5-HT receptor subtypes to test the presence of post-synaptic supersensitivity and to determine the receptor subtypes involved in mediating the effects of dieting. For example, animal studies have indicated the importance of 5-HT_{1B} receptors in the control of feeding behaviour (Leibowitz and Shor-Posner, 1986). In the absence of 5-HT_{1B} receptors in the human brain, it has been suggested that the 5-HT_{1D} receptor may play an important role in determining food intake in humans. Recently sumatriptan, a 5-HT_{1D} receptor agonist suitable for human work, has become available providing an opportunity to investigate this hypothesis.

In view of the methodological issues raised in Chapter Five and the paucity of published data examining the effects of TRP depletion in women, further TRP depletion studies in both healthy and recovered bulimic patients need to be carried out. Utilisation of a 100g amino acid dose would help ensure depletion of plasma TRP levels and an adequate fall in plasma TRP:LNAA thus maximising depletion of brain 5-HT, although such benefits would have to be weighed against an increased incidence of confounding side effects. In addition, the introduction of a standard diet for all subjects on the day prior to the study would help eliminate any effects of dietary variations on basal TRP levels. Alternatively, a low TRP diet utilised for several days prior to administration of the amino acid drink would ensure a standard diet while maximising brain 5-HT depletion. Such methodological refinements will in turn improve the chances of detecting any effects on mood and appetite changes in women that may follow acute TRP depletion.

Investigation into the role of serotonergic pathways in the control of human feeding behaviour is also in the early stages, but its development is hampered by a lack of suitable selective 5-HT agonists and antagonists challenge drugs available for use in humans. As new drugs do become available, however, a combined neuroendocrine and test meal approach similar to that outlined in Chapter Four, would enable further delineation of the 5-HT receptor subtypes involved in the control of human feeding behaviour. One immediate possibility would be to investigate the effects of the 5-HT_{1D} receptor agonist sumatriptan on food intake in humans. In addition, interpretation of the study carried out with 0.4mg/kg mCPP was complicated by the possibility of side effects having a direct effect on food intake. In future work using mCPP, a slightly reduced dose (eg. 0.35mg/kg) may be enough to eliminate this problem while still maintaining the anorectic effects of the drug.

In conclusion, investigations outlined in this thesis provide important data contributing to our understanding of the mechanisms underlying dieting-induced changes in brain 5-HT function and support earlier work indicating the increased vulnerability of women to these effects. In addition, abnormalities in ratings of appetite and satiety, along with abnormalities of food intake can be seen amongst women apparently recovered from
bulimia nervosa. Interpretation of these findings in terms of possible neurobiological processes underlying the disorder bulimia nervosa, is however, made difficult by the paucity of neurobiological research carried out on the disorder. It is the author's intention to investigate more closely the state of brain 5-HT function in recovered bulimic women (using a neuroendocrine approach) in order to determine whether or not abnormalities of brain 5-HT function persist beyond recovery from the clinical illness. Such research into the neurobiological basis of the eating disorders is required before a more accurate interpretation of the dieting and TRP depletion studies can be made and applied directly to the eating disorders.
APPENDICES AND REFERENCES
**APPENDIX 1**: Fenfluramine Challenge VA

Please place a mark in the line to indicate how you feel *right now*.

- **Not at all drowsy**
  - Extremely drowsy

- **No nausea at all**
  - Extreme nausea

- **Not at all dizzy**
  - Extremely dizzy

- **Not at all hungry**
  - Extremely hungry

- **Not at all light-headed**
  - Extremely light-headed

- **Not at all depressed**
  - Extremely depressed

- **Not at all happy**
  - Extremely happy
APPENDIX 2: mCPP Dieting Study VA

Please place a mark on the line to indicate how you feel right now.

Not at all light-headed

Extremely light-headed

No nausea at all

Extreme nausea

Not at all anxious

Extremely anxious

No feelings of unreality at all

Extreme feelings of unreality

Not at all sweaty

Extremely sweaty

Not at all hungry

Extremely hungry

Not at all happy

Extremely happy

Not at all mellow

Extremely mellow
APPENDIX 3: mCPP Appetite Study VA

Please place a mark on the line to indicate how you feel right now:

- No nausea at all
- Extreme nausea

- Not at all anxious
- Extremely anxious

- Not at all light-headed
- Extremely light-headed

- Not at all hungry
- Extremely hungry

- No desire at all to eat
- Extreme desire to eat

- Not at all happy
- Extremely happy

- Not at all depressed
- Extremely depressed
**APPENDIX 4: Food Item Checklist**

**FOOD PREFERENCE**

<table>
<thead>
<tr>
<th>Name</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please rate the following foods according to your preferences, using the scale below:

**Bread**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td></td>
</tr>
<tr>
<td>Soft Grain (Mighty White)</td>
<td></td>
</tr>
<tr>
<td>Wholemeal</td>
<td></td>
</tr>
</tbody>
</table>

**Sandwich Fillings and Cold Meats**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar Cheese</td>
<td></td>
</tr>
<tr>
<td>Cottage Cheese</td>
<td></td>
</tr>
<tr>
<td>Peanut Butter</td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td></td>
</tr>
<tr>
<td>Tuna Fish</td>
<td></td>
</tr>
<tr>
<td>Chicken Roll</td>
<td></td>
</tr>
<tr>
<td>Marmite</td>
<td></td>
</tr>
<tr>
<td>Strawberry Jam</td>
<td></td>
</tr>
</tbody>
</table>

**Biscuits**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rich Tea</td>
<td></td>
</tr>
<tr>
<td>Morning Coffee</td>
<td></td>
</tr>
<tr>
<td>Digestive</td>
<td></td>
</tr>
</tbody>
</table>

**Desserts**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry Yoghurt</td>
<td></td>
</tr>
<tr>
<td>Raspberry Yoghurt</td>
<td></td>
</tr>
<tr>
<td>Black Cherry Yoghurt</td>
<td></td>
</tr>
<tr>
<td>Fruit Salad (tinned)</td>
<td></td>
</tr>
<tr>
<td>Rice Pudding</td>
<td></td>
</tr>
</tbody>
</table>

**Crisps**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ready Salted</td>
<td></td>
</tr>
<tr>
<td>Smoky Bacon</td>
<td></td>
</tr>
<tr>
<td>Cheese and Onion</td>
<td></td>
</tr>
</tbody>
</table>

**Chocolate**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Chocolate</td>
<td></td>
</tr>
<tr>
<td>Plain Chocolate (Bournville)</td>
<td></td>
</tr>
<tr>
<td>White Chocolate (Milky Bar)</td>
<td></td>
</tr>
</tbody>
</table>

**Fruit**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td></td>
</tr>
</tbody>
</table>
**APPENDIX 5: Test Meal Food Items**

<table>
<thead>
<tr>
<th>Choice Range</th>
<th>Quantity</th>
<th>Approx. Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>8 slices of one type</td>
<td>270g</td>
</tr>
<tr>
<td>Margarine</td>
<td>Small tub</td>
<td>250g</td>
</tr>
<tr>
<td>Sandwich Fillings</td>
<td>4 choices (minimum of 2 to be protein)</td>
<td>eg. 230g Tuna; 250g cottage cheese; 100g ham; 340g peanut butter</td>
</tr>
<tr>
<td>Crisps</td>
<td>2 packets of one type</td>
<td>60g</td>
</tr>
<tr>
<td>Pudding</td>
<td>1 type</td>
<td>eg. 270g yoghurt</td>
</tr>
<tr>
<td>Fruit</td>
<td>1 item each of 2 types</td>
<td>eg. 120g apple; 100g banana</td>
</tr>
<tr>
<td>Biscuits</td>
<td>1 type</td>
<td>120g</td>
</tr>
<tr>
<td>Chocolate</td>
<td>12 squares of 1 type</td>
<td>75g</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>2 tomatoes</td>
<td>140g</td>
</tr>
</tbody>
</table>

Choice determined by food preference ratings completed by volunteer prior to study (Appendix 4).
APPENDIX 6: Food Record Sheet

RECORD SHEET

Subject Name: ..................................................................
Date: .................................................................
Code: ......................................................... Time Taken: .......

<table>
<thead>
<tr>
<th>Food</th>
<th>Initial Weight</th>
<th>Final Weight</th>
<th>Weight Consumed</th>
<th>Energy Consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filling 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filling 2:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein 1:</td>
<td></td>
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<td>Protein 2:</td>
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</tr>
<tr>
<td>Pudding</td>
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<tr>
<td>Crisps</td>
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</tr>
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<td>Fruit 1:</td>
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<td></td>
</tr>
<tr>
<td>Fruit 2:</td>
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</tr>
<tr>
<td>Biscuit:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Chocolate</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Tomato</td>
<td></td>
<td></td>
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<tr>
<td><strong>TOTALS</strong></td>
<td>**</td>
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</tr>
</tbody>
</table>
APPENDIX 7: TRP Depletion Study Appetite VA

Please place a mark on the line to indicate how you feel right now.

No wish at all to eat  Extreme wish to eat

Not at all hungry  Extremely hungry

Do not feel at all full  Feel extremely full

Do not expect to find the meal at all satisfactory  Expect to find the meal extremely satisfactory

Did not find the meal satisfactory at all  Found the meal extremely satisfactory
APPENDIX 8: TRP Depletion Study Mood VA

Please place a mark on the line to indicate how you feel right now:

Not at all sad/despondent

Extremely sad/despondent

Not at all anxious

Extremely anxious

Not at all tense

Extremely tense

Not at all irritable

Extremely irritable
**APPENDIX 9**: Profile of Mood State (POMS) Questionnaire

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>1. Friendly</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>9. Sorry for things done</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>2. Tense</td>
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<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>10. Shaky</td>
<td>0 0 0 0 0</td>
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<tr>
<td>3. Angry</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>11. Listless</td>
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<tr>
<td>4. Worn out</td>
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<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>12. Peaved</td>
<td>0 0 0 0 0</td>
</tr>
<tr>
<td>5. Unhappy</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>13. Considerate</td>
<td>0 0 0 0 0</td>
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<tr>
<td>6. Clear headed</td>
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<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>14. Sad</td>
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<tr>
<td>7. Lively</td>
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<td>0 1 2 3 4</td>
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<tr>
<td>8. Confused</td>
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<td>0 1 2 3 4</td>
<td>0 1 2 3 4</td>
<td>16. On Edge</td>
<td>0 0 0 0 0</td>
</tr>
</tbody>
</table>

Below is a list of words that describe feelings people have. Please read each carefully. Then fill in the circle under **ONE** of the numbers (0 to 4) to the right corresponding to the phase which best describes **HOW YOU ARE FEELING RIGHT NOW**.

The numbers refer to the phrases:

- 0 = Not at all
- 1 = A little
- 2 = Moderately
- 3 = Quite a bit
- 4 = Extremely
<table>
<thead>
<tr>
<th>Item</th>
<th>Score</th>
<th>Item</th>
<th>Score</th>
<th>Item</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>25. Sympathetic</td>
<td>0</td>
<td>29. Fatigued</td>
<td>0</td>
<td>33. Resentful</td>
<td>0</td>
</tr>
<tr>
<td>26. Uneasy</td>
<td>0</td>
<td>30. Helpful</td>
<td>0</td>
<td>34. Nervous</td>
<td>0</td>
</tr>
<tr>
<td>27. Restless</td>
<td>0</td>
<td>31. Annoyed</td>
<td>0</td>
<td>35. Lonely</td>
<td>0</td>
</tr>
<tr>
<td>28. Unable to concentrate</td>
<td>0</td>
<td>32. Discouraged</td>
<td>0</td>
<td>36. Miserable</td>
<td>0</td>
</tr>
<tr>
<td>29. Fatigued</td>
<td>0</td>
<td>30. Helpful</td>
<td>0</td>
<td>34. Nervous</td>
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</tr>
<tr>
<td>30. Helpful</td>
<td>0</td>
<td>31. Annoyed</td>
<td>0</td>
<td>35. Lonely</td>
<td>0</td>
</tr>
<tr>
<td>31. Annoyed</td>
<td>0</td>
<td>32. Discouraged</td>
<td>0</td>
<td>36. Miserable</td>
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</tr>
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<td>37. Muddled</td>
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<td>0</td>
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</tr>
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<td>39. Bitter</td>
<td>0</td>
<td>42. Ready to fight</td>
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</tr>
<tr>
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<td>0</td>
<td>40. Exhausted</td>
<td>0</td>
<td>43. Good Natured</td>
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</tr>
<tr>
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<td>0</td>
<td>41. Anxious</td>
<td>0</td>
<td>44. Gloomy</td>
<td>0</td>
</tr>
<tr>
<td>41. Anxious</td>
<td>0</td>
<td>42. Ready to fight</td>
<td>0</td>
<td>45. Desperate</td>
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</tr>
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<td>46. Sluggish</td>
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</tr>
<tr>
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<td>0</td>
<td>44. Gloomy</td>
<td>0</td>
<td>47. Rebellious</td>
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</tr>
<tr>
<td>44. Gloomy</td>
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<td>45. Desperate</td>
<td>0</td>
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<td>51. Alert</td>
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<tr>
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<td>49. Weary</td>
<td>0</td>
<td>52. Deceived</td>
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</tr>
<tr>
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<td>50. Bewildered</td>
<td>0</td>
<td>53. Furious</td>
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<tr>
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<td>51. Alert</td>
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<tr>
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<td>52. Deceived</td>
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<td>55. Trusting</td>
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<td>53. Furious</td>
<td>0</td>
<td>56. Full of pep</td>
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</tr>
<tr>
<td>53. Furious</td>
<td>0</td>
<td>54. Efficient</td>
<td>0</td>
<td>57. Bad tempered</td>
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<td>0</td>
<td>55. Trusting</td>
<td>0</td>
<td>58. Worthless</td>
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</tr>
<tr>
<td>55. Trusting</td>
<td>0</td>
<td>56. Full of pep</td>
<td>0</td>
<td>59. Forgetful</td>
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<tr>
<td>56. Full of pep</td>
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<td>57. Bad tempered</td>
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<td>0</td>
<td>61. Terrified</td>
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<tr>
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<td>0</td>
<td>59. Forgetful</td>
<td>0</td>
<td>62. Guilty</td>
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<td>60. Carefree</td>
<td>0</td>
<td>63. Vigorous</td>
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<tr>
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<td>61. Terrified</td>
<td>0</td>
<td>64. Uncertain about things</td>
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<tr>
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<td>62. Guilty</td>
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<td>66. Bushed</td>
<td>0</td>
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**MAKE SURE YOU HAVE ANSWERED ALL ITEMS**
APPENDIX 10: Beck Depression Inventory

On this questionnaire are groups of statements. Please read each group of statements carefully. Then pick out the one statement which best describes the way you have been feeling in the past week including today. Circle the number beside the statement you picked. If several statements apply equally well, circle each one. Be sure to read all the statements in each group before making your choice.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>I do not feel sad</th>
<th>1</th>
<th>I feel sad</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>I am sad all the time and can't snap out of it</td>
<td>2</td>
<td>I am so sad or unhappy I can't stand it</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I am not particularly discouraged about the future</td>
<td>0</td>
<td>I am not particularly discouraged about the future</td>
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<tr>
<td></td>
<td></td>
<td>I feel discouraged about the future</td>
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<td>I feel discouraged about the future</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I feel I have nothing to look forward to</td>
<td>2</td>
<td>I feel I have nothing to look forward to</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I feel the future is hopeless and that things cannot improve</td>
<td>3</td>
<td>I feel the future is hopeless and that things cannot improve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I do not feel like a failure</td>
<td>0</td>
<td>I do not feel like a failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I feel I have failed more than the average person</td>
<td>1</td>
<td>I feel I have failed more than the average person</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As I look back on my life, all I can see is a lot of failures</td>
<td>2</td>
<td>As I look back on my life, all I can see is a lot of failures</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I feel I am a complete failure as a person</td>
<td>3</td>
<td>I feel I am a complete failure as a person</td>
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<tr>
<td></td>
<td></td>
<td>I get as much satisfaction out of things as I used to</td>
<td>0</td>
<td>I get as much satisfaction out of things as I used to</td>
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<tr>
<td></td>
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<td>I don't enjoy things the way I used to</td>
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<td></td>
<td></td>
<td>I don't get real satisfaction out of anything anymore</td>
<td>2</td>
<td>I don't get real satisfaction out of anything anymore</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I am dissatisfied or bored with everything</td>
<td>3</td>
<td>I am dissatisfied or bored with everything</td>
</tr>
<tr>
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<td></td>
<td>I don't feel particularly guilty</td>
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<td></td>
<td></td>
<td>I feel guilty a good part of the time</td>
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</tr>
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<td>I feel guilty most of the time</td>
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</tr>
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<td>I feel guilty all the time</td>
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<td>I feel guilty all the time</td>
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<td>I don't feel I am being punished</td>
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<td>I don't feel I am being punished</td>
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<td>I feel I may be punished</td>
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<td>I expect to be punished</td>
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<td></td>
<td>I feel I am being punished</td>
<td>3</td>
<td>I feel I am being punished</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I don't feel disappointed in myself</td>
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<td>I don't feel disappointed in myself</td>
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<tr>
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<td>I am disappointed in myself</td>
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<td>I am disappointed in myself</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I am disgusted with myself</td>
<td>2</td>
<td>I am disgusted with myself</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I hate myself</td>
<td>3</td>
<td>I hate myself</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>8</td>
<td>I don't feel any worse than anybody else</td>
<td>I am critical of myself for my weaknesses or mistakes</td>
<td>I blame myself all the time for my faults</td>
<td>I blame myself for everything bad that happens</td>
</tr>
<tr>
<td>9</td>
<td>I don't have any thoughts of killing myself</td>
<td>I have thoughts of killing myself, but I would not carry them out</td>
<td>I would like to kill myself</td>
<td>I would kill myself if I had the chance</td>
</tr>
<tr>
<td>10</td>
<td>I don't cry any more than usual</td>
<td>I cry more than I used to</td>
<td>I cry all the time now</td>
<td>I used to be able to cry, but now I can't cry even though I want to</td>
</tr>
<tr>
<td>11</td>
<td>I am no more irritated now than I ever am</td>
<td>I get annoyed or irritated more easily than I used to</td>
<td>I feel irritated all the time now</td>
<td>I don't get irritated at all by things that used to irritate me</td>
</tr>
<tr>
<td>12</td>
<td>I have not lost interest in other people</td>
<td>I am less interested in other people than I used to be</td>
<td>I have lost most of my interest in other people</td>
<td>I have lost all of my interest in other people</td>
</tr>
<tr>
<td>13</td>
<td>I make decisions about as well as I ever could</td>
<td>I put off making decisions more than I used to</td>
<td>I have greater difficulty in making decisions than before</td>
<td>I can't make decisions anymore</td>
</tr>
<tr>
<td>14</td>
<td>I don't feel I look any worse than I used to</td>
<td>I am worried that I am looking old or unattractive</td>
<td>I feel there are permanent changes in my appearance that make me look old or unattractive</td>
<td>I believe that I look ugly</td>
</tr>
<tr>
<td>15</td>
<td>I can work as well as before</td>
<td>It takes an extra effort to get started at doing something</td>
<td>I have to push myself hard to do anything</td>
<td>I can't do any work at all</td>
</tr>
</tbody>
</table>
**APPENDIX 10 contd:**

<p>| | | |</p>
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<th></th>
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</thead>
<tbody>
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<td>I can sleep as well as usual</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>I don't sleep as well as I used to</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>I wake up 1-2 hours earlier than usual and find it hard to get back to sleep</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>I wake up several hours earlier than usual and cannot get back to sleep</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>I don't get any more tired than usual</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>I get tired more easily than I used to</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>I get tired from doing almost anything</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>I am too tired to do anything</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>My appetite is no worse than usual</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>My appetite is not as good as it used to be</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>My appetite is much worse now</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>I have no appetite at all anymore</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>I haven't lost much weight if any lately</td>
</tr>
<tr>
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<td>1</td>
<td>I have lost more than 5 pounds</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>I have lost more than 10 pounds</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>I have lost more than 15 pounds</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>I am no more worried about my health than usual</td>
</tr>
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<td></td>
<td>1</td>
<td>I am worried about aches and pains, upset stomach, constipation</td>
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<tr>
<td></td>
<td>2</td>
<td>I am very worried about physical problems and it is hard to think of much else</td>
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<td></td>
<td>3</td>
<td>I am so worried about my physical problems, that I can't think about anything else</td>
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<tr>
<td>21</td>
<td>0</td>
<td>I have not noticed any recent change in my interest in sex</td>
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<tr>
<td></td>
<td>1</td>
<td>I am less interested in sex than I used to be</td>
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<tr>
<td></td>
<td>2</td>
<td>I am less interested in sex now</td>
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<tr>
<td></td>
<td>3</td>
<td>I have lost interest in sex completely</td>
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</table>
**APPENDIX 11: Eating Attitudes Test**

For each of the numbered statements, place an 'X' in the column which applies best to how you have been over the PAST FOUR WEEKS. Most of the questions relate to food or eating, although other types of questions have been included. Please answer each question carefully.

<table>
<thead>
<tr>
<th>Always</th>
<th>Very Often</th>
<th>Often</th>
<th>Sometimes</th>
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</thead>
<tbody>
<tr>
<td>1. Like eating with other people</td>
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<td>2. Prepare food for others but do not eat what I cook</td>
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<td>3. Become anxious prior to eating</td>
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<td>4. Am terrified about being overweight</td>
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<td>5. Avoid eating when I am hungry</td>
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<td>6. Find myself preoccupied with food</td>
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<td>7. Have gone on eating binges where I feel I may not be able to stop</td>
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<td>8. Cut my food into small pieces</td>
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<td>9. Aware of the calorie content of the food I eat</td>
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<td>10. Particularly avoid foods with a high carbohydrate content (e.g. Bread, rice)</td>
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<td>11. Feel bloated after meals</td>
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<td>12. Feel that others would prefer if I ate more</td>
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<td>13. Vomit after I have eaten</td>
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<td>14. Feel extremely guilty after eating</td>
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<td>15. Am preoccupied with a desire to be thinner</td>
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<td>16. Exercise strenuously to burn off calories</td>
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<td>17. Weigh myself several times a day</td>
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<td>18. Like my clothes to fit tightly</td>
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<td>19. Enjoy eating meat</td>
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<td>20. Wake up early in the morning</td>
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<td>21. Eat the same food day after day</td>
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<td>22. Think about burning up calories when I exercise</td>
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<td>23. Have regular menstrual periods</td>
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<td>24. Other people think that I am too thin</td>
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<td>25. Am preoccupied with the thought of having fat on my body</td>
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<td>26. Take longer than others to eat my meals</td>
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<td>27. Enjoy eating at restaurants</td>
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<td>28. Take laxatives</td>
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<td>29. Avoid foods with sugar in them</td>
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<td>30. Eat diet foods</td>
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<td>31. Feel that food controls my life</td>
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### APPENDIX 11 contd:

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<tr>
<td>32. Display self control around food</td>
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<td>33. Feel that others pressure me to eat</td>
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<td>34. Give too much time and thought to food</td>
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<td>35. Suffer from constipation</td>
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<td>36. Feel uncomfortable after eating sweets</td>
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<td>37. Engage in dieting behaviour</td>
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<td>38. Like my stomach to be empty</td>
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<td>39. Enjoy trying rich new foods</td>
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<td>40. Have the impulse to vomit after meals</td>
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APPENDIX 12: Daily Eating Plan And 1000kcal Diet For Diet Study

1 pint skimmed milk
1/2oz (2 level teaspoons) of low fat spread
3 meat exchanges
4 bread exchanges
2 portions of fruit
Plenty of freely allowed foods

* * * * * * * * * * * * * * * * * * * * * * * * * * *

Daily Eating Plan:

Breakfast:

Breakfast cereal (or 1 bread exchange)
1 slice wholemeal bread (or 1 bread exchange)
Tea or coffee
Milk and fat allowance

Mid-morning:

Low calorie drink or tea or coffee with milk from allowance

Lunch:

2oz lean meat (or 1 meat exchange)
Large serving salad or freely allowed vegetables
1 slice wholemeal bread (or 1 bread exchange)
Fruit - fresh or stewed without sugar
Tea or coffee

Tea:

Low calorie drink or tea or coffee with milk from allowance

Evening Meal:

4oz lean meat (or 2 meat exchanges)
Large serving salad or freely allowed vegetables
1 small potato (or 1 bread exchange)
Fruit - fresh or stewed without sugar
Tea or coffee

Bed Time:

Tea or coffee with rest of milk allowance
APPENDIX 12 contd:

Meat Exchange:
2oz lean meat or chicken
20z oily fish (drain off excess oil)
4oz white fish
1oz (matchbox size) hard cheese
30z cottage cheese
5oz carton yoghurt

Bread Exchange:
1 small slice wholemeal bread
2 wholemeal crispbreads (eg Ryvita)
1 serving breakfast cereal (one weetbix; 1oz puffed wheat; shredded weat; 1oz bran flakes; 4oz porridge)
1 small potato
1 1/2oz (3 tablespoons) cooked brown rice
2 1/2oz (3 tablespoons) baked beans

Freely Available Foods:
Fruit: eg. grapefruit, melon, rhubarb, gooseberries,
blackberries
Vegetables: eg. asparagus, aubergine, french beans, celery,
cabbage
Condiments: eg. pepper, mustard, tomato juice, vinegar,
herbs, oxo
Drinks: eg. sugar free drinks, soda water, vichy, perrier water

Cooking Methods:
Grilling, poaching, casserole, bake, boiled
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