EARLY VASCULAR DISEASE IN CHILDREN WITH EPILEPSY

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Abstract

**Background:** Mortality from cardiovascular and cerebrovascular disease in patients with epilepsy is up to five times that seen in the general population. It is postulated that this elevation in cardiovascular disease is partly due to elevation of the cardiovascular risk factor Total Plasma Homocyst(e)ine (tHcy). Elevated tHcy is frequently elevated in adolescents and adults with epilepsy as a result of Antiepileptic Drug (AED) induced B-vitamin deficiencies, particularly folate, vitamin B12 and vitamin B6 that are important cofactors for homocysteine metabolism. It has been recommended by previous investigators that all children with epilepsy should receive vitamin supplementation to reduce their cardiovascular risk. Other cardiovascular risk factors, such as oxidative stress, hyperinsulinaemia and hyperlipidaemia are also frequently reported in patients with epilepsy treated with AEDs. As early cardiovascular disease, especially in children, is potentially reversible, we plan to investigate endothelial function and structure as well as biochemical cardiovascular risk factors, such as tHcy and lipid levels, in children with epilepsy treated with AED therapy.

**Methods:** Thirty children with idiopathic or symptomatic epilepsy who had been on AED treatment for at least twelve months were recruited from paediatric outpatient clinics in Wellington, New Zealand. Thirty age, sex and BMI matched healthy controls were also recruited. Fasting tHcy, serum folate, red blood cell folate, Pyridoxal-5-Phosphate (PLP), vitamin B12, plasma glucose and lipid levels were measured in each participant. Endothelial function and structure through Flow-Mediated Dilation (FMD) and Intima-Media Thickness (IMT) of the carotid and aortic arteries were measured in subjects and controls.
**Results:** No statistical differences in tHcy, serum folate, red blood cell folate and PLP concentrations were observed between the epilepsy and control groups. Sub group analysis of individual AED therapies also showed no differences. Vitamin B12 levels were elevated in children with epilepsy compared to controls, particularly in the Sodium Valproate (VPA) monotherapy group. Marginally significantly lower fasting glucose was apparent in children with epilepsy compared to controls. This was seen to be primarily due to VPA monotherapy. Lipid and lipoprotein concentrations in children with epilepsy were statistically comparable to controls. After analysis of individual AED treatments however elevated total cholesterol, total cholesterol/ HDL cholesterol ratio, free triglycerides and lipoprotein B levels were evident in children treated with Carbamazepine monotherapy. No statistical differences were apparent in FMD, carotid IMT and aortic IMT between children with epilepsy and controls.

**Conclusions:** We were unable to demonstrate elevated tHcy in our children with epilepsy and so not surprisingly their endothelial function and structure was also unremarkable. Given our findings vitamin supplementation in all children with epilepsy would appear unnecessary. It is likely that our population has diets with vitamin intakes adequate to compensate for any loss of vitamins induced by AED therapy and subsequently the threshold needed to produce elevated tHcy was not reached. Therefore, vitamin supplementation may only be indicated in populations with lower nutritional intakes and adults who naturally have lower B-vitamin levels compared to children. We conclude that recommendations of diets high in B-vitamins by paediatricians and neurologists would be of benefit.
Acknowledgements

I would like to thank my supervisors Dr Lynette Sadlier and Dr Esko Wiltshire who put in many days and nights of hard work to help me write this thesis and support me throughout this challenging research project. They taught me many important research and clinical skills that are valuable for my future. I would also like to thank Alison Notman, the sonographer who gave up mornings to scan each participant and the participants themselves and their parents who without their goodwill and sacrifice of breakfast and sleep, this study would not have been possible. Many thanks also to the Wellington Medical Research Foundation who funded this study. I would like to thank all the staff in the paediatric departments of Wellington and Hutt Valley Hospitals, who helped me recruit and organise the participants.

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Ehara taku toa, I te toa takitahi, katahi, ko taku toa he toa takitini
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Abbreviations

AED: Antiepileptic Drug
aIMT: Aortic Intima-Media Thickness
ANOVA: Analysis of Variance
Apo. A1: Apolipoprotein A1
Apo. B: Apolipoprotein B
BMI: Body Mass Index
BP: Blood Pressure
Ca: Calcium
CBS: Cystathionine β-Synthase
CBZ: Carbamazepine
CCA: Common Carotid Artery
CD: Compact Disk
cGMP: Cyclic Guanosine Monophosphate
cIMT: Carotid Intima-Media Thickness
Cl: Chloride
DVD: Digital Versatile Discs
ECG: Electrocardiogram
EDTA: Ethylene-Diamine-Tetraacetic Acid
EEG: Electroencephalogram
FMD: Flow-Mediated Dilatation
G-6-P: Glucose-6-Phosphate
GABA: γ-Aminobutyric Acid
GTN: Glyceryl Trinitrate
H2O2: Hydrogen Peroxide
HD: High Definition
HDL: High-Density Lipoprotein
IMT: Intima-Media Thickness
LDL: Low-Density Lipoprotein
LTG: Lamotrigine
MTHFR: Methylene tetrahydrofolate Reductase
Na: Sodium
NADPH: Nicotinamide Adenine Dinucleotide Phosphate
NO: Nitric Oxide
NS: Non-significant p > 0.05, patient group vs. control group
OXC: Oxcarbazepine
PB: Phenobarbital
PHT: Phenytoin
PLP: Pyridoxal-5-Phosphate
PML: Post-Methionine Load
PRD: Primidone
SAH: S-Adenosyl-L-Homocysteine
SAM: S-Adenosylmethionine
SMC: Smooth Muscle Cell
SPSS: Statistical Package for the Social Sciences Incorporated
SST: Serum Separated Tube
TG: Triglyceride
tHcy: Total Plasma Homocyst(e)ine
VLDL: Very Low-Density Lipoprotein
VPA: Sodium Valproate
vWF: von Willebrand Factor
ZNS: Zonisamide
Chapter One: Introduction

1.1 Problem Statement

Epilepsy is a common neurological condition that occurs in approximately 1-2% of the population (Menkes and Sankar 2000 p.919; Wallace and Farrell 2004 p.23). Epilepsy is treated with Antiepileptic Drug (AED) therapy. Some children outgrow their seizures, although in some patients life-long AED therapy is required (Wallace and Farrell 2004). In recent years, an increased risk of mortality due to vascular diseases in patients with epilepsy has become an increasing concern. The risk of cardiovascular and cerebrovascular disease in patients with epilepsy is reported to be up to five times greater than that of the general population (Hauser, Annegers et al. 1980; Cockerell, Johnson et al. 1994; Nilsson, Tomson et al. 1997) and the majority of patients with epilepsy have two or more cardiovascular risk factors (Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007).

It is postulated that this elevated cardiovascular risk in patients with epilepsy results from treatment with AEDs (Isojarvi, Pakarinen et al. 1993; Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Apeland, Mansoor et al. 2001; Bramswig, Kerksiek et al. 2002; Apeland, Mansoor et al. 2003; Attilakos, Papakonstantinou et al. 2006; Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007; Tomoum, Awadallah et al. 2008; Verrotti, Scardapane et al. 2008). Total Plasma Homocyst(e)ine (tHcy), an independent risk factor for atherosclerosis, is frequently elevated in patients treated with AED therapy.
(Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2003; Karabiber, Sonmezgoz et al. 2003; Attilakos, Papakonstantinou et al. 2006; Hamed, Hamed et al. 2007). Other cardiovascular risk factors, such as elevated oxidative stress, hyperinsulinaemia and hyperlipidaemia are also frequently associated with AED therapy (Isojarvi, Pakarinen et al. 1993; Isojarvi, Rattya et al. 1998; Apeland, Mansoor et al. 2002; Bramswig, Kerksiek et al. 2002; Verrotti, Basciani et al. 2002; Pylvanen, Knip et al. 2003; Aydin, Serdaroglu et al. 2005; Pylvanen, Pakarinen et al. 2006; Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007; Tomoum, Awadallah et al. 2008; Verrotti, Scardapane et al. 2008).

The association between AED therapy and cardiovascular risk factors in children with epilepsy has not been studied in great detail. Very few studies have investigated vascular structure in patients with epilepsy (Hamed, Hamed et al. 2007; Erdemir, Çullu et al. 2008; Tomoum, Awadallah et al. 2008). No studies at present have investigated endothelial function in epilepsy patients. As vascular disease begins in childhood (Ross 1993; Berenson, Srinivasan et al. 1998) and early vascular disease (especially in children) is potentially reversible (Celermajer, Sorensen et al. 1992; Widlansky, Gokce et al. 2003), it is important to understand the increased cardiovascular risk in children with epilepsy. Epilepsy may require long term AED therapy from a young age, thus treatment for abnormal tHcy or lipids, such as with vitamin supplementation, may be beneficial in preventing future cardiovascular or cerebrovascular disease.
1.2 Hypothesis

From the literature research discussed in the problem statement above and the background in chapter two I have developed three hypotheses:

1. Total plasma homocyst(e)ine, its determinants and lipid levels are abnormal in children with epilepsy receiving antiepileptic drug therapy.
2. Abnormal total plasma homocyst(e)ine, its vitamin determinants and lipid levels relate to abnormal vascular endothelial and smooth muscle function in children with epilepsy receiving AED therapy.
3. Carotid and aortic intima-media thickness is increased in children with epilepsy receiving antiepileptic drug therapy.

1.3 Aims

1. To determine whether vascular function is abnormal in children with epilepsy receiving antiepileptic drug therapy.
2. To measure the determinants of vascular endothelial and smooth muscle function in children with epilepsy receiving antiepileptic drug therapy.
3. To measure the determinants of carotid and aortic artery intima-media thickness in children with epilepsy.
1.4 Research Strategy

Due to the known elevated cardiovascular risk factors in patients with epilepsy I investigated the effect of AED therapy on endothelial function, vascular structure and biochemical risk factors in children with epilepsy. My research and findings are discussed in four chapters:

- Chapter two provides a detailed background on cardiovascular disease in patients with epilepsy. In this chapter the pathogenesis of atherosclerosis and its known risk factors are discussed. It also summarises tHcy metabolism and its proposed mechanism of action for its role in atherogenesis. Cardiovascular disease in patients with epilepsy is then discussed looking into the effects of AEDs on tHcy, lipids, oxidative stress and insulin levels.

- Chapter three is a description of the case-control study I conducted to investigate the effects of AED therapies on vascular structure, endothelial function and the biochemical variables tHcy, its vitamin determinants, glucose and lipid levels.

- Chapter four provides a description of the results found in this study. Controls are compared with all the children with epilepsy in this study, then with individual therapeutic treatment groups. Correlations between variables were also investigated.

- Chapter five summarises the results found and the associations of these results with past literature. It also discusses the strengths and weaknesses of this study and its implications for epilepsy and other diseases. Suggestions for future research are also discussed. Chapter five then ends with an overall conclusion of the study.
Chapter Two: Background

2.1 Classification and Treatment of Epilepsy

Epilepsy is a common neurological condition characterised by two or more seizures (Commission on Epidemiology and Prognosis International League Against Epilepsy 1993). Epilepsy is diagnosed in less than 1% of children under ten years old (Menkes and Sankar 2000 p.919; Wallace and Farrell 2004 p.23), with the incidence of epilepsy increasing to approximately 2% of adults (Menkes and Sankar 2000 p.919). The epilepsies are divided into two main classifications; idiopathic epilepsy and symptomatic (Menkes and Sankar 2000 pp.919-920; Wallace and Farrell 2004 p.2). Idiopathic epilepsies are presumed genetic and often have a better prognosis than symptomatic epilepsies (Menkes and Sankar 2000 p.919; Wallace and Farrell 2004 p.2). Symptomatic epilepsies are secondary to a structural or metabolic disorder such as a cerebrovascular accident, cerebral dysgenesis or cerebral tumour (Menkes and Sankar 2000 p.919; Wallace and Farrell 2004 p.2). Some children with epilepsy out grow their seizures, however in others, life long treatment is necessary (Menkes and Sankar 2000; Wallace and Farrell 2004).

Epilepsies are further classified into generalised or focal epilepsies. Focal epilepsies have focal seizures and generalised epilepsies have generalised seizures. Generalised seizures occur when a discharge of excitatory impulses spread simultaneously across the cortex. Common generalised seizures in children are generalised tonic-clonic, myoclonic and absence seizures (Menkes and Sankar 2000 pp.929-935). Focal seizures occur as a result of neuronal
excitation within an epileptic focus in only a small part of a cerebral hemisphere (Menkes and Sankar 2000 p.927). The seizure semiology reflects the function of the area of the cortex involved for example, visual symptoms when the occipital cortex is the focus. Awareness can be lost or maintained in focal seizures (Menkes and Sankar 2000 pp.935-939).

Seizures are prevented by AEDs. The aim is seizure control with no side effects. AEDs are prescribed based on epilepsy syndrome, seizure type and individual comorbidities (Menkes and Sankar 2000 p.958; Engel and Pedley 2008 pp.1117-1120). Two common AEDs used in children are Sodium Valproate (VPA) and Carbamazepine (CBZ) (Menkes and Sankar 2000 p.964; Wallace and Farrell 2004 p.397). VPA is an effective treatment for all types of seizures and epilepsy syndromes (Menkes and Sankar 2000 p.969; Wallace and Farrell 2004; Engel and Pedley 2008 pp.1674-1675). The mechanism of action of VPA is not completely understood although it is thought to act through several mechanisms. VPA primarily acts through enhancement of the inhibitory neurotransmitter γ-Aminobutyric Acid (GABA) (Menkes and Sankar 2000 p.969; Wallace and Farrell 2004 p.368; Engel and Pedley 2008 p.1673). Increased levels of GABA are found in the cerebrospinal fluid and the whole brain during VPA treatment. This increase may partly be due to increased GABA synthesis, function or reduced GABA degradation (Menkes and Sankar 2000 p.969; Wallace and Farrell 2004 p.368; Engel and Pedley 2008 p.1673). GABA reduces neuronal excitation via reduction of N-methyl-D-aspartate receptor mediated excitation (Engel and Pedley 2008 p.1673). A secondary action of VPA is inhibition of sodium (Na) channels preventing high-frequency repetitive firing of neuronal action potentials (Wallace and Farrell 2004 p.368; Engel and Pedley 2008 p.1673). VPA is also thought to affect Ca-channels and excitatory transmission although clinical
significance is not completely understood (Wallace and Farrell 2004 p.368; Engel and Pedley 2008 p.1673). The most common side effects of VPA are increased appetite and gastrointestinal upsets. Less common complications include hepatic failure, thrombocytopenia and tremor, which only occur in a small number of patients (Menkes and Sankar 2000 pp.969-971; Wallace and Farrell 2004 p.390; Engel and Pedley 2008 pp.1676-1677).

Carbamazepine is effective for focal seizures in symptomatic epilepsies and benign rolandic epilepsy, as well as secondarily generalised tonic clonic seizures (Menkes and Sankar 2000 p.967; Wallace and Farrell 2004 p.389; Engel and Pedley 2008 p.1548). The principle action of CBZ is use dependant inhibition of Na channels. This inhibition of Na channels leads to a reduction of sustained high-frequency repetitive neuronal firing and thus reduced neuronal excitation (Menkes and Sankar 2000 p.967; Wallace and Farrell 2004 p.363; Engel and Pedley 2008 p.1544). It is postulated that CBZ additionally acts on other sites of the central nervous system, such as calcium (Ca) channels, GABA, adenosine or acetylcholine systems, although significant evidence for this has yet to be reported (Wallace and Farrell 2004 p.363; Engel and Pedley 2008 p.1544). CBZ also induces hepatic enzymes resulting in folate deficiency in patients with epilepsy (Maxwell, Hunter et al. 1972; Reynolds 1975; Luoma, Sotaniemi et al. 1980; Menkes and Sankar 2000 p.968). Common complications of CBZ therapy include hypersensitive rashes, diplopia, vertigo, ataxia, gastrointestinal upsets and sedation (Menkes and Sankar 2000 p.968; Wallace and Farrell 2004 p.389; Engel and Pedley 2008 pp.1549-1551). These side effects however are usually prevented by gradual initiation of treatment (Menkes and Sankar 2000 p.968; Engel and Pedley 2008 p.1550). Weight gain and tremor are further common adverse effects although these are less common than treatment with VPA.
therapy (Engel and Pedley 2008 p.1550). Idiosyncratic adverse effects from CBZ therapy include aplastic anaemia, leukopenia, hepatic dysfunction and hyponatremia, which are uncommon and not always clinically significant (Menkes and Sankar 2000 p.968; Wallace and Farrell 2004 p.389; Engel and Pedley 2008 pp.1550-1551). The characteristics of these and other common AEDs are described in Table 1.
Table 1: Characteristics of AED treatments

<table>
<thead>
<tr>
<th>Antiepileptic Drug</th>
<th>Clinical Uses:</th>
<th>Important Side Effects</th>
<th>Best Known Mechanism of Action</th>
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<tr>
<td></td>
<td>Seizure Type</td>
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<td>Generalised:</td>
<td>Generalised:</td>
<td>Lethargy</td>
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<td>- Primary generalised tonic-clonic</td>
<td>- Idiopathic</td>
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<td>- Absence</td>
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<td>- Myoclonic</td>
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<td>Focal:</td>
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<td>- Complex focal</td>
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<td>Decreased libido</td>
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<td>- Secondarily generalised tonic-clonic</td>
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<td>Decreased appetite</td>
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<td>- Secondarily generalised tonic-clonic</td>
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<td>Hyper salivation</td>
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<td>- Primary generalised tonic-clonic</td>
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<td>Sedation</td>
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<td>- Absence</td>
<td>- Symptomatic</td>
<td>Tolerance</td>
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<td>- Myoclonic</td>
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<td>Muscle weakness</td>
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<td></td>
<td>Focal:</td>
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<td>Weight gain</td>
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<td></td>
<td>- Simple focal</td>
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<td>- Complex focal</td>
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<td></td>
<td>- Secondarily generalized tonic-clonic</td>
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(Menkes and Sankar 2000; Wallace and Farrell 2004; Engel and Pedley 2008)  
Na: Sodium; Ca: Calcium; Cl: Chloride; GABA: γ-Aminobutyric acid.
Table 1 (continued): Characteristics of AED treatments

<table>
<thead>
<tr>
<th>Antiepileptic Drug</th>
<th>Clinical Uses:</th>
<th>Common Side Effects</th>
<th>Best Known Mechanism of Action</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Seizure Type</td>
<td>Epilepsy Syndrome</td>
<td></td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>Generalised:</td>
<td>Generalised:</td>
<td>Ethosuxamide prevents absence seizures via inhibition of voltage-dependent T-type Ca-channels of thalamic neurons, the channels responsible for the 3 Hz spike and wave characteristic in absence seizures.</td>
</tr>
<tr>
<td></td>
<td>- Absence</td>
<td>- Childhood absence epilepsy</td>
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<td>- Juvenile absence epilepsy</td>
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<td></td>
<td>Generalised:</td>
<td>Insomnia</td>
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<td>Hiccups</td>
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<td>Gastrointestinal upsets</td>
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<td>Skin rashes</td>
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<td>Headaches</td>
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<td>Drowsiness</td>
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<td>Vertigo</td>
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<td>Ataxia</td>
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<td>Blood dyscrasias</td>
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<tr>
<td>Lamotrigine</td>
<td>Generalised:</td>
<td>Generalised:</td>
<td>Lamotrigine’s primary action is via presynaptic binding to fast voltage-gated Na-channels resulting in reduced release of the excitatory neurotransmitter glutamate. Inhibition of Ca-channels may also add to the broad anticonvulsant actions of Lamotrigine.</td>
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<td></td>
<td>- Primary generalised tonic-clonic</td>
<td>- Idiopathic</td>
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<td>- Absence</td>
<td>- Symptomatic</td>
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<td></td>
<td>- Myoclonic</td>
<td>Focal:</td>
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<td>- Tonic</td>
<td>- Idiopathic</td>
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<td>Focal:</td>
<td>- Symptomatic</td>
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<td>- Complex focal</td>
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<td>- Secondarily generalised tonic-clonic</td>
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<td></td>
<td>Generalised:</td>
<td>Rash</td>
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<td>- Idiopathic</td>
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<td>Ataxia</td>
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<td>Gastrointestinal upsets</td>
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<td></td>
<td>Diplopia</td>
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<td></td>
<td>Dizziness</td>
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<tr>
<td>Levetiracetam</td>
<td>Generalised:</td>
<td>Generalised:</td>
<td>The action of Levetiracetam is not completely understood although is thought to involve inhibition of Ca-channels as well as regulation of neurotransmitter release and GABA function.</td>
</tr>
<tr>
<td></td>
<td>- Primary generalised tonic-clonic</td>
<td>- Idiopathic</td>
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<td></td>
<td>- Absence</td>
<td>- Symptomatic</td>
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<td>- Myoclonic</td>
<td>Focal:</td>
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<td>- Complex focal</td>
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<td>- Secondarily generalised tonic-clonic</td>
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<td>Generalised:</td>
<td>Dizziness</td>
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<td>Drowsiness</td>
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<td></td>
<td>Behavioural problems</td>
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<td></td>
<td>Asthenia</td>
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<td></td>
<td>Infection</td>
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</table>

(Menkes and Sankar 2000; Wallace and Farrell 2004; Engel and Pedley 2008) Na: Sodium; Ca: Calcium; GABA: γ-Aminobutyric acid.)
Table 1 (continued): Characteristics of AED treatments

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</thead>
<tbody>
<tr>
<td></td>
<td>Seizure Type</td>
<td>Epilepsy Syndrome</td>
<td></td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Generalised:</td>
<td>Focal:</td>
<td>Increases the inhibitory action of GABA via elevated GABA turnover and function. Sodium Valproate further affects cellular function by inhibition of Na-channels. Ca-channels and excitatory transmission are also affected although clinical significance is not completely understood.</td>
</tr>
<tr>
<td></td>
<td>- Primary generalised tonic-clonic</td>
<td>- Idiopathic</td>
<td>Weight loss Cognitive impairment Drowsiness Asthenia Metabolic acidosis Paresthesias Nephrolithiasis</td>
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<tr>
<td></td>
<td>Focal:</td>
<td>- Symptomatic</td>
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<td>- Simple focal</td>
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<td>- Secondarily generalised tonic-clonic</td>
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<tr>
<td>Sodium Valproate</td>
<td>Generalised:</td>
<td>Generalised:</td>
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<td></td>
<td>- Primary generalised tonic-clonic</td>
<td>- Idiopathic</td>
<td>Sedation Tremor Hair loss Gastrointestinal upsets Weight Gain Thrombocytopenia Hepatotoxicity Hyperammonemia Teratogenicity</td>
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<td></td>
<td>Absence</td>
<td>- Symptomatic</td>
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<td>- Secondarily generalised tonic-clonic</td>
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<tr>
<td>Topiramate</td>
<td>Generalised:</td>
<td>Generalised:</td>
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<td></td>
<td>- Primary generalised tonic-clonic</td>
<td>- Idiopathic</td>
<td>Weight loss Cognitive impairment Drowsiness Asthenia Metabolic acidosis Paresthesias Nephrolithiasis</td>
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<td></td>
<td>Absence</td>
<td>- Symptomatic</td>
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<td>Topiramate</td>
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<td>- Simple focal</td>
<td>- Idiopathic</td>
<td>Weight loss Cognitive impairment Drowsiness Asthenia Metabolic acidosis Paresthesias Nephrolithiasis</td>
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<td>- Complex focal</td>
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<td>- Secondarily generalised tonic-clonic</td>
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(Menkes and Sankar 2000; Wallace and Farrell 2004; Engel and Pedley 2008)  

Na: Sodium; Ca: Calcium; GABA: γ-Aminobutyric acid.
2.2 **Cardiovascular Disease**

Cardiovascular disease, such as ischaemic heart disease and cerebrovascular accidents, are one of the leading causes of mortality in New Zealand (Robson and Harris 2007 p.39). Mortality due to cardiovascular disease is most pronounced in older populations with deaths due to the circulatory system being the leading cause of mortality in > 65 year olds (Robson and Harris 2007 p.59). Ethnic differences are observed with Māori populations having a two fold increased risk of circulatory diseases compared with non-Māori populations in New Zealand (Robson and Harris 2007 p.42). Endothelial dysfunction underlies the pathogenesis of atherosclerosis and over time results in arterial damage and development of cardiovascular or cerebrovascular disease (Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006).

2.2.1 **Endothelial Dysfunction**

The endothelium is a thin layer of endothelial cells, separating the lumen from the vascular wall of blood vessels. Under normal circumstances, endothelial cells sustain vascular homeostasis via regulation of blood viscosity, vascular tone, coagulation and vascular inflammation (Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006). Endothelial cells additionally act as a protective layer against atherosclerosis (Skilton and Celermajer 2006). Endothelial cells maintain vascular health through paracrine actions on surrounding cells (Vane, Anggard et al. 1990; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006). Nitric Oxide (NO), historically called endothelium-derived relaxing factor, is synthesised from L-arginine (using the cofactor nicotinamide adenine
dinucleotide phosphate (NADPH) in endothelial cells (Vane, Anggard et al. 1990; Boron and Boulpaep 2003 pp.478-480). The main function of NO is as a potent vasodilator (Vane, Anggard et al. 1990; Boron and Boulpaep 2003 pp.478-480; Skilton and Celermajer 2006). Relaxation of vascular smooth muscle is induced by NO via formation of the intracellular second messenger cyclic guanosine monophosphate (cGMP) (Vane, Anggard et al. 1990; Boron and Boulpaep 2003 p.480). cGMP –dependant protein kinase then phosphorylates myosin light-chain kinsae which results in reduced phosphorylation of the myosin light chain and thus reduced myosin-actin interaction(Boron and Boulpaep 2003 p.480). In response to endothelial stress, endothelial cells release NO, which acts on surrounding smooth muscle to produce arterial dilation (Vane, Anggard et al. 1990; Boron and Boulpaep 2003 p.478). Additionally, NO decreases platelet activation, smooth muscle proliferation and inhibits the binding of monocytes to endothelial cells (Vane, Anggard et al. 1990; Boron and Boulpaep 2003 p.478; Skilton and Celermajer 2006). Endothelial cells also generate vasoconstrictors (endothelin-1), cytokines, platelet regulating factors (tissue plasminogen activator) and further vasodilators (prostacyclin) that act to maintain healthy endothelium (Vane, Anggard et al. 1990; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006).

Chronic exposure to cardiovascular risk factors induces inflammation and the characteristic integrity of the endothelium is lost (Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006). Impaired endothelial cells express various inflammatory cytokines and adhesion molecules promoting vascular inflammation. Vascular inflammation enhances the formation of lipid filled foam cells, Smooth Muscle Cell (SMC) proliferation and binding of monocytes and T-cells which are important contributors to atherosclerosis (Widlansky, Gokce et al.
Endothelial cells also gain pro-thrombotic characteristics and abnormal vasoreactivity due to increased vasoconstrictor synthesis and decreased production of anti-thrombotic and vasodilator factors like NO. Consequently, in response to stress, inadequate NO is generated resulting in insufficient arterial vasodilation (Widlansky, Gokce et al. 2003). This altered vascular response can be measured by the ultrasound technique Flow-Mediated Dilatation (FMD) (Celermajer, Sorensen et al. 1992; Gokce, Keaney et al. 2003; Widlansky, Gokce et al. 2003; Yeboah, Crouse et al. 2007) which I will discuss later in the chapter.

Endothelial cells are vital in atherosclerotic prevention, thus, it is not unusual that the risk factors associated with vascular disease are also risk factors for endothelial injury. Common risk factors for endothelial injury include hypercholesterolaemia, cigarette smoking, hypertension and diabetes mellitus (Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006). Common interventions for endothelial dysfunction are also those valuable for atherosclerotic prevention. Lifestyle changes, such as a diet high in fruit and low in fat improve endothelial function (Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006). Exercise improves endothelial vasomotor function by increased production of NO and additionally aids in Body Mass Index (BMI) reduction (Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006). Drug interventions, such as statins, also improve endothelial function through the reduction of high plasma lipids and cholesterol (Widlansky, Gokce et al. 2003).
2.2.2 Pathogenesis of Atherosclerosis

Atherosclerosis, the underlying cause of cerebrovascular disease, coronary artery disease and peripheral vascular disease, develops silently from the first decade of life (Ross 1993; Berenson, Srinivasan et al. 1998). A study of autopsy specimens found fatty streaks, an early sign of atherosclerosis (Ross 1993), in the aorta and carotid arteries of children as young as two years old (Berenson, Srinivasan et al. 1998). They additionally reported an increased incidence of fatty streaks with age. The prevalence of fatty streaks in the carotid arteries increased from 50% in < 15 year olds, to 85% in adults aged 21-39 years (Berenson, Srinivasan et al. 1998). Chronic exposure to cardiovascular risk factors induces arterial inflammation and the instigation of atherosclerosis (Ross 1993; Libby, Ridker et al. 2002). Common cardiovascular risk factors include hypertension, hypercholesterolaemia, diabetes mellitus, obesity and cigarette smoking (Mayer, Jacobsen et al. 1996; Berenson, Srinivasan et al. 1998; Libby, Ridker et al. 2002; Raitakari, Juonala et al. 2003; Elliott, Jacobson et al. 2007). The number of cardiovascular risk factors a person possesses is strongly associated with the risk of vascular disease (Berenson, Srinivasan et al. 1998; Raitakari, Juonala et al. 2003). Berenson and colleagues reported a nine-fold increase of fatty streaks in children and young adults with three or four cardiovascular risk factors compared to patients with none (Berenson, Srinivasan et al. 1998). Cardiovascular risk factors in adolescence are also associated with increased Intima-Media Thickness (IMT) in adulthood (Raitakari, Juonala et al. 2003).

Under normal circumstances white blood cells do not bind to vascular endothelium (Libby, Ridker et al. 2002). Endothelial injury exposes subendothelial collagen (Harker, Ross et al. 1976) and promotes the expression of
specific adhesive glycoproteins (Ross 1993; Libby, Ridker et al. 2002). Inflammatory cells, for example T lymphocytes and monocytes, bind to these glycoproteins and under the influence of monocyte chemoattractant protein-1 they penetrate between endothelial cells towards the intima of the arterial blood vessel (Ross 1993; Libby, Ridker et al. 2002; Burtis, Ashwood et al. 2008 p.618). This accumulation of monocytes and T lymphocytes within the arterial intima is the fatty streak, the earliest visible sign of atherosclerosis (Ross 1993). Once the inflammatory cells are within the arterial wall, a local inflammatory response is initiated. T-cells further encourage inflammation by further promoting inflammatory cytokines (Libby, Ridker et al. 2002). Monocytes become macrophages, which ingest lipid and become foam cells (Ross 1993; Libby, Ridker et al. 2002). Fibrogenic mediators stimulate SMC proliferation and generation of the collagenous extracellular matrix (Libby, Ridker et al. 2002) which forms the thin cap of the atherosclerotic plaque (Ross 1993). Underlying the fibrous cap is a centre of lipid and necrotic debris (Ross 1993). As the atherosclerotic plaque progresses, macrophages release proteolytic enzymes that degrade the fibrous cap, making it vulnerable to rupture (Libby, Ridker et al. 2002). Ruptures of the atherosclerotic plaque lead to haemorrhage, and thrombus formation within the vessel lumen (Ross 1993; Libby, Ridker et al. 2002). Retracted endothelial cells expose underlying lipid filled macrophages providing a site for platelet aggregation thus enhancing thrombus formation (Ross 1993). As the thrombus enlarges, the arterial lumen becomes blocked. If this occurs in the coronary arteries, it subsequently leads to myocardial infarction and possible death (Ross 1993).
2.2.3 Measurement of Atherosclerosis

The measurement of vascular function is important for the assessment of cardiovascular risk. It is possible to detect early atherosclerosis before the irreversible development of vascular disease (Celermajer, Sorensen et al. 1992; Bots, Hoes et al. 1999; Jarvisalo, Jartti et al. 2001; Raitakari, Juonala et al. 2003; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006; Yeboah, Crouse et al. 2007). Old techniques for the measurement of atherosclerosis and endothelial function, for example autopsies, were invasive, expensive and not possible in the living (Salonen and Salonen 1991; Widlansky, Gokce et al. 2003). The most effective current techniques for the assessment of vascular function and structure are FMD and IMT (Salonen and Salonen 1991; Widlansky, Gokce et al. 2003).

Endothelial dysfunction is a fundamental event in the development of atherosclerosis and thus a useful barometer of vascular health (Widlansky, Gokce et al. 2003). Endothelial function can be measured by FMD which assesses the ability of endothelial cells to respond and dilate to stress (Celermajer, Sorensen et al. 1992; Gokce, Keaney et al. 2003; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006; Yeboah, Crouse et al. 2007). Using high-resolution ultrasound, arterial dilatation is assessed in two separate situations: firstly, endothelial dependent dilatation in response to a rapid increase in blood flow; and secondly, endothelial independent dilatation in response to sublingual administration of a synthetic nitrate. In the presence of endothelial injury, endothelial cells are less likely to produce the vasodilator NO, therefore resulting in reduced arterial vasodilation (Celermajer, Sorensen et al. 1992; Gokce, Keaney et al. 2003; Widlansky, Gokce et al. 2003; Yeboah, Crouse et al. 2007). Research looking at the relationship between FMD and vascular disease found that adults with low FMD
had a lower survival rate for cardiovascular events (Gokce, Keaney et al. 2003; Yeboah, Crouse et al. 2007). FMD is also highly correlated with the degree of coronary artery atherosclerosis at angiography (Neunteufl, Katzenschlager et al. 1997). Abnormal FMD is evident in children (Celermajer, Sorensen et al. 1992) before clinical signs of atherosclerosis are present (Celermajer, Sorensen et al. 1992; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006; Yeboah, Crouse et al. 2007). As early vascular disease is potentially reversible, early detection and intervention could improve vascular health in adulthood (Celermajer, Sorensen et al. 1992; Widlansky, Gokce et al. 2003).

The measurement of IMT is another useful way to assess arterial health. This technique assesses SMC proliferation and the deposition of lipids and inflammatory cells that accumulate within the arterial intima during atherogenesis (Skilton and Celermajer 2006). Cardiovascular risk factors, such as cigarette smoking, hypertension and a high BMI are associated with an increased Carotid Intima-Media Thickness (cIMT) in adults (Raitakari, Juonala et al. 2003). This association between cardiovascular risk factors and thickened IMT begins early in childhood before the appearance of clinical signs (Woo, Chook et al. 1999; Jarvisalo, Jartti et al. 2001; Raitakari, Juonala et al. 2003; Mackinnon, Jerrard-Dunne et al. 2004; Skilton and Celermajer 2006). Children and adolescence with diabetes mellitus and hypercholesterolaemia who have a high risk for vascular disease have been shown to have an increased aortic and carotid IMT compared to healthy children (Jarvisalo, Jartti et al. 2001). IMT is an important predictor of cardiovascular and cerebrovascular disease (Salonen and Salonen 1991; Bots, Hoes et al. 1999; Jarvisalo, Jartti et al. 2001; Raitakari, Juonala et al. 2003; Skilton and Celermajer 2006). A follow up study of 1600 adults with increased cIMT, observed an increased risk of stroke, coronary heart disease and
death (4.1%, 3.7% and 10.5% respectively) within around ten years (Bots, Hoes et al. 1999). Thus, IMT is a quick and effective measurement of atherosclerotic risk.

### 2.2.4 Lipids and Atherosclerosis

Lipid and lipoproteins have many important roles in humans. At abnormal levels, however, they are significant contributors to the development of atherosclerosis (Libby, Ridker et al. 2002; Burtis, Ashwood et al. 2008 p.402). Exogenous lipids are absorbed within the small intestine and transported via chylomicrons to the liver where they are a vital hepatic energy source (Burtis, Ashwood et al. 2008 p.403). Excess exogenous lipids are combined with endogenously formed hepatic lipids and transported to peripheral cells via Very Low-Density Lipoproteins (VLDL) or Low-Density Lipoproteins (LDL). VLDL mainly consists of plasma triglycerides whereas LDL is predominantly cholesterol with only a small amount of triglycerides. These lipoproteins play important roles in the maintenance of cholesterol by delivering lipids from the liver to peripheral cells (Burtis, Ashwood et al. 2008 pp.411-415). High-Density Lipoproteins (HDL) have the opposite effect to VLDL and LDL. They remove excess cholesterol from peripheral cells and transport it back to the liver where it is used in bile salts or incorporated back into LDL or VLDL (Burtis, Ashwood et al. 2008 pp.411-415). Apolipoproteins are proteins contained within lipoproteins. Apolipoprotein A1 is the major component of HDL and apolipoprotein B is a major component of LDL (Burtis, Ashwood et al. 2008 p.413). Plasma insulin levels contribute to lipid and lipoprotein levels by stimulating lipogenesis and inhibiting lipolysis in adipocytes (Kahn and Flier 2000).
Chronic elevation of lipids, especially of LDL, leads to lipid deposition within the intima of the arterial wall. Once within the arterial wall, lipoproteins are oxidised which in turn induce the expression of inflammatory markers. These inflammatory markers lead to inflammation, which plays a key role in the development of atherosclerosis (Libby, Ridker et al. 2002; Burtis, Ashwood et al. 2008 p.402). Conversely, HDL are protective of vascular disease as they remove cholesterol from the peripheral system (Libby, Ridker et al. 2002; Burtis, Ashwood et al. 2008 p.415). As LDL and HDL have opposing atherosclerotic actions, atherogenic ratios such as LDL to HDL or total cholesterol to HDL are additionally helpful in determining atherosclerotic risk.

2.2.5 Homocysteine and Atherosclerosis

Homocysteine is an important independent risk factor for atherosclerosis (Burtis, Ashwood et al. 2008 pp.428-429). Significant elevations in tHcy are associated with severe vascular disease at a young age (Welch and Loscalzo 1998; van Beynum, Smeitink et al. 1999; McCully 2007) with smaller elevations resulting in a 60-80% increased risk of coronary heart disease (Boushey, Beresford et al. 1995). The role of homocysteine in atherosclerosis is not completely understood although oxidation of homocysteine to Hydrogen Peroxide (H₂O₂) is considered an important factor in homocysteine induced endothelial damage (Wall, Harlan et al. 1980; Starkebaum and Harlan 1986; Stamler, Osborne et al. 1993; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Fowler 2005). Thrombus formation, lipid peroxidation and SMC proliferation are additional atherosclerotic effects associated with homocysteine (Harker, Ross et al. 1976; Heinecke, Kawamura et
al. 1993; Stamler, Osborne et al. 1993; Tsai, Perrella et al. 1994; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007).

2.2.5.1 Biochemistry of Homocysteine

Homocysteine is a sulphur containing amino acid formed as a result of methionine metabolism (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Fowler 2005). Methionine, an essential amino acid, is a vital methyl donor for protein synthesis and nucleic acids. Methionine supplies the carbon backbone for polyamine synthesis and provides an important source of sulphur (Fowler 2005). After donation of its methyl group, methionine is hydrolysed to homocysteine. Although being an amino acid, homocysteine is not incorporated into proteins and acts as an important regulator of essential compounds (Selhub 1999). Under physiological conditions, 70-80% of homocysteine is protein bound by disulfide bonds (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998). The remaining homocysteine is oxidised forming homocystine (disulphide of homocysteine) or mixed disulfides, including homocysteine thiolactone (Mayer, Jacobsen et al. 1996). Very little reduced or free homocysteine exists in blood plasma (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998). Total plasma homocyst(e)ine refers to the sum of these homocysteine species (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998). Throughout this thesis I refer to this combined pool of homocysteine as Total Plasma Homocyst(e)ine (tHcy) and the amino acid itself as homocysteine. tHcy is most effectively measured in a fasting state and frozen to prevent homocysteine binding or release (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998). The normal
adult reference range of fasting tHcy is 5-15 µmol/L with hyperhomocysteinaemia occurring at levels greater than 15 µmol/L (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998). Moderate hyperhomocysteinaemia is classified as fasting tHcy between 15-30 µmol/L, intermediate at 30-100 µmol/L, and severe at levels greater than 100 µmol/L (Refsum, Ueland et al. 1998; Welch and Loscalzo 1998).

Homocysteine is metabolised by two major pathways: the remethylation pathway and the transsulfuration pathway (Figure 1). Approximately half of the homocysteine enters the remethylation pathway and is converted to methionine via methyl donating enzymes methionine synthase (5-methyltetra-hydrofolate-homocysteine methyltransferase) or betaine methyltransferase (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Selhub 1999; Fowler 2005). Methionine synthase is common in all tissues and donates a methyl group to homocysteine in a vitamin B12 dependant reaction (Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Fowler 2005). Betaine methyltransferase, on the other hand, is only found in the liver (Refsum, Ueland et al. 1998; Selhub 1999; Fowler 2005), brain and kidneys (Fowler 2005), and is independent of vitamin B12 (Selhub 1999). Methionine is predominantly converted to S-adenosylmethionine (SAM), which after its use as a methyl donor, is hydrolysed back to homocysteine (Mayer, Jacobsen et al. 1996; Selhub 1999; Fowler 2005). SAM is an important regulator in methionine excess, promoting the transsulfuration pathway and inhibiting Methylenetetrahydrofolate reductase (MTHFR) required for remethylation (Selhub 1999).

During methionine excess (e.g. post-prandially), homocysteine principally enters the transsulfuration pathway (Refsum, Ueland et al. 1998; Welch and
Loscalzo 1998). Firstly, homocysteine irreversibly binds with serine producing cystathionine through a vitamin B6 dependant reaction (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Fowler 2005). Pyridoxal-5-phosphate (PLP), the active form of vitamin B6, catalyses the liver enzyme cystathionine β-synthase (CBS) (Welch and Loscalzo 1998; Selhub 1999; Fowler 2005). Cystathionine is then further broken down to cysteine by another PLP dependant enzyme, γ-cystathionase (Mayer, Jacobsen et al. 1996; Selhub 1999; Fowler 2005). In methionine surplus, cysteine forms sulphate or taurine and is excreted in urine (Mayer, Jacobsen et al. 1996; Welch and Loscalzo 1998; Selhub 1999).
Figure 1: Summary of pathways involved in homocysteine metabolism. From (Fowler 2005). Boxes with complete lines represent enzymes and those with dotted lines show cofactors. MAT: methionine adenosyltransferase; X-MT: various AdoMet dependent methyltransferases; SAHH: S-adenosylhomocysteine hydrolase; MS: methionine synthase (5-methyltetrahydrofolate - homocysteine methyltransferase); BMT: betaine methyltransferase; MTHFR: methylenetetrahydrofolate reductase; CBS: cystathionine β-synthase; γCL: γ-cystathionase; MeCbl: methylcobalamin; FAD: flavine adenine dinucleotide; PLP: pyridoxal-5-phosphate.
2.2.5.2 Determinants of Total Plasma Homocyst(e)ine concentration

tHcy concentrations are determined by a large number of acquired and inherited factors. Adult males characteristically have higher tHcy levels than females (Boushey, Beresford et al. 1995; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Selhub 1999) largely due to differing effects of sex hormones (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998). Additionally, varying vitamin status (Refsum, Ueland et al. 1998) and greater muscle mass in men (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998) influence sex related tHcy levels. Prior to puberty, tHcy levels are lower and gender differences are not apparent (Refsum, Ueland et al. 1998). tHcy concentrations are half that expected in adults (Refsum, Ueland et al. 1998) and increase steadily after the onset of puberty with age (Boushey, Beresford et al. 1995; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Selhub 1999). A low dietary intake of essential cofactors involved in homocysteine metabolism, for instance B-vitamins, is the most frequent lifestyle cause of elevated tHcy (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Fowler 2005; McCully 2007). The optimal dietary intake to prevent hyperhomocysteinaemia is 400µg folic acid and 3mg vitamin B6 (McCully 2007). Conversely, vitamin B12, which only occurs in animal products, is often only inadequate in vegans that consume no meat or dairy products. Insufficient vitamin B12 absorption may also occur in elderly due to reduced gastric acidity (McCully 2007). Processed food and additives further reduce vitamin intake via > 85% loss of B-vitamins during processing (McCully 2007). The association between elevations in tHcy and B-vitamin deficiencies is not linearly related. B-vitamin deficiencies demonstrate a threshold phenomenon where an elevation in tHcy does not occur until a certain level of cofactor deficiency is reached. This is best demonstrated in homozygous
MTHFR deficiency where elevated tHcy levels are only seen when low plasma folate concentrations are present (Jacques, Bostom et al. 1996). Caffeinated drinks and chronic ethanol consumption results in raised tHcy, whereas modest ethanol consumption and exercise improve tHcy levels (Refsum, Ueland et al. 1998). Thus a diet high in fruits, grains and meat (Boushey, Beresford et al. 1995; McCully 2007) or vitamin supplementation is recommended to prevent raised tHcy (Boushey, Beresford et al. 1995; Welch and Loscalzo 1998; Selhub 1999; McCully 2007). Disease states, such as renal failure also influence tHcy levels via inadequate homocysteine clearance through metabolism rather than excretion (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007).

Genetic aetiologies of elevated tHcy involve mutations in the genes coding enzymes of homocysteine metabolism. The most common of these has been localised to chromosome 21 (Mayer, Jacobsen et al. 1996; Fowler 2005), and results in cystathionine β-synthase (CBS) deficiency, which is clinically known as homocystinuria (Refsum, Ueland et al. 1998; Welch and Loscalzo 1998). Insufficient CBS activity results in redirection of homocysteine from the transsulfuration pathway to be remethylated back into methionine. Severe CBS deficiency saturates the remethylation pathway therefore elevating tHcy concentrations as high as 400-µmol/L (Welch and Loscalzo 1998; Selhub 1999). The homozygous form of CBS deficiency is rare occurring in approximately 1/200,000 births (Welch and Loscalzo 1998), leading to premature vascular disease in young adult hood. Fifty percent of untreated patients will have their first thromboembolism before the age of 30 years (Welch and Loscalzo 1998). Mental retardation, lens dislocation, skeletal deformities and neurological abnormalities also frequently occur in homozygous CBS deficiency (Welch and
Loscalzo 1998; Fowler 2005). Heterozygotes for CBS deficiency have normal fasting tHcy, yet elevated post-methionine loaded tHcy (Refsum, Ueland et al. 1998). Homocystinuria can also be caused by homozygous mutations in the remethylation enzymes MTHFR or methionine synthase, even more rarely than for homozygous CBS deficiency (Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Fowler 2005). In addition there is a common polymorphism in the MTHFR gene, C677T, which is a common cause of mild or moderate hyperhomocysteinaemia (Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999). This single nucleotide polymorphism leads to the substitution of a valine for an alanine (Welch and Loscalzo 1998; Selhub 1999; Fowler 2005), resulting in 50% lower enzyme activity and creating hyperhomocysteinaemia (Selhub 1999; Fowler 2005). The MTHFR C677T mutation occurs in 5-15% of people (Welch and Loscalzo 1998; Selhub 1999) with ethnic (Refsum, Ueland et al. 1998) and geographic (Selhub 1999) diversity. Like CBS deficiency, a single allele is not a significant risk for vascular disease (Welch and Loscalzo 1998; Fowler 2005).

2.2.5.3 Role of Homocysteine in Atherosclerosis

The association between homocysteine and atherosclerosis was first proposed by McCully in 1969. McCully observed severe atherosclerotic plaques in children with methionine synthase, CBS and MTHFR deficiency. As these enzymes are essential in homocysteine metabolism, McCully proposed elevated tHcy to be a factor in atherosclerosis (McCully 2007). Following this, the relationship between tHcy and atherosclerosis has been further delineated. In a meta-analysis of 27 studies, Boushey and colleagues found a 90% increased risk of
cerebrovascular disease for a 5 µmol/L change in tHcy. There was additionally a 60% and 80% increased risk for coronary heart disease for each 5 µmol/L increase in tHcy in men and women respectively (Boushey, Beresford et al. 1995). In a separate study investigating tHcy and stroke, raised tHcy was found in 18% of children with a recent ischaemic stroke compared to 5% of controls (van Beynum, Smeitink et al. 1999). Mild hyperhomocysteinaemia was associated with a 4-fold risk of ischaemic stroke in these children (van Beynum, Smeitink et al. 1999).

Homocysteine induced endothelial injury promotes atherosclerotic development within arterial walls. In vivo, copper catalysed homocysteine incubation induces oxidative lysis of endothelial cells (Wall, Harlan et al. 1980; Starkebaum and Harlan 1986). 51-Cr labelled human (Wall, Harlan et al. 1980; Starkebaum and Harlan 1986) and bovine (Starkebaum and Harlan 1986) endothelial cells both demonstrate dose dependant 51-Cr release during homocysteine incubation. Visually, the endothelium was patchy, with resemblance to vasculature during early atherosclerosis with 18% - 82% of endothelial cell detachment in tHcy concentrations between 2.5 mM – 10 mM (Wall, Harlan et al. 1980). Patchy endothelium in vivo is also reported in hyperhomocysteinemic baboons (Harker, Ross et al. 1976). The mechanism of hyperhomocysteinaemia induced endothelial injury is via auto-oxidation of homocysteine releasing the by-product H₂O₂ (Wall, Harlan et al. 1980; Starkebaum and Harlan 1986; Stamler, Osborne et al. 1993; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Fowler 2005). An early study in 1980 demonstrated a 90 - 97% reduction in endothelial cell lysis in the presence of catalase (Wall, Harlan et al. 1980). Catalase reduces H₂O₂ to oxygen and water. This study suggests an important role of H₂O₂ in endothelial dysfunction rather than just the amino acid itself (Wall, Harlan et al. 1980).
Endothelial dysfunction in hyperhomocysteinaemia is demonstrated by reduced FMD (Tawakol, Omland et al. 1997; Woo, Chook et al. 1997). Following increased vascular flow to the hand after occlusion by a sphygmomanometer, healthy adults with raised tHcy concentrations had decreased arterial dilation in contrast to patients with lower tHcy (6.5 ± 1.7% versus 10.8 ± 1.7%; p < 0.001). Hyperhomocysteinaemia had no effect on Glyceryl Trinitrate (GTN) induced dilation (p = 0.90). Despite the small population size (n = 81), the exclusion of participants with cardiovascular risk factors allowed investigation of the independent effect of tHcy on endothelium, therefore demonstrating a significant association between tHcy and endothelial dysfunction in humans (Woo, Chook et al. 1997). In vitro, homocysteine incubation induces lipid peroxidation (Heinecke, Kawamura et al. 1993; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007), although is not observed in hyperhomocysteinemic humans (Refsum, Ueland et al. 1998).

Hyperhomocysteinaemia induces thrombus formation as a result of endothelial injury (Harker, Ross et al. 1976; Welch and Loscalzo 1998) and the powerful procoagulant effects of homocysteine (Mayer, Jacobsen et al. 1996; Welch and Loscalzo 1998; McCully 2007). In hyperhomocysteinemic induced baboons, Harker and colleagues observed platelet accumulation on abnormal endothelium, and concluded this was the result of sub-endothelial exposure from endothelial loss. Furthermore, platelet turnover increased three-fold along with decreased platelet survival demonstrating increased platelet consumption and thus thrombus formation in hyperhomocysteinaemia (Harker, Ross et al. 1976). Reduced NO synthesis also plays an essential role in thrombus formation. Under normal circumstances, homocysteine injury is neutralised by S-nitrosation of
homocysteine (Stamler, Osborne et al. 1993; Welch and Loscalzo 1998). Oxides of nitrogen bind to homocysteine to form S-nitroso-homocysteine, which like NO has antiplatelet properties (Stamler, Osborne et al. 1993; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998). Consequently, the –SH group of homocysteine is blocked thereby reducing H$_2$O$_2$ generation (Stamler, Osborne et al. 1993; Welch and Loscalzo 1998). In the presence of chronic hyperhomocysteinaemia, however, the balance between tHcy concentration and NO synthesis is disturbed. Endothelial dysfunction precipitated by homocysteine causes NO depletion therefore minimising endothelial protection and permitting further endothelial injury (Stamler, Osborne et al. 1993; Mayer, Jacobsen et al. 1996; Welch and Loscalzo 1998).

Unlike endothelial cells, homocysteine induces SMC proliferation (Harker, Ross et al. 1976; Tsai, Perrella et al. 1994; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007) rather than cell lysis (Wall, Harlan et al. 1980; Starkebaum and Harlan 1986). In hyperhomocysteinemic baboons intimal fibromusculoelastic lesions similar to that seen during early atherosclerosis are seen (Harker, Ross et al. 1976). In human and rat aortic SMC, homocysteine increased DNA synthesis in a dose dependant manner with a 4.5 fold increase in thymidine incorporation at 1mM homocysteine. Elevated thymidine incorporation, however, was not observed within human endothelial cells (Tsai, Perrella et al. 1994).

In summary, tHcy is an important independent risk factor for atherosclerosis (Boushey, Beresford et al. 1995; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007). Although the molecular mechanism behind homocysteine induced atherogenesis is not completely
understood, experimental evidence suggests the oxidation of homocysteine and endothelial dysfunction are important contributors (Harker, Ross et al. 1976; Wall, Harlan et al. 1980; Starkebaum and Harlan 1986; Stamler, Osborne et al. 1993; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Fowler 2005). Other important characteristics of atherosclerosis such as thrombus formation (Harker, Ross et al. 1976; Stamler, Osborne et al. 1993; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007), lipid peroxidation (Heinecke, Kawamura et al. 1993; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007) and SMC proliferation (Harker, Ross et al. 1976; Tsai, Perrella et al. 1994; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007), additionally occur in hyperhomocysteinaemia. Elevated tHcy results in an increased risk of cerebrovascular and cardiovascular disease (Boushey, Beresford et al. 1995; van Beynum, Smeitink et al. 1999), therefore tHcy monitoring and treatment may be valuable in the prevention of vascular disease.

2.3 Epilepsy and Cardiovascular Disease

2.3.1 Mortality and Cardiovascular Disease in Epilepsy

Patients with epilepsy have an increased risk of premature death. A Swedish study of 9000 epilepsy patients reported a mortality rate 3.6 times higher than the general population (Nilsson, Tomson et al. 1997). This confirmed previous studies that found mortality risk over two times that expected (Hauser, Annegers et al. 1980; Annegers, Hauser et al. 1984; Cockerell, Johnson et al. 1994). Increased mortality is most pronounced in younger populations, with mortality
rates levelling off to that of the general population as age increases (Cockerell, Johnson et al. 1994; Nilsson, Tomson et al. 1997). These study populations include both idiopathic and symptomatic epilepsy.

In studies including only idiopathic epilepsies, the reported mortality is more variable. Hauser and colleagues studied 415 patients with idiopathic epilepsy and reported the risk of death was 80% greater than the general population (Hauser, Annegers et al. 1980). Annegers and colleagues found a 57% increased risk (Annegers, Hauser et al. 1984). Conversely, Olafsson and colleagues demonstrated no statistically significant evidence of an increased mortality risk in patients with epilepsy (standardised mortality rate =1.3 [0.8-1.9]), however the study had small numbers (n=182) (Olafsson, Hauser et al. 1998). Therefore, early death among patients with epilepsy is a growing topic of concern throughout the western world.

The three most common causes of early death in patients with epilepsy are malignant neoplasms, pneumonia and vascular disease (Cockerell, Johnson et al. 1994; Nilsson, Tomson et al. 1997). Vascular disease is estimated to be up to five times more common in patients with epilepsy than the general population (Hauser, Annegers et al. 1980; Cockerell, Johnson et al. 1994; Nilsson, Tomson et al. 1997). Nilsson and colleagues reported a 2.5 times greater incidence of ischaemic heart disease, a five times increase in cerebrovascular disease and a three times increase in circulatory disease in epilepsy patients compared to a control population (Nilsson, Tomson et al. 1997). Studies investigating mortality rates in patients with only idiopathic epilepsies report similar trends, although statistical significance was not achieved due to smaller sample sizes (Hauser, Annegers et al. 1980; Annegers, Hauser et al. 1984). The inclusion of participants
with symptomatic epilepsies may overestimate standardised mortality rates due to epilepsy, as the mortality may relate to the underlying aetiology rather than the epilepsy per say (Nilsson, Tomson et al. 1997).

Cardiovascular risk factors reported in patients with epilepsy include abnormal lipid profiles, increased oxidative stress, elevated insulin resistance and elevated fibrinogen, von Willebrand factor (vWF) and tHcy (Isojarvi, Pakarinen et al. 1993; Isojarvi, Rattya et al. 1998; Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2002; Verrotti, Basciani et al. 2002; Apeland, Mansoor et al. 2003; Karabiber, Sonmezgoz et al. 2003; Pylvanen, Knip et al. 2003; Aydin, Serdaroglu et al. 2005; Attilakos, Papakonstantinou et al. 2006; Pylvanen, Pakarinen et al. 2006; Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007; Tomoum, Awadallah et al. 2008; Verrotti, Scardapane et al. 2008). Evaluation of cardiovascular risk among patients with epilepsy reveals multiple risk factors in the majority of patients (Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007). A large study of patients with epilepsy observed two or more cardiovascular risk factors in 53% of patients with epilepsy, versus 28% expected in the general population. Of these, 8% had a medium to moderate ten-year risk of coronary heart disease (Elliott, Jacobson et al. 2007).

### 2.3.2 Lipid and Lipoproteins in Epilepsy

There have been conflicting reports regarding lipoproteins and cholesterol levels in patients with epilepsy. The reasons for this are unclear but may relate to small sample sizes and differing methodologies (Tables 2 - 4). The majority of
case-control studies observe no abnormalities for total cholesterol, LDL cholesterol, HDL cholesterol and free triglycerides in patients with epilepsy (Apeland, Mansoor et al. 2001; Hamed, Hamed et al. 2007; Erdemir, Çullu et al. 2008; Tomoum, Awadallah et al. 2008). When subgroups of patients on individual AEDs are analysed, however, elevated lipids and lipoproteins are frequently reported in patients treated with enzyme inducers such as CBZ (Isojarvi, Pakarinen et al. 1993; Verrotti, Basciani et al. 1998; Bramswig, Kerksiek et al. 2002; Tomoum, Awadallah et al. 2008). Paradoxically VPA treated patients often demonstrate normal or improved lipid and lipoprotein levels compared to healthy controls (Verrotti, Basciani et al. 1998; Apeland, Mansoor et al. 2001; Hamed, Hamed et al. 2007; Erdemir, Çullu et al. 2008; Tomoum, Awadallah et al. 2008; Verrotti, Scardapane et al. 2008). There is one contradictory study of women with epilepsy treated with VPA that showed elevated triglycerides and decreased HDL cholesterol levels although, the women in this study had high BMIs which may explain this difference (Isojarvi, Ratty et al. 1998).

Normal lipid profiles are found in children and adolescents with epilepsy prior to the commencement of AEDs with lipid levels normalizing after cessation of the AED therapy (Verrotti, Basciani et al. 1998). Verrotti and colleagues measured lipid and lipoprotein levels before, during and after treatment with AEDs. Altered lipids were apparent in subjects treated with either CBZ or VPA, although after AED therapy was concluded, all lipid and lipoprotein variables returned to levels comparable to baseline and control concentrations (Verrotti, Basciani et al. 1998). Altered lipid and lipoprotein levels are also observed in healthy volunteers after treatment with CBZ therapy (Bramswig, Kerksiek et al.
These findings suggest the abnormalities in lipid profiles in patients on anticonvulsants are due to the AED itself rather than the epilepsy.

Elevated lipids lead to increased intimal lipid deposition in the intima of arteries and development of atherosclerotic plaques (Ross 1993; Libby, Ridker et al. 2002). As discussed above, lipid deposition can be assessed through ultrasound measurement of arterial IMT (Salonen and Salonen 1991; Bots, Hoes et al. 1999; Jarvisalo, Jartti et al. 2001; Raitakari, Juonala et al. 2003). Only three studies have assessed IMT in patients with epilepsy (Hamed, Hamed et al. 2007; Erdemir, Çullu et al. 2008; Tomoum, Awadallah et al. 2008). Hamed and colleagues demonstrated significantly thickened IMT in the common carotid artery (CCA), area of bifurcation and internal carotids in 51%, 73% and 44% of adult epilepsy patients respectively (Hamed, Hamed et al. 2007). This difference was particularly noted in subsets of individuals treated with CBZ or multiple drug therapy (Hamed, Hamed et al. 2007). Erdemir and colleagues additionally demonstrated increased IMT in children treated with VPA therapy (Erdemir, Çullu et al. 2008). A further study evaluating IMT in a small group of young children (N = 22) found a trend for increased carotid artery IMT although statistical significance was not reached (Tomoum, Awadallah et al. 2008). This may be due to the small numbers, the differing AED treatments or that these children have not had time to develop lipid deposition that is of a level that can be detected by IMT measurements (Berenson, Srinivasan et al. 1998; Tomoum, Awadallah et al. 2008).
<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subjects (controls)</th>
<th>Study Characteristics</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
<th>TG</th>
<th>Apo. A1</th>
<th>Apo. B</th>
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</thead>
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<td>2001</td>
<td>Apeland et al</td>
<td>45 (45)</td>
<td>- Adults with symptomatic, cryptogenic or primary generalised epilepsy.</td>
<td>Inducers: NS</td>
<td>Inducers: NS</td>
<td>Inducers: NS</td>
<td>Inducers: ↓</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Unchanged AEDs for &gt; 6 months.</td>
<td>VPA: NS</td>
<td>VPA: NS</td>
<td>VPA: NS</td>
<td>VPA: NS</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Inducers include PHT, PB and PRD.</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>Hamed et al</td>
<td>225 (60)</td>
<td>- Adults with primary epilepsy.</td>
<td>CBZ: NS</td>
<td>CBZ: ↓</td>
<td>CBZ: NS</td>
<td>CBZ: NS</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- VPA: NS</td>
<td>VPA: ↓</td>
<td>VPA: NS</td>
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<td></td>
<td></td>
<td></td>
<td>- Multi: NS</td>
<td>Multi: ↓</td>
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<td>Multi: ↓</td>
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<tr>
<td></td>
<td></td>
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<td>- Untreated: NS</td>
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<td>Untreated: NS</td>
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<tr>
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<td>Erdemir et al</td>
<td>44 (40)</td>
<td>- Children with primary epilepsy.</td>
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<td>VPA: NS</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- VPA monotherapy &gt; 1 year.</td>
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<td></td>
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<td>2008</td>
<td>Tomoum et al</td>
<td>22 (15)</td>
<td>- Children and adolescents with idiopathic epilepsy.</td>
<td>All: NS</td>
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<td>All: NS</td>
<td>All: NS</td>
<td>All: ↓</td>
<td>All: NS</td>
</tr>
<tr>
<td></td>
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<td>- AED treatment &gt; 6 months.</td>
<td>CBZ: ↑</td>
<td>CBZ: ↑</td>
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<td></td>
<td></td>
<td>- VPA treatment &gt; 6 months.</td>
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<td></td>
<td></td>
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<td>- VPA &lt; CBZ</td>
<td>VPA &lt; CBZ</td>
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<td>VPA &lt; CBZ</td>
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</tr>
</tbody>
</table>

NS: Non-significant p > 0.05, patient group vs. control group.
AED: Antiepileptic drug; All: All subjects with epilepsy; Apo. A1: Apolipoprotein A1; Apo. B: Apolipoprotein B; CBZ: Carbamazepine;
HDL Cholesterol: High-density cholesterol; LDL Cholesterol: Low-density cholesterol; Multi: Multiple drug therapy; PB: Phenobarbital;
PHT: Phenytoin; PRD: Primidone; TG: Triglycerides; VPA: Sodium Valproate.
Table 3: Follow-up studies assessing lipid and lipoprotein levels in epilepsy

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subjects (controls)</th>
<th>Study Characteristics</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
<th>TG</th>
<th>Apo. A1</th>
<th>Apo. B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>Isojarvi et al</td>
<td>19 (-)</td>
<td>Five year follow up of CBZ monotherapy in adults with idiopathic epilepsy</td>
<td>After: - 2 months: ↑ - 1 year: ↑ - 5 years: ↑</td>
<td>After: - 2 months: ↑ - 1 year: ↑ - 5 years: ↑</td>
<td>After: - 2 months: NS - 1 year: ↑ - 5 years: NS</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2002</td>
<td>Brämswig et al</td>
<td>21 (-)</td>
<td>Healthy adult males with normal lipids. - Treated with CBZ for an average of 70 ± 18 days.</td>
<td>↑ NS ↑ ↑ ↑</td>
<td>-</td>
<td>-</td>
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<tr>
<td>2008</td>
<td>Verrotti et al</td>
<td>31 (31)</td>
<td>One year follow up of VPA monotherapy in children with cryptogenic epilepsy.</td>
<td>-</td>
<td>-</td>
<td>NS</td>
<td>-</td>
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</tbody>
</table>

NS: Non-significant p > 0.05, patient group vs. control group; * p < 0.05, during treatment vs. after treatment.
**Table 4:** Intervention study assessing lipid and lipoprotein levels in epilepsy

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subjects (controls)</th>
<th>Study Characteristics</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
<th>TG</th>
<th>Apo. A1</th>
<th>Apo. B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Isojärvi <em>et al</em></td>
<td>12 (24)</td>
<td>- Adult women with idiopathic generalised or cryptogenic symptomatic epilepsy.</td>
<td>VPA: NS</td>
<td>VPA: ↓</td>
<td></td>
<td>VPA: ↑</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Treated with VPA</td>
<td>LTG: NS</td>
<td>LTG: NS&lt;sup&gt;p&lt;/sup&gt;</td>
<td></td>
<td>LTG: NS&lt;sup&gt;p&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Previous history of an endocrine disorder.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- VPA was replaced by LTG therapy.</td>
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</tbody>
</table>

NS: Non-significant p > 0.05, patient group vs. control group; <sup>p</sup> p < 0.05, VPA therapy vs. 12 months of LTG therapy.

**Apo. A1:** Apolipoprotein A1; **Apo. B:** Apolipoprotein B; **HDL Cholesterol:** High-density cholesterol; **LDL Cholesterol:** Low-density cholesterol; **LTG:** Lamotrigine; **TG:** Triglycerides; **VPA:** Sodium Valproate.
2.3.3 **Oxidative Stress in Epilepsy**

Oxidative stress, another contributor to atherosclerosis, is observed in epilepsy patients (Hamed, Hamed et al. 2007; Verrotti, Scardapane et al. 2008). Oxidative stress arises when oxygen free radical production outweighs antioxidant protection, resulting in the alteration of DNA, cellular components and lipids. Oxygen free radicals are caused by many factors including ultraviolet light, vitamins and homocysteine (Finkel and Holbrook 2000). Elevated homocysteine, which can be observed in patients with epilepsy (Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2002; Apeland, Mansoor et al. 2003; Karabiber, Sonmezgoz et al. 2003; Hamed, Hamed et al. 2007), can result in increased oxidative stress as it is easily oxidised producing the reactive oxygen specie $\text{H}_2\text{O}_2$ (Wall, Harlan et al. 1980; Starkebaum and Harlan 1986; Stamler, Osborne et al. 1993; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Fowler 2005). $\text{H}_2\text{O}_2$ induces endothelial cell injury and LDL oxidation which both promote atherogenesis (Wall, Harlan et al. 1980; Starkebaum and Harlan 1986; Heinecke, Kawamura et al. 1993; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007). Findings in patients with epilepsy potentially contributing to increased oxidative stress include: elevated malondialdehyde, the end product for an important free radical reaction; increased susceptibility to LDL oxidation (lag phase); and decreased antioxidants such as vitamin E (Hamed, Hamed et al. 2007; Verrotti, Scardapane et al. 2008). In a large follow-up study of VPA treated patients, makers of oxidative stress were only reported to be abnormal in
patients with an increased BMI (Verrotti, Scardapane et al. 2008). Weight gain is a common side effect of VPA treatment (Menkes and Sankar 2000 p.969; Wallace and Farrell 2004 p.390; Engel and Pedley 2008 p.1676) and less commonly seen with other AEDs (Engel and Pedley 2008 pp.1549-1550). As oxidative stress is associated with obesity, the altered oxidant status observed in epilepsy patients on VPA may be secondary to weight gain rather than a direct AED effect (Verrotti, Scardapane et al. 2008). Altered oxidative status has also been reported in patients on AEDs other than VPA although the effect of weight on oxidative stress in these patients was not considered (Hamed, Hamed et al. 2007).

2.3.4 Hyperinsulinemia in Epilepsy

Hyperinsulinemia, an independent risk factor for coronary heart disease (Verrotti, Basciani et al. 2002), is increasingly recognised in epilepsy patients treated with VPA. The cause of elevated insulin levels in VPA treatment is not clear, although it is proposed to result from either insulin resistance (Verrotti, Basciani et al. 2002) or reduced insulin extraction by the liver (Pylvanen, Pakarinen et al. 2006). Altered insulin is reported after only three months of VPA therapy (Aydin, Serdaroglu et al. 2005), and is often associated with obesity (Isojarvi, Rattya et al. 1998; Verrotti, Basciani et al. 2002; Pylvanen, Knip et al. 2003). As insulin stimulates glucose uptake into adipocytes, it has been proposed that hyperinsulinemia may contribute to weight gain, and thus obesity, in VPA treated patients. In addition, increased glucose uptake stimulated by elevated insulin results in reduced glucose levels in patients on VPA (Aydin, Serdaroglu et al. 2005; Pylvanen, Pakarinen et al. 2006). Insulin resistance, which can also
cause hyperinsulinaemia, however, complicates this interaction and may explain why normal glucose concentrations are reported in some studies (Verrotti, Basciani et al. 2002). Treatment with other AEDs such as CBZ or Lamotrigine (LTG), conversely, have no effect on insulin levels in patients with epilepsy (Isojarvi, Rattya et al. 1998; Pylvanen, Knip et al. 2003)(Pylvanen 2003, Isojarvi 1997) and indeed when LTG is substituted for VPA, insulin, lipids, and BMI in women normalise (Isojarvi, Rattya et al. 1998).

2.3.5 Total Plasma Homocysteine in Epilepsy

Elevated tHcy is postulated to be a significant contributor to early vascular disease in patients with epilepsy. Hyperhomocysteinaemia occurs in roughly 13-81% of patients with epilepsy (Schwaninger, Ringleb et al. 1999; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2002; Karabiber, Sonmezgoz et al. 2003; Huemer, Ausserer et al. 2005; Attilakos, Papakonstantinou et al. 2006; Hamed, Hamed et al. 2007; Kurul, Unalp et al. 2007). This variation may be due to differing methodology in tHcy measurement, for instance fasting versus Post-Methionine Loading (PML) or non-fasting. It is well known that tHcy above 15µmol/L is associated with an increased risk of vascular disease (Boushey, Beresford et al. 1995; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007). Elevated tHcy at the upper end of the reference range, however, is also associated with an increased risk of atherosclerosis. An increased tHcy concentration of 5 µmol/L is linked to a 60-90% increase in cardiovascular and cerebrovascular disease (Boushey, Beresford et al. 1995). Therefore even in the
absence of hyperhomocysteinaemia, patients with epilepsy may still be at risk of early vascular disease. Studies assessing tHcy, its B-vitamin cofactors and other cardiovascular risk factors are summarised in Tables 5 - 6.

Elevated tHcy concentrations are constantly observed in children, adolescents and adults with epilepsy (Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2002; Apeland, Mansoor et al. 2003; Karabiber, Sonmezgoz et al. 2003; Hamed, Hamed et al. 2007). In 1999, Schwaninger and colleagues found tHcy in patients with epilepsy to be 14.7 ± 3.0 µM versus 9.5 ± 0.5 µM in matched controls (p < 0.05) (Schwaninger, Ringleb et al. 1999). Along with elevated tHcy concentrations, B-vitamin deficiencies are also frequently observed amongst patients with epilepsy. The most common of these B-vitamin deficiencies include reduced levels of folate and vitamin B6 (pyridoxal-5-phosphate), essential cofactors involved in homocysteine metabolism (Kishi, Fujita et al. 1997; Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2002; Apeland, Mansoor et al. 2003; Karabiber, Sonmezgoz et al. 2003). In 2003, Apeland and colleagues reported fasting serum folate and PLP levels to be 9.8 nmol/L (8.8 - 10.8) and 39.7 nmol/L (36.4 – 43.3) compared to 11.0 nmol/L (10.4 – 11.7) and 47.5 nmol/L (43.0 – 52.6) respectively, in controls (Apeland, Mansoor et al. 2003). Significant negative correlations between tHcy and folate (-0.2 to -0.58) are additionally observed in many investigations (Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2003; Huemer, Ausserer et al. 2005). PLP demonstrates a negative correlation to tHcy (Apeland, Mansoor et al. 2003) although possibly due to smaller study populations, significance was not always achieved (Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000;
Attilakos, Papakonstantinou et al. 2006). Vitamin B12, another cofactor involved in homocysteine metabolism, largely shows no significant concentration differences between patients with epilepsy and healthy controls (Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Apeland, Mansoor et al. 2003). Data involving vitamin B12, however, is contradictory with both positive (Attilakos, Papakonstantinou et al. 2006) and negative (Vilaseca, Monros et al. 2000; Huemer, Ausserer et al. 2005) correlations with tHcy being found. Vitamin B2, involved in the activation of vitamin B6 to PLP, was found in one study to have a negative correlation with tHcy after adjustment for age and gender (Apeland, Mansoor et al. 2003). Evidence therefore suggests that hyperhomocysteinaemia in patients with epilepsy is induced by deficiencies of essential B-vitamins involved with homocysteine metabolism.
Table 5: Case-control studies assessing tHcy, its B-vitamin cofactors and other cardiovascular risk factors in epilepsy

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subjects (controls)</th>
<th>Study Population</th>
<th>tHcy</th>
<th>PLP</th>
<th>Serum Folate</th>
<th>Red blood cell folate</th>
<th>Vitamin B12</th>
<th>Other Cardiovascular Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Kishi et al</td>
<td>135 (74)</td>
<td>- Children and adolescents with epilepsy. - AED treatment &gt; 6 months</td>
<td>-</td>
<td>-</td>
<td>PB: ↓</td>
<td>CBZ: ↓</td>
<td>VPA: NS</td>
<td>ZNS: NS</td>
</tr>
<tr>
<td>1999</td>
<td>Schwaninger et al</td>
<td>51(51)</td>
<td>- Adults with symptomatic, cryptogenic or idiopathic epilepsy. - AED treatment &gt; 1 month</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td></td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>2000</td>
<td>Vilaseca et al</td>
<td>136 (185)</td>
<td>- Children with focal or generalised epilepsy. - AED treatment &gt; 10 months - Treated with CBZ (62) or VPA (74) monotherapy</td>
<td>All: ↑</td>
<td>CBZ: ↑</td>
<td>VPA: ↑</td>
<td>All: ↓</td>
<td>CBZ: ↓</td>
<td>All: NS CBZ: NS VPA: ↑</td>
</tr>
</tbody>
</table>

NS: Non-significant; ^ p < 0.05 only in 1-15 year olds.

AED: Antiepileptic drug; CBZ: Carbamazepine; PB: Phenobarbital; PLP: Pyridoxal-5-Phosphate; tHcy: Total plasma homocysteine; VPA: Sodium Valproate; ZNS: Zonisamide.
Table 5 (continued): Case-control studies assessing tHcy, its B-vitamin cofactors and other cardiovascular risk factors in epilepsy

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subjects (controls)</th>
<th>Study Population</th>
<th>tHcy</th>
<th>PLP</th>
<th>Serum Folate</th>
<th>Red blood cell folate</th>
<th>Vitamin B12</th>
<th>Other Cardiovascular Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Apeland et al</td>
<td>45 (45)</td>
<td>- Adults with symptomatic, cryptogenic or primary generalised epilepsy.</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Unchanged AEDs for &gt; 6 months.</td>
<td>Inducers: ↑</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Inducers include PHT, PB and PRD.</td>
<td>VPA: NS</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>2002</td>
<td>Karabiber et al</td>
<td>66 (29)</td>
<td>- Children with epilepsy treated with CBZ or VPA.</td>
<td>CBZ: ↑</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CBZ: ↓ VPA: NS VPA &gt; CBZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Unchanged AEDs for &gt; 12 months.</td>
<td>VPA: ↓</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Apeland et al</td>
<td>101 (101)</td>
<td>- Adults with symptomatic, cryptogenic or primary generalised epilepsy.</td>
<td>All: ↑</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Unchanged AEDs for &gt; 6 months.</td>
<td>CBZ: ↑</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- CBZ (43), VPA (24) and multi (13). Inducers (21) include PHT, PB and PRD</td>
<td>Inducers: ↑</td>
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<td></td>
<td></td>
<td>- Inducers: ↑ Multi: NS</td>
<td>VPA: NS</td>
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</tbody>
</table>

NS: Non-significant; * p < 0.05 after exclusion of subjects on multi-vitamins.
AED: Antiepileptic drug; All: All subjects with epilepsy; CBZ: Carbamazepine; Multi: Multiple drug therapy; PB: Phenobarbital; PHT: Phenytoin; PLP: Pyridoxal-5-Phosphate; PRD: Primidone; tHcy: Total plasma homocysteine; VPA: Sodium Valproate.
Table 5 (continued): Case-control studies assessing tHcy, its B-vitamin cofactors and other cardiovascular risk factors in epilepsy

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subjects (controls)</th>
<th>Study Population</th>
<th>tHcy</th>
<th>PLP</th>
<th>Serum Folate</th>
<th>Red blood cell folate</th>
<th>Vitamin B12</th>
<th>Other Cardiovascular Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Kurul et al</td>
<td>25 (10)</td>
<td>- Children with idiopathic epilepsy. - Unchanged AEDs for &gt; 12 months. - Treated with CBZ (11), OXC (6) or VPA (8).</td>
<td>All: NS CBZ: NS OXC: NS VPA: NS</td>
<td>All: NS CBZ: NS OXC: NS VPA: NS</td>
<td>-</td>
<td>-</td>
<td>All: NS CBZ: NS OXC: NS VPA: NS</td>
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</tr>
</tbody>
</table>

NS: Non-significant.
AED: Antiepileptic drug; All: All subjects with epilepsy; CBZ: Carbamazepine; Multi: Multiple drug therapy; OXC: Oxcarbazepine; PLP: Pyridoxal-5-Phosphate; tHcy: Total plasma homocysteine; VPA: Sodium Valproate; vWF: von Willebrand factor.
<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Subjects (controls)</th>
<th>Study Population</th>
<th>tHcy</th>
<th>PLP</th>
<th>Serum Folate</th>
<th>Red blood cell folate</th>
<th>Vitamin B12</th>
<th>Other Cardiovascular Risk Factors</th>
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<tr>
<td></td>
<td>Follow-up studies</td>
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<td></td>
<td>After:</td>
<td>- CBZ: ↑</td>
<td>After:</td>
<td>- CBZ: ↓</td>
<td>- CBZ: NS</td>
<td>- CBZ: NS</td>
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<td>Before:</td>
<td>After: NS</td>
<td>Before: NS</td>
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<td>- CBZ: ↑</td>
<td>After: NS</td>
<td>Before: NS</td>
<td>After: NS</td>
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<td></td>
<td>Intervention studies</td>
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<td>After: ↓**</td>
<td>↓</td>
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<td>After: NS**</td>
<td>After: NS**</td>
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<td></td>
<td>After:</td>
<td>↑**</td>
<td>After: ↑**</td>
<td>After: ↑**</td>
</tr>
<tr>
<td>2005</td>
<td>Huemer et al</td>
<td>10 (9)</td>
<td>- Children and adolescents with epilepsy</td>
<td>Before: NS</td>
<td>-</td>
<td>Before:</td>
<td>-</td>
<td>Before: NS</td>
<td>Before: NS</td>
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<td></td>
<td></td>
<td>After: ↓***</td>
<td>-</td>
<td>After:</td>
<td>↑***</td>
<td>After: ↑***</td>
<td>After: NS</td>
</tr>
</tbody>
</table>

NS: Non-significant; ** p < 0.05 compared with subjects before intervention; *** p < 0.05 compared with placebo group.
AED: Antiepileptic drug; CBZ: Carbamazepine; PLP: Pyridoxal-5-Phosphate; tHcy: Total plasma homocysteine; VPA: Sodium Valproate.
2.3.5.1 Total Plasma Homocyst(e)ine and Antiepileptic Drug Treatment

Antiepileptic drug treatment influences the degree of tHcy and B-vitamin abnormalities in patients with epilepsy. Enzyme inducers, for instance CBZ and Phenytoin (PHT), result in greater thiol and B-vitamin changes in contrast to enzyme inhibitors such as VPA (Kishi, Fujita et al. 1997; Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2003; Karabiber, Sonmezgoz et al. 2003; Attilakos, Papakonstantinou et al. 2006; Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007). Investigations of AED monotherapy on folate deficiency show more pronounced folate reductions in children and adolescents treated with PHT (Kishi, Fujita et al. 1997) and CBZ (Verrotti, Pascarella et al. 2000; Apeland, Mansoor et al. 2003; Attilakos, Papakonstantinou et al. 2006) in contrast to VPA treatment. The molecular mechanism producing folate reductions is not well understood, but is thought to result from four possible causes: (a) abnormal folic acid absorption; (b) competitive interactions between folate co-enzymes and AEDs; (c) an increased demand of folate for the hydroxylation of natural compounds and AEDs; and (d) the induction of hepatic microsomal enzymes (Reynolds 1975; Kishi, Fujita et al. 1997). Recent investigations of AEDs and vitamin status suggest enzyme induction is the principal cause of vitamin abnormalities (Kishi, Fujita et al. 1997; Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2003; Attilakos, Papakonstantinou et al. 2006). In 1972, an English study involving 75 children demonstrated greater hepatic microsomal enzyme activity in patients with epilepsy compared to controls. Hepatic enzyme activity was also associated with folate depletion. This investigation,
however, lacked a feasible control group and the type of AED treatment was not indicated (Maxwell, Hunter et al. 1972). Nevertheless, further research has found increased hepatic cytochrome P450 concentration in patients with epilepsy in relation to matched controls (Luoma, Sotaniemi et al. 1980). As folate is an essential cofactor in the hydroxylation of many natural and synthetic substances, enzyme induction would increase folate demand and therefore result in folate depletion (Maxwell, Hunter et al. 1972). PLP deficiencies are also more pronounced in patients on enzyme inducing AEDs compared to VPA (Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Apeland, Mansoor et al. 2003; Attilakos, Papakonstantinou et al. 2006). As both folate and PLP are found in lower concentrations in children, adolescents and adults on enzyme inducers, evidence suggests enzyme induction generates vitamin deficiencies in patients with epilepsy.

Given that folate and PLP depletion due to enzyme induction is frequently observed in patients with epilepsy, it is not surprising that more pronounced tHcy elevation is seen in treatment with enzyme inducing AEDs (James, Jones et al. 1997; Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2003; Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007). In 225 adults with epilepsy, tHcy was raised in patients treated with CBZ, in contrast to VPA or untreated participants. Other cardiovascular risk factors, fibrinogen and von willebrand factor, were also increased in patients on CBZ treatment versus controls whereas patients on VPA showed no considerable plasma differences (Hamed, Hamed et al. 2007). Elevated fibrinogen and vWF are markers of endothelial activation and may result in greater thrombosis due to elevated tHcy and therefore endothelial injury (Boron and Boulpaep 2003 p.432; Hamed, Hamed et al. 2007). tHcy was also elevated in patients on PHT therapy, although interestingly folate levels were normal (James, Jones et al.
This therefore suggests that tHcy elevation is not strictly due vitamin depletion. Multiple AED treatment is reported in some literature to be associated with more pronounced tHcy and vitamin abnormalities (Huemer, Ausserer et al. 2005; Hamed, Hamed et al. 2007), possibly due to stronger enzyme induction observed in multi-drug therapy (Luoma, Sotaniemi et al. 1980). One study on the other hand reported normal tHcy levels in adults treated with multiple AED treatments (Apeland, Mansoor et al. 2003). Comparisons of patients treated with multiple AED therapy however are difficult as the proportions of patients on various AEDs and the combinations of AEDs in each study will vary considerably.

Hyperhomocysteinaemia in itself can result in epilepsy. High homocysteine concentrations have been found to induce seizures in both immature and mature rats (Kubova, Folbergrova et al. 1995) and mice (Marangos, Loftus et al. 1990). One possible epileptogenic mechanism of homocyst(e)ine is the reduction of adenosine, a natural anticonvulsant. Homocysteine binds to adenosine reducing its anticonvulsant actions and therefore increasing seizure susceptibility (Marangos, Loftus et al. 1990).

Supporting the effects of AEDs on tHcy, tHcy and vitamin concentrations alter with the addition of AED treatment (Verrotti, Pascarella et al. 2000; Attilakos, Papakonstantinou et al. 2006). Two follow up studies reported no differences of tHcy, folate, vitamin B12 and PLP concentrations between children and adolescents with untreated epilepsy and matched controls. After 1 year of AED treatment there were elevated tHcy levels in adolescents treated with VPA or CBZ in contrast to baseline levels or controls (Verrotti, Pascarella et al. 2000; Attilakos, Papakonstantinou et al. 2006). Furthermore, plasma folate and PLP concentrations significantly decreased in all patients with epilepsy, while vitamin B12 remained unchanged (Verrotti,
Pascarella et al. 2000). Attilakos and colleagues also found raised tHcy for both VPA and CBZ treated children with epilepsy, although reduced folate and PLP was only seen in CBZ treated patients (Attilakos, Papakonstantinou et al. 2006). Patients on VPA, interestingly had raised serum folate and vitamin B12 compared to baseline levels. This suggests that tHcy elevation in patients on VPA may not be due to cofactor deficiency (Attilakos, Papakonstantinou et al. 2006). As this study lacked a control group however, it could not be concluded that these changes were abnormal (compared to healthy children) only that the tHcy and B-vitamin levels were either raised or reduced compared to a baseline level before AED treatment (Attilakos, Papakonstantinou et al. 2006). In spite of this, these studies suggest that altered plasma thiol and B-vitamin status is in fact due to AED treatment, rather than seizure exposure or epilepsy itself (Verrotti, Pascarella et al. 2000; Attilakos, Papakonstantinou et al. 2006).

### 2.3.5.2 Vitamin Supplementation in Epilepsy

Vitamin supplementation is one of the most effective treatments for hyperhomocysteinaemia. Oral vitamin replacement with folic acid and B-vitamins normalises tHcy and vitamin concentrations to levels similar to healthy controls (Boushey, Beresford et al. 1995; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Apeland, Mansoor et al. 2002; Huemer, Ausserer et al. 2005; McCully 2007). Supplementation of folic acid alone is sufficient to improve tHcy concentrations, and produces similar tHcy reductions as combined B-vitamin and folic acid supplementation (Boushey, Beresford et al. 1995). McCully recommends improved diets and/or vitamin supplementation in patients with tHcy levels above 8-µmol/L (McCully 2007). A meta analysis of 27 studies has estimated that vitamin supplementation resulting in a 5 µmmol/L
reduction of tHcy would decrease 6-10% of deaths due to coronary artery disease per year in the USA (Boushey, Beresford et al. 1995). As vitamins are relatively inexpensive and improve tHcy within six weeks (Boushey, Beresford et al. 1995; Welch and Loscalzo 1998) oral vitamins are therefore a useful method for tHcy reduction.

Vitamin supplementation in patients with epilepsy on AEDs is effective in lowering tHcy concentrations. A 2002 study investigating the effect of vitamin supplementation in fasting and PML hyperhomocysteinemic patients with epilepsy found that after 30 days of vitamin supplementation with folic acid (0.4 mg once daily), pyridoxine hydrochloride (vitamin B6) (40 mg three times daily) and riboflavin (25 mg three times daily), fasting and PML tHcy decreased by 36% and 29% respectively, to concentrations similar to levels found in controls (Apeland, Mansoor et al. 2002). Additionally, vitamin intervention increased serum folate levels (7.6 (6.3-9.2) nmol/l to 12.2 (10.4-14.3) nmol/l) to concentrations comparable to controls (11.3 (10.3-12.3) nmol/l). Post-methionine load PLP increased eight fold, whereas fasting PLP dropped even lower than baseline levels (Apeland, Mansoor et al. 2002). A further investigation using only folic acid supplementation (1 mg daily) observed normalised tHcy and folate concentrations but these results are based on a very small sample size (Huemer, Ausserer et al. 2005).

A recent study that investigated vitamin supplementation in patients with epilepsy, looked at the oxidising properties of homocyst(e)ine. After 30 days of vitamin intervention, homocyst(e)ine redox-species normalised lowering reduced, oxidised and total homocyst(e)ine concentrations by 17%, 22% and 28% respectively, with an unchanged reduced/ total homocysteine ratio. Protein bound cysteine was also reduced in patients with epilepsy after vitamin intervention (Apeland, Frøyland et al. 2008). With lower levels of tHcy and reduced homocysteine, homocysteine oxidation and thus H2O2
would significantly reduce. Vitamin intervention is therefore effective in improving the redox-status in patients with epilepsy on AEDs. Investigations of vitamin supplementation in patients with epilepsy have only included hyperhomocysteinemic patients. tHcy concentrations in the upper end of the normal range are also associated with an increased risk of vascular disease (Boushey, Beresford et al. 1995) therefore future research is needed to determine the effect of vitamin intervention in a general population with epilepsy.

Nevertheless, evidence supports the importance of vitamin supplementation in patients with epilepsy on AEDs (Apeland, Mansoor et al. 2002; Huemer, Ausserer et al. 2005; Apeland, Frøyland et al. 2008). An additional possible benefit to reduced tHcy is improving seizure control. Homocysteine reduces the natural anticonvulsant adenosine, thus reducing seizure threshold (Marangos, Loftus et al. 1990). Long-term AED treatment resulting in elevated tHcy may therefore reduce the anticonvulsant effects of AED treatment. As patients with epilepsy frequently have elevated tHcy compared to healthy controls (Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2002; Apeland, Mansoor et al. 2003; Karabiber, Sonmezgoz et al. 2003; Hamed, Hamed et al. 2007), vitamin supplementation has been recommended to prevent cardiovascular and cerebrovascular disease later in life (Boushey, Beresford et al. 1995; Apeland, Mansoor et al. 2002; Huemer, Ausserer et al. 2005; Apeland, Frøyland et al. 2008).

2.4 Summary

In conclusion, death due to cerebrovascular or cardiovascular disease is an increasing concern amongst patients with epilepsy. The majority of
patients with epilepsy have two or more cardiovascular risk factors including abnormal lipids, and various plasma components such as insulin, tHcy and B-vitamins. The mechanism behind vascular disease in patients with epilepsy is not understood, although experimental evidence suggests anticonvulsants play a significant role. As early vascular disease, especially in childhood, is potentially reversible, investigations of vascular disease in patients with epilepsy on AEDs may be beneficial in preventing cerebrovascular or cardiovascular disease later in life.
Chapter Three: Methodology

3.1 Ethical Approval

Ethical approval was provided by the Central Regional Ethics Committee, Ministry of Health, Wellington (CEN/07/10/071). Information sheets were given to each subject and the study explained in full. Written consent was obtained for each participant and/ or their parent/ guardian. Support from local iwi was additionally provided by Ngāi Tahu Research Consultation Committee who considered the research to be of importance to Māori Health. Full information sheets and consent forms are provided in the appendices.

3.2 Participants

3.2.1 Epilepsy Subjects

Children with epilepsy were consecutively recruited from paediatric neurology and general paediatric clinics at Capital and Coast and Hutt Valley District Health Boards in Wellington, New Zealand.

Inclusion criteria:

- Children aged between 8 and 18 years (younger children were not recruited as they were unable to tolerate the ultrasound investigations).
- Two or more unprovoked epileptic seizures (Commission on Epidemiology and Prognosis International League Against Epilepsy 1993).
- Twelve or more months of consistent therapy with an anticonvulsant

Exclusion criteria:
- Symptomatic epilepsy due to cerebral vascular disorders.
- Poor medication compliance. Information on compliance was obtained from paediatric clinical records and verbal confirmation at the time of testing.
- History of smoking.
- Regular vitamin supplementation.
- A degree of mental-motor retardation with an unknown aetiology that made them unable to tolerate the ultrasound investigations.
- A growth disorder.
- A metabolic disorder (particularly inborn errors of homocysteine, cobalamin, folate metabolism or any condition known to interfere with homocysteine metabolism).
- Medication other than anticonvulsants known to interfere with homocysteine metabolism.
- Clinical evidence of acute illness, renal dysfunction, thyroid dysfunction or chronic inflammatory disease.

These exclusion criteria were adopted due to the known effects of the above factors on vascular function or its assessment, homocysteine metabolism or its determinants, or both.
### 3.2.2 Controls

Control subjects were healthy children between eight and eighteen years of age with no history or current evidence of medical problems. The exclusion criteria above for the subjects were also applied.

Three different methods were used in the recruitment of control subjects:

1. Each subject with epilepsy was asked to bring a friend or family member. These controls were therefore from similar demographics as the study subjects.
2. Staff from the Paediatric department at Wellington Hospital asked friends or family to participate
3. Controls from a previous study assessing vascular function in children with diabetes were used (Wiltshire, Gent et al. 2002). These controls were recruited by the same two methods described above. IMT measurements were not performed in these subjects.

### 3.3 Clinical Information

Clinical records were reviewed for all subjects with epilepsy. I performed an interview with all subjects and controls prior to ultrasound and blood testing. Information on seizure type, epileptic syndrome, aetiology, age of diagnosis, current AED treatment, duration of current AED treatment, previous AED treatments and seizure control were obtained for the subjects with epilepsy. Dr Sadleir, a paediatric neurologist, reviewed this clinical information to ensure diagnostic accuracy.
Information on age, sex, ethnic background, vitamin supplementation, and family history of high cholesterol or premature cardiovascular disease was recorded on all subjects with epilepsy and controls. Premature cardiovascular disease was classified as high cholesterol at any age or peripheral vascular disease, stroke, myocardial infarction or any other atherosclerotic condition before the age of 55 years (this corresponded with the definition used by Tonstad and colleagues) (Tonstad, Refsum et al. 1996).

Height was measured with a wall-mounted Harpenden stadiometer (Holtain, Wales, United Kingdom). Weight was measured using electronic scales. BMI was subsequently calculated using the equation BMI = Weight (Kg) / [Height (m)]². Self reported pubertal stage was recorded on all subjects using Tanner illustrations. Resting blood pressure was measured in the sitting position using a Dinamap automatic blood pressure device (ProCare100). Information on medication other than anticonvulsants, such as the oral contraceptive pill was additionally recorded on all subjects.

### 3.4 Biochemical Methods

Venous blood samples were collected in all subjects after an overnight fast between 8am and 10.30am. Anticonvulsant medication could be taken prior to testing and breakfast was provided after completion of the investigations. Serum AED levels were not measured, as there is no evidence to suggest a direct association with vascular function.
3.4.1 *Glucose, Total Plasma Homocyst(e)ine and its Determinants*

Total plasma homocyst(e)ine samples were collected in a tube containing ethylene-diamine-tetraacetic acid (EDTA) and placed on ice. Plasma was then separated within 20 mins by centrifugation at 3000 rpm to minimise homocysteine accumulation from cells following sample collection (Burtis, Ashwood et al. 2008 p.429). tHcy levels were determined using the Abbott AxSYM Homocysteine assay. Firstly homocysteine adducts were reduced to free homocysteine by the use of dithiothreitol. Free homocysteine was then enzymatically converted to S-adenosyl-L-homocysteine (SAH), via SAH hydrolase, and then measured using fluorescent polarization immunoassay (Burtis, Ashwood et al. 2008 p.429). Total plasma homocyst(e)ine sample were analysed at Canterbury Health Laboratories. The interassay coefficient for the measurement of tHcy was 3.8% in this laboratory. Although PML tHcy, which stresses the transsulfuration pathway, may have provided further information on hyperhomocysteinaemia, this was not tested as it involves up to a 16 hour fast and is difficult to test on children. Furthermore, fasting tHcy (compared to PML tHcy) provides the most valuable association with vascular function (Welch and Loscalzo 1998).

Serum vitamin B12 was collected in a Serum Separated Tube (SST) and analysed when received using the Roche electrochemiluminescence method (Roche modulator E170, Roche diagnostics) (Burtis, Ashwood et al. 2008 p.489). The coefficient of variation for vitamin B12 in our laboratory (Laboratory Services, Wellington Hospital Capital Coast Health Ltd) is 5%. Serum Vitamin B6 was collected in an EDTA tube and protected from light. Serum vitamin B6 was then measured using a Chromsystem Isocratic High-Performance Liquid Chromatography system with fluorescence detection.
Serum Vitamin B6 samples were analysed at Canterbury Health Laboratories.

Serum folate was collected in a SST tube and measured using the Roche E170 electrochemiluminescence method (Roche Diagnostics) (Burtis, Ashwood et al. 2008 pp.489, 494). The interassay coefficient for the measurement of serum folate in our laboratory (Laboratory Services, Wellington Hospital Capital Coast Health Ltd) is 5%. Red blood cell folate was collected in an EDTA tube and analysed using a Chemiluminescent Microparticle Immunoassay (Architect system, Abbott Diagnostics) (Burtis, Ashwood et al. 2008 pp.489, 494). Red blood cell folate samples were analysed at Canterbury Health Laboratories. The coefficient of variation value for this assay was 11.1%.

Fasting plasma glucose levels were collected in a SST tube and analysed using the Roche hexokinase method (Roche Diagnostics). After the addition of two buffers (Tris(hydroxymethyl)-aminomethane and 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethane sulfonic acid), glucose is firstly converted to Glucose-6-Phosphate (G-6-P) via the enzyme hexokinase. Glucose-6-phosphate dehydrogenase then oxidises G-6-P to gluconate-6-phosphate and NADPH. NADPH formed through this reaction is then measured photometrically. These NADPH levels are directly proportional to the glucose concentration (Burtis, Ashwood et al. 2008 pp.389-390). The coefficient of variation for the measurement of plasma glucose in our laboratory (Laboratory Services, Wellington Hospital Capital Coast Health Ltd) is 1.6%.
3.4.2 Lipids and Lipoproteins

Fasting lipids and apolipoproteins were measured. Fasting lipids were analysed by Laboratory Services, Wellington Hospital Capital Coast Health Ltd. Apolipoproteins were analysed by Canterbury Health Laboratories. Total cholesterol blood samples were collected in a SST tube and measured enzymatically using the Roche P800 cholesterol esterase/oxidase method (Roche/Hitachi diagnostics). This method involved hydrolysis of cholesterol esters to form cholesterol and fatty acids (using the catalyst cholesterol esterase), oxidation of cholesterol forming cholest-4-en-3-one and H₂O₂ (cholesterol oxidase) and complexion of H₂O₂ with phenol and 4-aminophenazone, producing a red 4-(p-benzoquinone-monoimino)-phenazone solution (peroxidise). Total cholesterol concentration is directly proportional to the colour intensity of the red phenazone solution that was subsequently measured photometrically at 505nm (Burtis, Ashwood et al. 2008 p.422). The coefficient of variation for the measurement of total cholesterol in our laboratory is 2%.

Triglycerides were collected in a SST tube and measured with an enzymatic colorimetric test using the Roche P800 modulator. This involved the hydrolysis of triglycerides to form glycerol and carboxylic acids (using lipase), the conversion of glycerol to glycerol-3-phosphate (glycerol kinase), and then further oxidation of glycerol-3-phosphate forming dihydroxyacetone phosphate and H₂O₂ (glycerophosphate oxidase). H₂O₂ then reacts with 4-aminophenazone and 4-chlorophenol forming a 4(p-benzoquinone-monoimino)-phenazone solution (Peroxidase) that was subsequently analysed photometrically at 505nm (Roche/Hitachi Diagnostics) (Burtis, Ashwood et al. 2008 p.422-423). Triglyceride measurements in our laboratory had a limitation of ± 0.1 mmol/L.
HDL cholesterol was collected in a SST tube and measured with a homogeneous enzymatic colorimetric test using the Roche P800 modulator (Roche/Hitachi diagnostics). This method involved hydrolysis of HDL-cholesterol esters to free cholesterol and fatty acids (using the catalyst PEG-cholesterol esterase), oxidation of HDL-cholesterol to Δ4-cholestenone and H₂O₂ (PEG-cholesterol oxidase). The H₂O₂ formed then reacts with 4-aminoantipyrine and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline to form a purple-blue solution. HDL-cholesterol concentration is directly proportional to the colour intensity of the purple-blue pigment which was measured photometrically at 600nm (Burtis, Ashwood et al. 2008 p.424). The interassay limitation for HDL cholesterol measurements in our laboratory is ± 0.2mmol/L.

LDL was subsequently calculated using the Friedewald equation:

\[
LDL \text{ cholesterol} = [Total \text{ cholesterol}] - [HDL \text{ cholesterol}] - \frac{[Triglycerides]}{2.22}
\]

(Burtis, Ashwood et al. 2008 p.424)

Apolipoprotein A1 and Apolipoprotein B blood samples were collected in a SST tube and measured by immunonephelometry using a BN* system (Dade Behring) (Burtis, Ashwood et al. 2008 p.425). The coefficients of variation for these assays were 7% for Apolipoprotein A1 and 5% for Apolipoprotein B.
3.5 Ultrasound Methods for Vascular Function and Structure

An experienced sonographer blinded to the subject groups performed all the ultrasound measurements. All scans were measured with a broadband linear 5-12 MHz probe using an ATL HDI 5000 ultrasound system. An electrocardiogram was recorded on each scan. Ultrasound testing was performed at Wellington Hospital in a temperature-controlled room between 7.30am and 10am following an overnight fast.

3.5.1 Flow-Mediated Dilation

Endothelial vasomotor function was assessed using FMD and GTN-induced dilation as described in chapter one (Salonen and Salonen 1991; Celermajer, Sorensen et al. 1992; Jarvisalo, Jartti et al. 2001; Gokce, Keaney et al. 2003; Raitakari, Juonala et al. 2003; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006; Yeboah, Crouse et al. 2007). FMD was measured using high-resolution ultrasound and vessel wall tracking. The diameter of the right brachial artery was measured in a longitudinal section from two dimensional ultrasound images with a broadband linear 5-12 MHz probe. The focal zone was set to the depth of the artery and depth and gain settings adjusted ensuring optimal images of the lumen/arterial wall interface. To maximise the size of the vessel all images were set in the minimal field of view. A suitable site for imaging the vessel, 2-15cm above the elbow was selected and two marks placed on the arm to ensure consistent recordings. Machine settings were not altered throughout the study. An electrocardiogram was measured along with each ultrasonic image. The first scan was taken at rest, providing a baseline for consecutive scans. Reactive hyperaemia was then induced by occlusion of arterial blood flow using a sphygmomanometer
inflated above 250mmHg on the lower arm, for four minutes. Following deflation of the cuff, increased blood flow to the hand (shear stress) lead to endothelial-dependant dilation. Arterial flow velocity was measured during the resting scan and the first 15 seconds after deflation of the cuff using a pulsed Doppler signal 60° to the vessel. The second scan with reactive hyperaemia (percentage increase in blood flow) was taken following cuff deflation for 90 seconds. After 15 minutes of arterial recovery, an additional resting (third) scan was recorded. Endothelial-dependant dilation was then compared to the vessel’s response to sublingual nitroglycerin, an endothelium independent dilator. Sublingual GTN spray (0.4mg, Nitrolingual Pumpspray, POHL) was administered and the last (fourth) scan recorded after four minutes.

Ultrasound images were recorded onto high quality videotape or Digital Versatile Discs (DVD) and measured by an independent investigator (Dr Esko Wiltshire or Ngaire Keenan) blinded to the stage of the study and subject group. Electronic callipers were used to measure the vessel diameter from the proximal “m” line to the distal “m” line (the “m” line is an echodense ultrasonic line produced by the media-adventitia interface). Four end-diastolic measurements within seven cardiac cycles (concurrently with the Electrocardiogram (ECG) R wave) were made for each scan and then the average calculated. Reactive hyperaemia was determined by division of blood flow in the first 15 seconds after cuff deflation, by blood flow measured in the resting scan. The vessel diameters after reactive hyperaemia (third scan) and GTN administration (fourth scan) were then expressed as a percentage of the first baseline scan. Scans were excluded from analysis if the arterial diameters in the first (resting) and third (re-control) scans differed by more than 3%. The coefficient of variation between two scans on twenty subjects in our unit is
3.7% (Wiltshire, Gent et al. 2002). Images of the scans are shown in Figures 17 - 22 in chapter four.

Arterial vasomotor function is dependant on the generation and release of NO by vascular endothelial cells (Vane, Anggard et al. 1990; Widlansky, Gokce et al. 2003). Arterial dilation in response to GTN occurs independently of NO. Comparison of the two therefore provide an accurate measurement of endothelial function. Other techniques for the measurement of endothelial function in vivo are invasive (Salonen and Salonen 1991; Widlansky, Gokce et al. 2003), thus FMD is the best assessment of endothelial function in children. GTN-induced dilatation provides additional information on vascular smooth muscle function.

3.5.2 Carotid Artery and Aortic Intima-Media Thickness

The aortic and CCA IMT was measured with sono CT using a broadband linear 5-12 MHz probe. The left and right CCAs were imaged in a standard magnification (2 x 2cm) slightly inferior to the carotid bulb (distal 10mm of the CCA). A vascular preset was used and the focal zone set slightly below the distal wall. A High Definition (HD) zoom was used to achieve a large field of view and to allow imaging of solely the vessel. Three scanning angles were recorded, anterior oblique, lateral and posterior oblique. Four to five still images were taken concurrently with the ECG R wave and stored on a high quality Compact Disk (CD). The aorta was visualized immediately superior to the aortic bifurcation. The ultrasound machine was set to a small bowel preset. An HD zoom was then used to include the posterior lumen and the gain altered to visualize the posterior wall intima. Four still images were recorded at the ECG R wave and stored on a high quality CD.
Using automatic edge detection and a measurement computer software package (Woo, Chook et al. 1999; Mackinnon, Jerrard-Dunne et al. 2004), the average, maximum and minimum distances between the lumen-intima and media-adventitia interface (IMT) was measured in the posterior (far) wall of the CCA and aorta. Analysis of each ultrasound image occurred offline in a random blinded fashion.

### 3.6 Statistical Methods

Power calculations for the study population determined that 30 subjects in each group (epilepsy and controls) would have 80% power at the 5% level of significance to detect a difference in FMD of $2.9 \pm 4\%$ between children with epilepsy and controls (i.e. 9% increase in FMD in controls versus 6.1% in children with epilepsy). This calculation was based on a similar standard deviation in other studies and a smaller difference in FMD, but which is still clinically relevant.

Analyses were performed using SPSS (Statistical Package for the Social Sciences Incorporated) version 15 software. First of all descriptive data (mean, median, standard deviation, quartiles, inter-quartile range) were calculated and then normality for each continuous variable assessed with the Kolmogorov-Smirnov test. Data are presented as mean and standard deviation for normally distributed variables and median and interquartile range ($25^{\text{th}} - 75^{\text{th}}$ percentiles) for non-normally distributed variables. The two groups (epilepsy subjects and controls) are then compared using the chi-squared test for ordinal data, student t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. For comparisons between AED treatment groups, one way ANOVA (Analysis of Variance) was
used for normally distributed data and the Kruskal-Wallis test in non-normally distributed data.

Correlations were calculated using Pearson’s correlation test for normally distributed data and Spearman’s rank correlation test for non-normally distributed data. We considered a p-value < 0.05 to be statistically significant.
Chapter Four: Results

4.1 Participants

Thirty children with epilepsy and thirty healthy controls aged between 8 – 18 years were included in this study. The demographic characteristics of subjects and controls are presented in Table 7. No statistical difference was found between groups for gender, age, pubertal stage, weight, height, BMI, systolic blood pressure and diastolic blood pressure. Information on tanner stage was unavailable in four controls.

Table 7: Demographic characteristics of children with epilepsy and controls

<table>
<thead>
<tr>
<th>Demographic Data</th>
<th>Controls Sample size, (%)</th>
<th>All Epilepsy Subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Sex: Male</td>
<td></td>
<td>14 (47)</td>
<td>14 (47)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16 (53)</td>
<td>16 (53)</td>
</tr>
<tr>
<td>Ethnicity:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European Māori descent</td>
<td></td>
<td>30 (100)</td>
<td>25 (83)</td>
</tr>
<tr>
<td>Samoan</td>
<td></td>
<td>-</td>
<td>4 (13)</td>
</tr>
<tr>
<td>Age (years)*</td>
<td></td>
<td>13.33 ± 2.30</td>
<td>13.92 ± 2.91</td>
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<tr>
<td>Tanner Stage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>6 (22)</td>
<td>6 (20)</td>
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<td>9 (30)</td>
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<td>5</td>
<td></td>
<td>4 (15)</td>
<td>6 (20)</td>
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<td>Weight (Kg)*</td>
<td></td>
<td>56.08 ± 13.94</td>
<td>57.77 ± 22.87</td>
</tr>
<tr>
<td>Height (m)*</td>
<td></td>
<td>1.62 ± 0.14</td>
<td>1.62 ± 0.18</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)*</td>
<td></td>
<td>21.17 ± 3.88</td>
<td>21.20 ± 5.59</td>
</tr>
<tr>
<td>Systolic Blood Pressure (mmHg)*</td>
<td></td>
<td>113.43 ± 14.92</td>
<td>114.72 ± 16.01</td>
</tr>
<tr>
<td>Diastolic Blood Pressure (mmHg)*</td>
<td></td>
<td>63.65 ± 9.29</td>
<td>64.21 ± 7.41</td>
</tr>
</tbody>
</table>

* Data expressed as mean ± standard deviation, students t-test
4.1.1 Epilepsy subjects

Initial search of Electroencephalogram (EEG) referrals and outpatient clinic lists ascertained seventy-seven potentially eligible children with epilepsy from the Wellington region, New Zealand. Of these children, sixty-nine was eligible with the remaining eight children either off AED therapy (3) or had been treated with AEDs for less than 12 months (5). Fifty-one children were then contacted, twenty-one of which elected not to participate for various reasons described in Figure 2. Eighteen children with epilepsy were unable to be contacted. Thirty patients with epilepsy consented to participate in our study with a recruitment rate of 59%. No patients elected to withdraw after commencement of testing (Figure 2).

The children’s epilepsy syndrome and the AED they were on are described in Table 8. The median age at diagnosis was 8.0 years and the median duration of epilepsy was 4.8 years. The children with epilepsy were diagnosed with idiopathic generalised epilepsy (8), idiopathic focal epilepsy (8), symptomatic focal epilepsy (9), cryptogenic focal epilepsy (3) and two were unable to be classified. None of the symptomatic epilepsies were secondarily to vascular disorders. Four children identified themselves as from Māori descent, one belonged to the iwi Tuhoe, one belonged to both Waikato and Ngati Maniapoto and two did not know their tribal affiliations.

Children were on the same AED therapy for one to ten years with a median duration of treatment of 2.8 years. Children were divided into four treatment groups: VPA monotherapy (12); CBZ monotherapy (7); other AED monotherapy (7); and polytherapy (4). The other AEDs used were Clobazam (2), LTG (2), Levetiracetam (2) and Topiramate (1). Subjects in the polytherapy group received CBZ and Acetazolamide (1), Topiramate and Ethosuxamide (1), VPA and LTG (1) or VPA and CBZ (1).
Figure 2: Flow chart demonstrating recruitment and testing of patients with epilepsy in the Wellington region, New Zealand
**Table 8:** Epilepsy syndrome and AEDs of children with epilepsy

<table>
<thead>
<tr>
<th>Epilepsy Syndrome, sample size (%)</th>
<th>All Epilepsy Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Idiopathic generalised:</strong></td>
<td></td>
</tr>
<tr>
<td>Childhood absence epilepsy</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Epilepsy with tonic-clonic seizures only</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Gastaut’s benign circling epilepsy</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Juvenile myoclonic epilepsy</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Idiopathic generalised epilepsy not otherwise specified</td>
<td>1 (3)</td>
</tr>
<tr>
<td><strong>Idiopathic focal:</strong></td>
<td>8 (27)</td>
</tr>
<tr>
<td>Benign childhood epilepsy with centrottemporal spikes</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Early onset benign childhood occipital epilepsy</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Late onset childhood occipital epilepsy</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Familial temporal lobe epilepsy</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Idiopathic focal epilepsy not otherwise specified</td>
<td>2 (7)</td>
</tr>
<tr>
<td><strong>Cryptogenic focal:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 (10)</td>
</tr>
<tr>
<td><strong>Symptomatic focal:</strong></td>
<td>9 (30)</td>
</tr>
<tr>
<td><strong>Unclassified:</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (7)</td>
</tr>
<tr>
<td><strong>Age of diagnosis (years)</strong></td>
<td>8.0 (4.0 – 12.3)</td>
</tr>
<tr>
<td><strong>Duration of disease (years)</strong></td>
<td>4.8 (2.5 – 8.2)</td>
</tr>
<tr>
<td><strong>AED treatment, sample size (%)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Monotherapy:</strong></td>
<td>26 (87)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>7 (23)</td>
</tr>
<tr>
<td>Clobazam</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Sodium Valproate</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Topiramate</td>
<td>1 (3)</td>
</tr>
<tr>
<td><strong>Polytherapy</strong></td>
<td>4 (13)</td>
</tr>
<tr>
<td><strong>Length of treatment (years)</strong></td>
<td>2.8 (1.5 – 3.4)</td>
</tr>
</tbody>
</table>

* Mann Whitney U test, Median (25th – 75th percentiles)
### 4.1.2 Controls

Controls were healthy children with no current or previous history of medical problems as per methods. Seventeen percent (5) of controls were recruited from friends or family of epilepsy subjects, seventeen percent (5) recruited from friends or family of staff at the Paediatric department of Wellington Hospital and sixty-seven percent (20) of controls were from a previous study assessing vascular function in children with diabetes (Wiltshire et al 2002). The same methodology was used for recruitment in this and the previous research study. No statistical difference in gender, age, pubertal stage, weight, height, BMI, systolic blood pressure or diastolic blood pressure was evident between the New Zealand and Australian controls.

### 4.2 Biochemistry Results

Blood samples were collected from 30 subjects with epilepsy and 30 controls. tHcy was the only laboratory variable measured in one subject due to insufficient blood collection. Due to a laboratory error, PLP was unable to be measured in one other epilepsy subject.

#### 4.2.1 Glucose, Total Plasma Homocyst(e)ine and its Determinants

Tables 9 and 10 summarise glucose, tHcy, and it determinants. Glucose, PLP, serum folate and red blood cell folate demonstrated a normal distribution and were analysed using a student’s t-test (Table 9). tHcy and vitamin B12 were not normally distributed and analysed using a Mann-Whitney statistical test (Table 9). For comparison of separate treatment
groups, normally distributed data was analysed using an ANOVA and post-hoc Tukey test and non-normally distributed data analysed with a Kruskal–Wallis statistical test (Table 10).

Table 9: Glucose, tHcy and its determinants

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Epilepsy Patients</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)*</td>
<td>5.09 ± 0.37</td>
<td>4.87 ± 0.43</td>
<td>0.059</td>
</tr>
<tr>
<td>tHcy (µmol/L)**</td>
<td>6.20 (5.45 – 7.70)</td>
<td>6.50 (5.45 – 8.40)</td>
<td>0.883</td>
</tr>
<tr>
<td>Pyridoxal-5-Phosphate (nmol/L)*</td>
<td>96.13 ± 41.76</td>
<td>89.04 ± 48.97</td>
<td>0.717</td>
</tr>
<tr>
<td>Serum Folate (nmol/L)*</td>
<td>23.95 ± 8.47</td>
<td>25.62 ± 6.73</td>
<td>0.430</td>
</tr>
<tr>
<td>Red Cell Folate (nmol/L)*</td>
<td>609.92 ± 339.11</td>
<td>532.73 ± 179.81</td>
<td>0.314</td>
</tr>
<tr>
<td>Serum Vitamin B12 (pmol/L)**</td>
<td>378.0 (255.0 – 498.0)</td>
<td>459.0 (367.5 – 567.0)</td>
<td>0.019</td>
</tr>
</tbody>
</table>

* Data expressed as mean ± standard deviation, students t-test; ** Data expressed as median (25th – 75th percentile), Mann-Whitney test.

tHcy: Total plasma homocyst(e)ine.
<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>VPA Monotherapy</th>
<th>CBZ Monotherapy</th>
<th>Other AED Treatments</th>
<th>Polytherapy</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose (mmol/L)</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.09 ± 0.37</td>
<td>4.64 ± 0.29&lt;sup&gt;a,c&lt;/sup&gt;</td>
<td>5.17 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.07 ± 0.39</td>
<td>4.70 ± 0.52</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>tHcy (µmol/L)</strong>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.20 (5.45 - 7.70)</td>
<td>6.55 (5.30 – 8.65)</td>
<td>6.50 (4.70 – 9.00)</td>
<td>6.40 (5.60 – 8.95)</td>
<td>6.50 (6.03 – 10.20)</td>
<td>0.830</td>
</tr>
<tr>
<td><strong>Pyridoxal-5-Phosphate (nmol/L)</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>96.13 ± 41.76</td>
<td>108.11 ± 58.13</td>
<td>82.17 ± 43.42</td>
<td>93.60 ± 45.11</td>
<td>50.75 ± 18.46</td>
<td>0.356</td>
</tr>
<tr>
<td><strong>Serum Folate (nmol/L)</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.95 ± 8.47</td>
<td>25.37 ± 3.32</td>
<td>24.98 ± 6.83</td>
<td>27.12 ± 8.59</td>
<td>25.08 ± 12.67</td>
<td>0.924</td>
</tr>
<tr>
<td><strong>Red Cell Folate (nmol/L)</strong>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>609.92 ± 339.11</td>
<td>576.75 ± 144.10</td>
<td>583.00 ± 58.02</td>
<td>506.67 ± 242.11</td>
<td>196.00 ± 26.87</td>
<td>0.318</td>
</tr>
<tr>
<td><strong>Serum Vitamin B12 (pmol/L)</strong>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>378.00 (255.00 – 468.00)</td>
<td>500 (441.25 – 730.25)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>376.00 (350.00 – 459.00)</td>
<td>417.50 (312.75 – 497.50)</td>
<td>571.00 (291.50 – 754.50)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

* Data expressed as means ± standard deviation, ANOVA test; ** Data expressed as median (25<sup>th</sup> – 75<sup>th</sup> percentile), Kruska - Wallis test.

**AED**: Antiepileptic drug; **CBZ**: Carbamazepine; **tHcy**: Total plasma homocyst(e)ine; **VPA**: Sodium Valproate.

<sup>a</sup>- Significance versus controls, p < 0.05; <sup>b</sup>- Significance versus VPA, p < 0.05; <sup>c</sup>- Significance versus CBZ, p < 0.05; <sup>d</sup>- Significance versus other AED treatments, p < 0.05
Glucose

Lower glucose levels were observed in all patients with epilepsy (4.87 ± 0.43 mmol/L) compared with controls (5.09 ± 0.37 mmol/L), although statistical significance was not achieved (p = 0.059) (Table 9). There was a significant difference in glucose values between the treatment groups (Table 10). This difference reflected lower glucose values in subjects treated with VPA, as using a post-hoc statistical test to compare individual treatment groups, glucose concentrations were significantly reduced in subjects treated with VPA monotherapy (4.64 ± 0.29) compared with controls (5.09 ± 0.37; p = 0.014) and CBZ monotherapy treated subjects (5.17 ± 0.41; p = 0.035). No statistical differences, however, were observed between controls (5.09 ± 0.37) and CBZ monotherapy (5.17 ± 0.41; P= 0.984), other AED therapies (5.07 ± 0.39; P= 1.00) or polytherapy (4.70 ± 0.52; P= 0.329). No differences were also observed between CBZ monotherapy, other AEDs and polytherapy treatment groups (Table 10).

The polytherapy group contained two subjects who received VPA as part of their AED therapy, and it is noteworthy that this group also had lower glucose values than controls (although the difference was not significant, probably because there are only 4 subjects in this group). If the data is analysed comparing subjects who received valproate (either as monotherapy or combined with other AEDs), glucose was significantly lower in subjects receiving valproate (valproate 4.68 ± 0.34 mmol/L, controls 5.09 ± 0.37; p = 0.01). Box and whisker plots comparing glucose concentrations in controls and various AED treatment groups are demonstrated in Figure 3.
Figure 3: Box and whisker plots comparing glucose concentrations in various AED treatment groups and controls. For this and subsequent box and whisker plots, the horizontal line represents the median, the boundary of the box represents the 25th and 75th percentiles and the bars represent values within 1.5 times the interquartile range. Outliers are shown as white circles.
Total Plasma Homocyst(e)ine

There was no significant difference in total plasma homocyst(e)ine concentrations between children with epilepsy (6.50 µmol/L) and controls (6.20 µmol/L; p = 0.883). Analysis of the subgroups on various AED therapies also demonstrated no significant differences (p = 0.830). A tHcy level consistent with a diagnosis of hyperhomocysteinaemia was found in one subject with epilepsy (tHcy = 16 µmol/L) but no controls. Figure 4 demonstrates box and whisker plots comparing tHcy concentrations in controls and various AED treatment groups.

![Figure 4](image_url)

**Figure 4:** Box and whisker plots comparing tHcy concentrations in various AED treatment groups and controls
Pyridoxal-5-Phosphate

The mean pyridoxal-5-phosphate levels in children with epilepsy (89.04 ± 48.97) were statistically no different than the mean value found in healthy controls (96.13 ± 41.76; p = 0.717). Using ANOVA, there was no statistical difference between treatment groups (p = 0.356). Figure 5 demonstrates box and whisker plots comparing PLP concentrations in controls and various AED treatment groups.

Figure 5: Box and whisker plots comparing PLP concentrations in various AED treatment groups and controls
Serum and red blood cell folate

The average serum folate concentration in children with epilepsy (25.62 ± 6.73 nmol/L) was not statistically different from controls (23.95 ± 8.47 nmol/L; p = 0.430). There was also no difference between therapeutic subgroups (p = 0.924). Figure 6 demonstrates box and whisker plots comparing serum folate concentrations in controls and various AED treatment groups.

Figure 6: Box and whisker plots comparing serum folate concentrations in various AED treatment groups and controls
Red blood cell folate in epilepsy subjects (532.73 ± 179.81 nmol/L) similarly was statistically no different from controls (609.92 ± 339.11 nmol/L; p = 0.314). Individual analysis of AED treatment groups additionally demonstrated no statistical differences (p = 0.318). Box and whisker plots comparing red blood cell folate concentrations in controls and various AED treatment groups are demonstrated in Figure 7.

**Figure 7:** Box and whisker plots comparing red blood cell folate concentrations in various AED treatment groups and controls
Vitamin B12

The median concentration of vitamin B12 in epilepsy patients (459.0 pmol/L) was significantly elevated compared with healthy controls (378.0 pmol/L; p = 0.019). This elevation was specifically observed in epilepsy subjects treated with VPA monotherapy who had a median vitamin B12 concentration of 500 pmol/L compared with 378.0 pmol/L observed in controls (p = 0.006). Compared to controls, however, no differences were evident with CBZ monotherapy (376.00 pmol/L; p = 1.00), other AEDs (417.50 pmol/L; p = 0.993) and polytherapy (571.00 pmol/L; p = 0.418). No statistical differences were also observed between VPA monotherapy, CBZ monotherapy, other AEDs and polytherapy treatments. The 2 subjects receiving VPA as part of polytherapy had vitamin B12 values equivalent to those receiving VPA monotherapy (404 and 738 pmol/L), further suggesting VPA is responsible for the difference. Figure 8 demonstrates box and whisker plots comparing vitamin B12 concentrations in controls and various AED treatment groups.
Figure 8: Box and whisker plots comparing vitamin B12 concentrations in various AED treatment groups and controls
4.2.2 Lipids and Lipoproteins

Lipid and lipoprotein levels in epilepsy patients and controls are seen in Tables 11 and 12. Total cholesterol, HDL cholesterol, LDL cholesterol, Apolipoprotein A1, Apolipoprotein B and Apolipoprotein B/ Apolipoprotein A1 was normally distributed and analysed using a student’s t-test. Total cholesterol/ HDL cholesterol and free triglycerides were not normally distributed and analysed using a Mann-Whitney statistical test (Table 11). For comparison of AED treatment groups, normally distributed data was analysed using an ANOVA and post-hoc Tukey test while non-normally distributed data analysed using the Kruskal-Wallis statistical test (Table 12).

Table 11: Lipid and lipoprotein levels in epilepsy patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 30)</th>
<th>Epilepsy patients (N = 29)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cholesterol (mmol/L)</strong></td>
<td>3.92 ± 0.73</td>
<td>4.16 ± 1.14</td>
<td>0.365</td>
</tr>
<tr>
<td><strong>HDL Cholesterol (mmol/L)</strong></td>
<td>1.44 ± 0.30</td>
<td>1.28 ± 0.35</td>
<td>0.079</td>
</tr>
<tr>
<td><strong>Total Cholesterol/ HDL Cholesterol</strong></td>
<td>2.54 (2.29 – 3.42)</td>
<td>3.10 (2.70 – 4.03)</td>
<td>0.144</td>
</tr>
<tr>
<td><strong>LDL Cholesterol (mmol/L)</strong></td>
<td>2.20 ± 0.65</td>
<td>2.42 ± 0.90</td>
<td>0.300</td>
</tr>
<tr>
<td><strong>Free Triglycerides (mmol/L)</strong></td>
<td>0.69 (0.38 – 0.91)</td>
<td>0.75 (0.66 - 0.98)</td>
<td>0.068</td>
</tr>
<tr>
<td><strong>Apolipoprotein A1 (g/L)</strong></td>
<td>1.34 ± 0.23</td>
<td>1.36 ± 0.22</td>
<td>0.748</td>
</tr>
<tr>
<td><strong>Apolipoprotein B (g/L)</strong></td>
<td>0.66 ± 0.17</td>
<td>0.74 ± 0.25</td>
<td>0.226</td>
</tr>
<tr>
<td><strong>Apolipoprotein B/ Apolipoprotein A1</strong></td>
<td>0.50 ± 0.16</td>
<td>0.55 ± 0.18</td>
<td>0.319</td>
</tr>
</tbody>
</table>

* Data expressed as mean ± standard deviation, students t-test;
** Data expressed as median (25th – 75th percentiles), Mann-Whitney test.

**HDL Cholesterol**: High-density lipoprotein cholesterol; **LDL Cholesterol**: Low-density lipoprotein cholesterol.
Table 12: Lipid and lipoprotein levels in children with epilepsy in individual AED treatment groups and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 30)</th>
<th>VPA monotherapy (N = 12)</th>
<th>CBZ monotherapy (N = 7)</th>
<th>Other AED treatments (N = 6)</th>
<th>Polytherapy (N = 4)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Cholesterol (mmol/L)</strong></td>
<td>3.92 ± 0.73</td>
<td>4.01 ± 0.78</td>
<td>5.16 ± 1.40&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;d&lt;/sup&gt;&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.68 ± 1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.58 ± 0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.015</td>
</tr>
<tr>
<td><strong>HDL Cholesterol (mmol/L)</strong></td>
<td>1.44 ± 0.30</td>
<td>1.28 ± 0.29</td>
<td>1.36 ± 0.41</td>
<td>1.24 ± 0.36</td>
<td>1.19 ± 0.51</td>
<td>0.437</td>
</tr>
<tr>
<td><strong>Total Cholesterol/ HDL Cholesterol</strong>**</td>
<td>2.54 (2.29 – 3.42)</td>
<td>3.10 (2.60 – 4.40)</td>
<td>3.30 (0.77 – 4.70)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.95 (2.75 – 3.28)</td>
<td>2.85 (2.45 – 4.90)</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>LDL Cholesterol (mmol/L)</strong></td>
<td>2.20 ± 0.65</td>
<td>2.24 ± 0.75</td>
<td>3.00 ± 1.25</td>
<td>2.32 ± 0.76</td>
<td>2.05 ± 0.37</td>
<td>0.168</td>
</tr>
<tr>
<td><strong>Free Triglycerides (mmol/L)</strong>**</td>
<td>0.69 (0.38 – 0.91)</td>
<td>0.70 (0.67 – 1.33)</td>
<td>0.93 (0.77 – 1.53)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64 (0.40 – 0.74)</td>
<td>0.78 (0.61 – 0.85)</td>
<td>0.083</td>
</tr>
<tr>
<td><strong>Apolipoprotein A1 (g/L)</strong></td>
<td>1.34 ± 0.23</td>
<td>1.36 ± 0.14</td>
<td>1.53 ± 0.25</td>
<td>1.26 ± 0.25</td>
<td>1.23 ± 0.26</td>
<td>0.192</td>
</tr>
<tr>
<td><strong>Apolipoprotein B (g/L)</strong></td>
<td>0.66 ± 0.17</td>
<td>0.68 ± 0.21</td>
<td>0.93 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.69 ± 0.19</td>
<td>0.67 ± 0.15</td>
<td>0.087</td>
</tr>
<tr>
<td><strong>Apolipoprotein B/ Apolipoprotein A1</strong></td>
<td>0.50 ± 0.16</td>
<td>0.51 ± 0.17</td>
<td>0.61 ± 0.22</td>
<td>0.54 ± 0.09</td>
<td>0.57 ± 0.23</td>
<td>0.635</td>
</tr>
</tbody>
</table>

* Data expressed as means ± standard deviation, ANOVA test; ** Data expressed as median (25<sup>th</sup> – 75<sup>th</sup> percentile), Kruskal - Wallis test.

** HDL Cholesterol**: High-density lipoprotein cholesterol; ** LDL Cholesterol**: Low-density lipoprotein cholesterol;

** AED**: Antiepileptic drug; ** CBZ**: Carbamazepine; ** VPA**: Sodium Valproate.

<sup>a</sup>- Significance versus controls, p < 0.05;  
<sup>b</sup>- Significance versus VPA, p < 0.05;  
<sup>c</sup>- Significance versus CBZ, p < 0.05

<sup>d</sup>- Significance versus other AED treatments, p < 0.05;  
<sup>e</sup>- Significance versus polytherapy, p < 0.05
Total cholesterol in children with epilepsy (4.16 ± 1.14 mmol/L) was not statistically different from controls (3.92 ± 0.73 mmol/L; p = 0.365). Total cholesterol was significantly higher in children on CBZ monotherapy compared to controls (p = 0.016), other AEDs (p= 0.034) and polytherapy (p = 0.048). No other statistical differences were found in other treatment subgroups (Table 12). Box and whisker plots comparing total cholesterol concentrations in controls and various AED treatment groups are demonstrated in Figure 9.
HDL cholesterol levels in children with epilepsy (1.28 ± 0.35 mmol/L) were reduced compared to control children (1.44 ± 0.30 mmol/L) although statistical significance was not reached (p = 0.079). There was no statistically significant difference in HDL cholesterol levels in controls compared with VPA monotherapy (p = 0.677), CBZ monotherapy (p = 0.982), other AEDs (p = 0.683) or polytherapy treatments (p = 0.653). Figure 10 demonstrates box and whisker plots of HDL cholesterol concentrations in various treatment groups and controls.

**Figure 10:** Box and whisker plots comparing HDL cholesterol concentrations in various AED treatment groups and controls
The median ratio of total cholesterol to HDL cholesterol in children with epilepsy (3.10) was not different than controls (2.54; \( p = 0.144 \)). Analysis of individual treatment groups, however, found a significantly elevated total cholesterol/ HDL cholesterol in epilepsy subjects treated with CBZ monotherapy (3.30) compared to controls (2.54; \( p = 0.024 \)). No statistical differences were observed between controls and the other treatment groups (Figure 11).

**Figure 11:** Box and whisker plots comparing total cholesterol/ HDL cholesterol in various AED treatment groups and controls
LDL cholesterol levels in children with epilepsy (2.42 ± 0.90 mmol/L) were statistically no different than controls (2.20 ± 0.65 mmol/L; p = 0.300). There were no significant differences in LDL concentrations between the therapeutic subgroups (p = 0.168). Box and whisker plots comparing LDL cholesterol concentrations in controls and various AED treatment groups are demonstrated in Figure 12.

**Figure 12:** Box and whisker plots comparing LDL cholesterol concentrations in various AED treatment groups and controls
Free Triglycerides

The median free triglyceride levels in all subjects with epilepsy (0.75 mmol/L) were not different from those observed in controls (0.69 mmol/L; p = 0.068). Analysis of individual treatment groups however demonstrated statistically significantly elevated free triglyceride levels in epilepsy patients treated with CBZ monotherapy (0.93 mmol/L) compared with controls (0.69 mmol/L; p = 0.028). There were no other statistical differences in the analyses of the other AED subgroups. Figure 13 demonstrates box and whisker plots of free triglyceride concentrations in various AED treatment groups and controls.

Figure 13: Box and whisker plots comparing free triglyceride concentrations in various AED treatment groups and controls
Apolipoprotein A1 and Apolipoprotein B

Apolipoprotein A1 levels in children with epilepsy (1.36 ± 0.22 g/L) were statistically comparable to levels observed in controls (1.34 ± 0.23 g/L; p = 0.748) and there were, no differences between the AED subgroups (p = 0.192). Box and whisker plots of apolipoprotein A1 concentrations in various AED treatment groups and controls are shown in Figure 14.

![Box and whisker plots](image.png)

**Figure 14:** Box and whisker plots comparing apolipoprotein A1 concentrations in various AED treatment groups and controls
Similarly, mean apolipoprotein B concentration in all children with epilepsy (0.74 ± 0.25 g/L) were not different from controls (0.66 ± 0.17 g/L, p=0.226). Analysis of individual AED treatment groups, however, demonstrated elevated apolipoprotein B in subjects treated with CBZ monotherapy (0.93 ± 0.34 g/L) compared with controls (0.66 ± 0.17 g/L; p = 0.044). No differences in Apolipoprotein B levels were observed between any of the other AED subgroups. Figure 15 demonstrates box and whisker plots of apolipoprotein B concentrations in various AED treatment groups and controls.

Figure 15: Box and whisker plots comparing apolipoprotein B concentrations in various AED treatment groups and controls
The ratio of apolipoprotein B/apolipoprotein A1 demonstrated no statistical differences between children with epilepsy (0.55 ± 0.18) and controls (0.50 ± 0.16; p = 0.319). Furthermore, no differences were apparent between controls, VPA monotherapy, CBZ monotherapy, other AEDs and polytherapy treatment (p = 0.635; Figure 16).

**Figure 16:** Box and whisker plots comparing apolipoprotein B/apolipoprotein A1 in various AED treatment groups and controls
4.3 Ultrasound Investigation of Vascular Function and Structure

4.3.1 Flow-Mediated Dilation

Flow-mediated dilation was measured in thirty subjects with epilepsy and thirty matched controls. Two epilepsy subjects and two controls were excluded from FMD analysis due to baseline scans (scans one and three) differing by more than three percent, as explained in the chapter three. Examples of ultrasound scans in a FMD study are demonstrated in Figures 17 to 22. In the first four figures, the brachial artery is shown with a measurement at each of the four stages of the study. The first scan shows a resting arterial diameter of 0.30 cm (Figure 17). This is the same diameter as measured in the re-control scan (Figure 19). The second and fourth scans demonstrate a 7% dilatation after reactive hyperaemia (from 0.30 to 0.32) and a 23% dilatation in response to GTN (from 0.30 to 0.37). The figures also demonstrate that each measurement was made at the same point of the artery using ultrasonic markers. In this study, the fascial interface and the upper and lower echodense lines were used as ultrasonic markers. Doppler tracings are demonstrated in Figures 21 and 22. The change in flow rate is calculated by the ratio of the area of the Doppler trace (velocity-time integral) multiplied by the vessel area ($\pi r^2$) and the heart rate for the baseline and reactive hyperaemia scans. For example, in the scans shown reactive hyperaemia is 973% ($100 \times ((0.32 \times 62)/(0.04 \times 51))$ which is the Doppler area times the heart rate after cuff deflation divided by the Doppler area times the heart rate at rest. This is then multiplied by 100).
Figure 17: Baseline (resting) scan of the brachial artery. The ultrasound image was recorded on a high quality DVD. The brachial artery is shown in the centre of the figure with echodense (white) walls and an echolucent (black) centre as it contains blood. The distance in centimetres was measured by electronic callipers (seen as two crosses at the vessel edges with connecting dots) from the proximal “m” line to the distal “m” line. The measured distance is shown in the bottom left corner (0.30cm). The thin vertical line on the right of the image is the calibration line for measurement. The measurement is made concurrently with the ECG R wave (shown at the bottom of the screen). The heart rate is shown at the end of the ECG strip (51 beats per minute). Ultrasonic markers are used to ensure that measurements are made at the same place in each scan. The markers used in this FMD study were the fascial interface and upper and lower echodense lines (shown by arrows).
Figure 18: Ultrasound scan of the brachial artery after reactive hyperaemia. The measurement was taken concurrently with the ECG R wave (shown at the bottom of the figure). The vessel diameter is measured using electronic callipers and ultrasonic markers (fascial interface and upper and lower echodense lines) are used to ensure that each measurement is in the same site of the artery. The vessel diameter has increased from 0.30cm to 0.32 cm indicating a 7% increase. This is a normal endothelial response to reactive hyperaemia.
Figure 19: Re-control scan of the brachial artery. This scan was taken 15 minutes after cuff deflation. The measurement was taken concurrently with the ECG R wave (shown at the bottom of the figure). The vessel diameter is measured using electronic callipers and ultrasonic markers (fascial interface and upper and lower echodense lines) are used to ensure that each measurement is in the same site of the artery. The vessel diameter has returned to the same diameter as in the baseline scan, 0.30cm.
Figure 20: Ultrasound scan of the brachial artery after GTN. The measurement was taken concurrently with the ECG R wave (shown at the bottom of the figure). The vessel diameter is measured using electronic callipers and ultrasonic markers (fascial interface and upper and lower echodense lines) are used to ensure that each measurement is in the same site of the artery. The vessel diameter has increased from 0.30 cm to 0.37 cm indicating a 23% increase. This is a normal endothelial response to GTN.
**Figure 21:** Baseline Doppler scan. The brachial artery is shown in the top left corner. The area of one peak is measured by tracing around it (shown as a thin white line surrounding the middle peak). The measured area is shown in the bottom left corner (0.04cm²). The area of the Doppler trace is equivalent to blood flow.
Figure 22: Doppler scan during cuff deflation. The brachial artery is shown in the top left corner. The increase in blood flow is shown by the smaller peak on the left (systole prior to cuff deflation), followed by larger peaks after cuff deflation. The area of one peak is measured by tracing around it (shown as a thin white line surrounding the second large peak). The measured area is shown in the bottom left corner (0.32cm²). The area of the Doppler trace is equivalent to blood flow. The change in flow is then calculated by the ratio of the Doppler area multiplied by the vessel area and the heart rate for the baseline and reactive hyperaemia scans. The change in flow for this study was 973%.
The average percentage increase of the brachial artery diameter in both FMD and GTN-induced dilatation was not different between epilepsy and control groups (Table 13). Individual analysis of various AED therapies additionally showed no statistically significant differences between controls, VPA monotherapy, CBZ monotherapy, other AEDs or polytherapy in either FMD (p = 0.893) or GTN-induced dilatation (p = 0.399) (Table 14). The increase in blood flow to induce NO release, measured by percentage flow rate, was additionally comparable between the two groups. Figures 23 and 24 demonstrate box and whisker plots comparing FMD and GNT-induced dilations in various AED treatment groups and controls.

**Table 13:** Percentage increase of the brachial artery diameter in children with epilepsy and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 28)</th>
<th>All epilepsy patients (N = 28)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow-mediated Dilation(%)</td>
<td>8.20 ± 4.3%</td>
<td>7.57 ± 5.0%</td>
<td>0.605</td>
</tr>
<tr>
<td>GTN-induced Dilatation(%)</td>
<td>23.48 ± 7.0%</td>
<td>25.14 ± 8.4%</td>
<td>0.419</td>
</tr>
<tr>
<td>% Flow Change</td>
<td>628.3 ± 225%</td>
<td>716.6 ± 342 %</td>
<td>0.473</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation, students t-test. GTN: Glyceryl trinitrate.
Table 14: Percentage increase of the brachial artery diameter in children with epilepsy in individual AED treatment groups and controls

<table>
<thead>
<tr>
<th></th>
<th>Control (N = 28)</th>
<th>VPA monotherapy (N = 12)</th>
<th>CBZ monotherapy (N= 7)</th>
<th>Other AED treatments (N = 6)</th>
<th>Polytherapy (N = 3)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow-mediated Dilation (%)</td>
<td>8.20 ± 4.3%</td>
<td>6.58 ± 5.0%</td>
<td>7.17 ± 7.2%</td>
<td>7.68 ± 2.4%</td>
<td>7.70 ± 2.2%</td>
<td>0.893</td>
</tr>
<tr>
<td>GTN-induced Dilation (%)</td>
<td>23.48 ± 7.0%</td>
<td>22.49 ± 9.8%</td>
<td>29.38 ± 8.5%</td>
<td>25.38 ± 6.8%</td>
<td>22.80 ± 6.4%</td>
<td>0.399</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation, students t-test.

GTN: Glyceryl trinitrate.
Figure 23: Box and whisker plots comparing FMD in various AED treatment groups and controls
Figure 24: Box and whisker plots comparing GTN-induced dilatation in various AED treatment groups and controls
4.3.2 Carotid Artery and Aortic Intima-Media Thickness

Carotid and aortic IMT images were available in the New Zealand controls only (10) and due to a technical error, carotid and aortic IMT images were unable to be obtained in one child with epilepsy. The measurement of Aortic Intima-Media Thickness (aIMT) scans was difficult, due to the discomfort in testing and we were therefore only able to obtain good quality images from 8 of 10 controls and 13 of the 29 children with epilepsy. The aIMT results need to be interpreted with caution as the quality was less than ideal (judged by the number of pixel points used for the measurements). Examples of carotid and aortic IMT scans are demonstrated in Figures 25 and 26.
Figure 25: Ultrasound scan of the left CCA. The ultrasound image was recorded on a high quality CD. The carotid artery is shown in the centre of the figure with echodense (white) walls and an echolucent (black) centre as it contains blood. The carotid bulb is seen on the left. The mean, maximum and minimum distances between the lumen-intima and media-adventitia interface (IMT) is measured using automatic edge detection in a 1cm area of the posterior (far) wall. The intima and media of the vessel wall is demonstrated by arrows. Each measurement is made concurrently with the ECG R wave (shown at the bottom of the screen next to the heart rate). Ultrasound images were also taken in the right carotid artery then an average of the right and left common carotid arteries were taken.
Figure 26: Ultrasound scan of the abdominal aorta. The aorta is shown in the centre of the figure with echodense (white) walls and a echolucent (black) centre as it contains blood. The aortic bifurcation is to the right of the image (not shown in the figure). The mean, maximum and minimum distances between the lumen-intima and media-adventitia interface (IMT) is measured using automatic edge detection in a 1 cm area of the posterior (far) wall. The intima and media of the vessel wall is demonstrated by arrows. Each measurement is made concurrently with the ECG R wave (shown at the bottom of the screen next to the heart rate).
Carotid and aortic IMT in epilepsy subjects and controls are summarised in Table 15. Tables 16 and 17 summarise carotid and aortic IMT in individual AED treatment groups and controls. The mean value is the average diameter over a 1 cm section. The max value is the maximum diameter measured.

Table 15: Carotid and aortic IMT

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Epilepsy patients</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cIMT (mm)</td>
<td>0.495 ± 0.04</td>
<td>0.474 ± 0.039</td>
<td>0.152</td>
</tr>
<tr>
<td>Max cIMT (mm)</td>
<td>0.597 ± 0.047</td>
<td>0.576 ± 0.049</td>
<td>0.231</td>
</tr>
<tr>
<td>Mean aIMT (mm)</td>
<td>0.544 ± 0.036</td>
<td>0.563 ± 0.065</td>
<td>0.437</td>
</tr>
<tr>
<td>Max aIMT (mm)</td>
<td>0.696 ± 0.036</td>
<td>0.708 ± 0.084</td>
<td>0.695</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation, students t-test
Table 16: Carotid IMT in children with epilepsy in individual AED treatment groups and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 10)</th>
<th>VPA monotherapy (N = 11)</th>
<th>CBZ monotherapy (N = 7)</th>
<th>Other AED treatments (N = 7)</th>
<th>Polytherapy (N = 4)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cIMT (mm)</td>
<td>0.495 ± 0.045</td>
<td>0.477 ± 0.033</td>
<td>0.466 ± 0.039</td>
<td>0.431 ± 0.051</td>
<td>0.471 ± 0.042</td>
<td>0.664</td>
</tr>
<tr>
<td>Max cIMT (mm)</td>
<td>0.597 ± 0.047</td>
<td>0.584 ± 0.053</td>
<td>0.56 ± 0.042</td>
<td>0.574 ± 0.06</td>
<td>0.580 ± 0.42</td>
<td>0.658</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation, ANOVA test
The mean and max IMT diameters of the common carotid arteries were not different between epilepsy and control groups (Table 15). After analysis of treatment groups, no statistically significant differences were found between controls, VPA monotherapy, CBZ monotherapy, other AEDs and polytherapy in both mean \( (p = 0.664) \) and max \( (p = 0.658) \) cIMT (Table 16). Box and whisker plots comparing mean cIMT in controls and various AED treatment groups are demonstrated in Figure 27.

**Figure 27:** Box and whisker plots comparing mean cIMT measurements in various AED treatment groups and controls
Table 17: Aortic IMT in children with epilepsy in individual AED treatment groups and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (N =8)</th>
<th>VPA monotherapy (N =6)</th>
<th>CBZ monotherapy (N =3)</th>
<th>Other AED treatments (N =3)</th>
<th>Polytherapy (N =1)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean aIMT (mm)</td>
<td>0.544 ± 0.036</td>
<td>0.563 ± 0.055</td>
<td>0.581 ± 0.093</td>
<td>0.579 ± 0.066</td>
<td>0.47</td>
<td>0.461</td>
</tr>
<tr>
<td>Max aIMT (mm)</td>
<td>0.696 ± 0.036</td>
<td>0.721 ± 0.076</td>
<td>0.686 ± 0.056</td>
<td>0.741 ± 0.126</td>
<td>0.6</td>
<td>0.446</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation, ANOVA test

* Result in single subject
The mean and max IMT diameters of the aortic arteries similarly were not different between the epilepsy and control groups. Individual analysis of AED treatment groups additionally demonstrated no statistical difference in mean \((p = 0.461)\) and max \((p = 0.446)\) aIMT (Table 17). Box and whisker plots comparing mean aIMT in controls and various AED treatment groups are demonstrated in Figure 28.

**Figure 28:** Box and whisker plots comparing mean aIMT measurements in various AED treatment groups and controls
4.4 Correlations

Correlations observed between different primary outcome variables in all children in this study are summarised in Table 18. FMD, GTN, cIMT, aIMT and Glucose were analysed using Pearson correlation coefficient. tHcy was analysed using Spearman’s rank correlation coefficient.

4.4.1 Biochemistry Correlations

tHcy positively correlated with systolic blood pressure (r = 0.32; p = 0.02) and negatively correlated with serum folate levels (r = 0.44; p = 0.001). Glucose levels positively correlated with red blood cell folate (r = 0.41; p = 0.005). Negative correlations between glucose and serum vitamin B12 was also observed in all subjects (r = -0.29; p = 0.04). No other statistically significant correlations were observed in glucose, tHcy and its determinants or lipids and lipoproteins.

4.4.2 Ultrasound Correlations

GTN-induced dilation correlated with FMD as expected (r = 0.32, p = 0.002). Positive correlations were demonstrated in GTN with total cholesterol (r = 0.28; p = 0.05) and LDL cholesterol (r = 0.35; p = 0.01) in all children. GTN demonstrated a negative correlation with systolic blood pressure (r = -0.29; p = 0.04). No other statistically significant correlations with FMD, GTN or cIMT were observed in this study. Mean cIMT did not statistically correlate to any other variable.
Although, as noted in the vascular structure and function section above, the aIMT results need to be interpreted with caution, mean aIMT showed significant correlations with age \((r = 0.75, \ p < 0.001)\), weight \((r = 0.75, \ p < 0.001)\), BMI \((r = 0.54, \ p = 0.01)\), HDL cholesterol \((r = -0.58, \ p = 0.006)\), total cholesterol/ HDL cholesterol \((r = 0.48, \ p = 0.03)\) and red blood cell folate \((r = 0.65, \ p = 0.003)\).
**Table 18:** Correlations between variables in all subjects

<table>
<thead>
<tr>
<th></th>
<th>FMD</th>
<th>GTN</th>
<th>Mean cIMT</th>
<th>Glucose</th>
<th>tHcy**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>FMD</td>
<td>-</td>
<td>-</td>
<td>0.32 0.002</td>
<td>0.03 0.88</td>
<td>-0.04 0.78</td>
</tr>
<tr>
<td>GTN</td>
<td>0.32 0.002</td>
<td>-</td>
<td>-14 0.41</td>
<td>-0.10 0.49</td>
<td>-0.19 0.08</td>
</tr>
<tr>
<td>Mean cIMT</td>
<td>0.03 0.88</td>
<td>-14 0.41</td>
<td>-</td>
<td>0.035 0.84</td>
<td>0.05 0.76</td>
</tr>
<tr>
<td>Glucose</td>
<td>-0.04 0.78</td>
<td>-0.10 0.49</td>
<td>0.035 0.84</td>
<td>-</td>
<td>0.21 0.14</td>
</tr>
<tr>
<td>tHcy</td>
<td>0.07 0.59</td>
<td>-19 0.17</td>
<td>0.19 0.24</td>
<td>0.22 0.13</td>
<td>-</td>
</tr>
<tr>
<td>Pyridoxal-5-Phosphate</td>
<td>0.21 0.26</td>
<td>-0.05 0.78</td>
<td>-0.04 0.84</td>
<td>0.15 0.42</td>
<td>-0.22 0.24</td>
</tr>
<tr>
<td>Serum Folate</td>
<td>-0.18 0.21</td>
<td>0.12 0.42</td>
<td>-0.17 0.31</td>
<td>-0.16 0.26</td>
<td>-0.437 0.001</td>
</tr>
<tr>
<td>Red Cell Folate</td>
<td>-0.13 0.38</td>
<td>-0.07 0.65</td>
<td>-0.22 0.21</td>
<td>0.41 0.005</td>
<td>-0.15 0.30</td>
</tr>
<tr>
<td>Serum Vitamin B12</td>
<td>0.01 0.97</td>
<td>0.002 0.99</td>
<td>0.01 0.94</td>
<td>-0.29 0.04</td>
<td>-0.16 0.25</td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>0.20 0.16</td>
<td>0.28 0.05</td>
<td>-0.16 0.34</td>
<td>-0.03 0.81</td>
<td>-0.18 0.21</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>0.03 0.82</td>
<td>0.17 0.24</td>
<td>0.06 0.72</td>
<td>-0.17 0.25</td>
<td>-0.13 0.37</td>
</tr>
<tr>
<td>Total Cholesterol: HDL</td>
<td>0.12 0.41</td>
<td>-0.01 0.96</td>
<td>-0.11 0.50</td>
<td>0.12 0.42</td>
<td>0.16 0.25</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.19 0.19</td>
<td>0.35 0.01</td>
<td>-0.29 0.08</td>
<td>-0.01 0.94</td>
<td>-0.04 0.78</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>0.08 0.56</td>
<td>-0.09 0.54</td>
<td>-0.02 0.92</td>
<td>0.07 0.62</td>
<td>0.12 0.38</td>
</tr>
<tr>
<td>Free Triglycerides</td>
<td>0.00 1.00</td>
<td>0.23 0.11</td>
<td>0.06 0.75</td>
<td>-0.22 0.14</td>
<td>-0.231 0.1</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>0.20 0.16</td>
<td>0.23 0.12</td>
<td>-0.24 0.17</td>
<td>0.01 0.95</td>
<td>-0.12 0.39</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>0.19 0.27</td>
<td>0.05 0.75</td>
<td>-0.24 0.152</td>
<td>0.11 0.48</td>
<td>0.038 0.79</td>
</tr>
<tr>
<td>Apolipoprotein B: A1</td>
<td>-0.14 0.23</td>
<td>-0.24 0.08</td>
<td>-0.06 0.71</td>
<td>0.16 0.27</td>
<td>0.25 0.07</td>
</tr>
<tr>
<td>Age</td>
<td>0.24 0.08</td>
<td>-0.07 0.65</td>
<td>-0.12 0.34</td>
<td>0.24 0.10</td>
<td>0.17 0.22</td>
</tr>
<tr>
<td>BMI</td>
<td>0.17 0.25</td>
<td>-0.29 0.04</td>
<td>-0.24 0.16</td>
<td>0.28 0.06</td>
<td>0.32 0.02</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-0.10 0.47</td>
<td>-0.05 0.71</td>
<td>0.10 0.57</td>
<td>-0.13 0.38</td>
<td>-0.04 0.80</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-0.04 0.78</td>
<td>-0.14 0.41</td>
<td>-0.03 0.88</td>
<td>-0.04 0.78</td>
<td>-0.08 0.49</td>
</tr>
</tbody>
</table>

cIMT: Carotid intima-media thickness; FMD: Flow-mediated dilation; GTN: Glyceryl trinitrate; tHcy: Total plasma homocyst(e)ine.
Chapter Five: Discussion

5.1 Introduction

The risk of cardiovascular and cerebrovascular disease in patients with epilepsy has been reported to be up to five times more than that of the general populations (Hauser, Annegers et al. 1980; Cockerell, Johnson et al. 1994; Nilsson, Tomson et al. 1997). It has been postulated that elevated tHcy and lipid plasma concentrations caused by AED therapies may contribute to this increased incidence of vascular disease (Isojarvi, Pakarinen et al. 1993; Schwaninger, Ringleb et al. 1999; Verrotti, Pascarella et al. 2000; Apeland, Mansoor et al. 2001; Bramswig, Kerksiek et al. 2002; Apeland, Mansoor et al. 2003; Attilakos, Papakonstantinou et al. 2006; Elliott, Jacobson et al. 2007; Hamed, Hamed et al. 2007; Tomoum, Awadallah et al. 2008). As early vascular disease is potentially reversible it is important to understand the contributors and risk factors in childhood to prevent its development later in life (Celermajer, Sorensen et al. 1992; Widlansky, Gokce et al. 2003). This study investigates the effects of AEDs on atherosclerotic biochemical risk factors and vascular structure in children with epilepsy and also uniquely investigates endothelial and smooth muscle vascular function.

The major findings in this study were:

1. Total plasma homocyst(e)ine, folate and pyridoxal-5-phosphate concentrations were no different between children with epilepsy and controls.

2. Vitamin B12 levels were raised in children with epilepsy compared to controls. This finding was primarily due to the effect of VPA and not seen in the other AED therapies.
3. Fasting glucose levels were lower in children with epilepsy than the control group, although only marginal significance at the 5% level was achieved. After analysis of individual AED treatments, it appears this was also primarily due to the effect of VPA.

4. Lipid and lipoprotein concentrations in children with epilepsy were statistically comparable to controls. Comparisons of individual AED treatments, demonstrated significantly elevated total cholesterol, total cholesterol/ HDL cholesterol ratio, free triglycerides and lipoprotein B concentrations in children treated with CBZ monotherapy compared to controls. Furthermore, total cholesterol levels in CBZ treated children were statistically elevated compared to VPA monotherapy.

5. Vascular function and structure, via FMD and IMT, was not different between children with epilepsy and controls.

Significant correlations observed in this study included positive correlations between tHcy and systolic blood pressure, glucose with red blood cell folate and GTN-induced dilatation with total cholesterol and LDL cholesterol. Negative correlations were found between glucose and vitamin B12, tHcy and serum folate and GTN-induced dilatation with systolic blood pressure. aIMT showed significant correlations with age, weight, BMI, HDL cholesterol and red blood cell folate as expected, although the quality of the images restricts interpretation of these significant correlations. These expected correlations between known cardiovascular risk factors and early atherosclerotic development (which commences first in the aorta (Berenson, Srinivasan et al. 1998) suggest our methodology is robust. A significant correlation between FMD and GTN-induced dilatation, in this study, further validates the methodology.
5.2 Strengths and Weaknesses

A prospective case-control approach was used in this study to test the association between AED therapy and cardiovascular disease in children and adolescents. Case-control studies provide a quick and economical method of looking at associations with diseases. They are particularly useful in the assessment of diseases with many risk factors and long latent periods, such as cardiovascular disease. A weakness of case-control studies, however, is that they are more susceptible to bias and misguided inference (Rothman and Greenland 1998 p.114). Evaluation of the strengths and weaknesses of case-control studies can be divided into three components: precision, internal validity and external validity (generalizability).

5.2.1 Precision

Rothman and Greenland describe study precision as a lack of random error influenced by sample size and study efficiency (Rothman and Greenland 1998 p.116). Power calculations for FMD measurements were based on clinically significant differences found in previous case control studies of children with diabetes and obesity. A population size of thirty children in each group (epilepsy and controls) was calculated to have 80% power at the 5% level of significance to detect a difference in FMD of 2.9 ± 4% between children with epilepsy and controls. The differences in FMD and IMT between controls and children with epilepsy in this study, however, were less than previously seen in obesity and diabetes. A larger study population would therefore be required to produce a statistically significant result based on the value this study detected. We presumed based on past literature, that tHcy and its vitamin cofactor levels would be abnormal in our
children with epilepsy compared to controls. The difference in tHcy concentrations between children with epilepsy and controls, however, was also less than previous literature (as discussed below). Retrospective power calculations determined 233 children in each group would have needed to be recruited for a difference of 0.63 µmol/L at the 5% significance level. Even so, a significant difference of 0.63 µmol/L would not be clinically significant as a rise in tHcy of this amount would not substantially increase cardiovascular risk.

A possible reason for our finding of no statistical difference between controls and children with epilepsy was that we looked at children with epilepsy regardless of AED therapy. Therefore our children were on many different types of AEDs, some of which have questionable effects on tHcy, its vitamin cofactors and lipid levels (i.e. VPA and LTG). The size of the individual treatment groups however was not large enough to give sufficient statistical power to compare each treatment group with each other and controls in most of our analyses. The small sample size of each treatment group, particularly CBZ, other AEDs and polytherapy, has resulted in wide confidence intervals for each variable. These wide confidence intervals suggest that there may be true differences between various AED therapies and controls.

5.2.2 Internal Validity

Internal validity refers to the validity of conclusions made for a certain study population (Rothman and Greenland 1998 pp.118-119). One important aspect of internal validity is the methodology used to minimise information bias. The methodology used for this study was appropriate for the assessment
of atherosclerotic risk in children with epilepsy. Based on the metabolism of homocysteine, we investigated all the relevant biochemical factors associated with homocysteine. We also looked at lipid and lipoproteins that are well known atherogenic risk factors. The biochemical tests used to measure tHcy, its vitamin cofactors and lipid levels are well established with acceptable coefficient of variation values (Burtis, Ashwood et al. 2008). FMD and IMT measurements are sensitive measurements of vascular function and structure that are the earliest findings in cardiovascular disease (Salonen and Salonen 1991; Celermajer, Sorensen et al. 1992; Jarvisalo, Jartti et al. 2001; Gokce, Keaney et al. 2003; Raitakari, Juonala et al. 2003; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006; Yeboah, Crouse et al. 2007). These ultrasound techniques are well established with a reported coefficient of variation of 3.7% for FMD and 4.0% for GTN-induced dilation (Wiltshire, Gent et al. 2002). The ultrasonographer involved in this study was experienced in FMD and cIMT having used these techniques in a previous study of children with diabetes.

One weakness of this study was the small number of aIMT that could be performed. The measurement of aortic aIMT is difficult due to the posterior position of the aorta. To gain good images of the aortic vessel wall requires significant pressure to be applied with the ultrasound probe onto the abdomen, which can result in discomfort for the child. The ultrasonographer in our study had no previous experience with this procedure and was not able to obtain aIMT in all subjects. Additionally, the quality of some of the images that were obtained, judged by the number of pixel points used for measurements, was less than ideal. Adding to the weakness, aIMT in the controls from the previous Australian study was not attempted. Measurement of aIMT was easier in subjects with a smaller BMI, due to the reduced distance from the abdominal wall to the aorta, and therefore a reduced amount of pressure needed to image the aorta. An aIMT measurement was
not obtained in one subject who had a full bladder preventing imaging of the aorta. Possible strategies to improve the yield of this procedure could include sending the ultrasonographer to a centre with good yield of this technique for training and requiring the children to empty their bladder prior to testing.

Another important contributor to internal validity is the way the subjects and controls are selected. A potential weakness of this study is the selection of the control group. Only 33% of the control group was recruited from the base population. The remaining 67% were Australian controls used in a previous research study on early vascular disease in children with type I diabetes (Wiltshire, Gent et al. 2002). The controls from this diabetic study were recruited from friends and family of participants and colleagues as was done here. Differences between New Zealand and Australian populations in regards to potential confounding factors such as diet however are considered minimal. Furthermore, there was no significant difference between the Australian and New Zealand controls in the atherosclerotic risk factors we could record; gender, age, pubertal stage, weight, height, BMI, systolic blood pressure or diastolic blood pressure. It is therefore unlikely that this selection of controls had a significant impact on the results.

The control group of children was not matched for ethnicity with the subjects. There were four children with epilepsy of Māori descent and one of Samoan decent, whereas the control group consisted of only Europeans. New Zealand Māori and Samoans have a known increased risk factor for cardiovascular disease compared to Non-Māoris (Chan, Wright et al. 2008). This could have resulted in an overestimation of the cardiovascular risk in epilepsy subjects compared with controls. As the only risk for cardiovascular disease observed in the children with epilepsy was abnormal lipid levels in the CBZ treated children and the Māori or Samoan children were represented
in the CBZ, VPA, newer AEDs and polytherapy groups this overestimation is unlikely.

It is conceivable that the increased risk of vascular death in adults with epilepsy is due to something other than the effect of AEDs. It may be that seizures themselves or the underlying pathophysiology of the epilepsy have an effect on atherosclerotic development. It would have made the study stronger if we could have used a control group of children with epilepsy not on AEDs to eliminate the potential confounding factor of the epilepsy itself. Unfortunately this is not feasible. Inclusion of an untreated control group would be unethical, as children without AED therapy would continue to have seizures, which would impact on their quality of life and potentially result in significant morbidity and possibly mortality. Although there are some types of epilepsy that are often not treated, such as Benign Epilepsy of Childhood with Centro-temporal Spikes, these children have infrequent seizures and all out grow their epilepsy by the age of 18 years. It needs to be emphasised that epilepsy is a group of disorders with different aetiologies and the common symptom of seizures. Therefore having a control group of one syndrome and a subject group with other syndromes would introduce its own bias.

Another possible control group may have been children with epilepsy taking newer AED therapies such as LTG. Based on the pathophysiology of these AEDs it is hypothesised that cardiovascular risk may be less than that of VPA and CBZ therapy (Isojarvi, Rattya et al. 1998). To date, however, there have been no studies assessing cardiovascular risk with newer AED therapies in children or adults, therefore this may not be the case. Our study had only small numbers of children on these medications, which meant we could not answer this question definitively. It did appear though from our minimal data
that these AEDs may have less of an effect. It should be noted this study did not find many effects however of any of the AEDs.

The only subjects with epilepsy excluded from this study were those with epilepsy due to a cardiovascular aetiology, or those with mental-motor retardation of an unknown cause. The exclusion of the latter of these was two fold: (1) Due to the perceived intolerance of the testing procedures; and (2) To avoid inclusion of epilepsy subjects with a metabolic aetiology. Metabolic epilepsies have an association of elevated cardiovascular risk, therefore this exclusion criterion was important to rule out possible confounding effects.

A strength of the study is that epilepsy subjects were age, sex and BMI matched with controls to avoid confounding due to these known atherosclerotic risk factors. Pubertal hormones are also known to affect tHcy levels (Boushey, Beresford et al. 1995; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Selhub 1999). Our subjects, however, were age and pubertally matched, via tanner staging, thus puberty is unlikely to have influenced our results. Other factors that can have had an effect on atherosclerosis development are diet, AED compliance, family history of cardiovascular disease and cigarette smoking. The children and families were asked about AED compliance and cigarette smoking at the initial interview. Although this is obviously not as good as serum AED levels or serum cotinine levels we did not find any reason to suspect there was AED non-compliance or that any child smoked in a regular fashion. We did not do a dietary history from either group but as the controls came from friends and family of the subjects it is likely the diets were similar.
5.2.3 **External Validity (Generalizability)**

Generalizability (external validity) refers to the ability of a study population to produce unbiased inferences to a target population (Rothman and Greenland 1998 pp.133-134). One way to gain effective generalisability is through inclusion of a wide range of subjects allowing thorough representation of a target population. This, however, often decreases internal validity by inclusion of subjects with potential biases and confounders. Study designs are typically stronger if the subject selection is narrowed allowing unbiased testing and inference (Rothman and Greenland 1998 pp.133-134).

The population of this study is representative of the general population for four main reasons: Firstly, this study includes children of different ethnicities including European, Māori and Samoan descendants. The proportion of European and Māori children in this study was similar to that of the general New Zealand population (Statistics New Zealand 2006). Only one Samoan child however was included in this study limiting generalisability to this population; Secondarily, the children in our study had a variety of epilepsy syndromes similar to what would be seen in a general population (Berg, Shinnar et al. 1999; Berg, Shinnar et al. 2000); Thirdly, children in our study were treated with either CBZ (7), VPA (12), newer AEDs (7) and polytherapy (4). These anticonvulsants represent the main AEDs used in children with epilepsy in New Zealand (Lagan and Brydon 2009). Other inducing AEDs such as PHT and Phenobarbital are rarely used for any period of time in children in New Zealand; and fourthly, expected correlations such as aIMT with total cholesterol and tHcy with serum folate, were demonstrated. This suggests that the techniques used in this study were robust producing results consistent with previous literature. Therefore, as this
study is representative of the ethnicities, epilepsy syndromes, AED treatments and demonstrated known associations between different variables, this study is able to be accurately generalised to the wider population of children with epilepsy.

5.3 Study Results

5.3.1 Biochemical Results

_Total Plasma Homocyst(e)ine and its determinants_

tHcy, a known risk factor for atherosclerosis, has been found to be elevated in adults (Schwaninger, Ringleb et al. 1999; Apeland, Mansoor et al. 2001; Apeland, Mansoor et al. 2002; Apeland, Mansoor et al. 2003; Hamed, Hamed et al. 2007) and adolescents (Verrotti, Pascarella et al. 2000) treated with AED therapies. We were unable to replicate this finding in our children and found no significant difference in tHcy between the children with epilepsy and the controls.

Most of the previous research showing an increased tHcy concentration associated with AED therapy is in adult populations. Adult and child populations are not comparable. Firstly, folate and vitamin B12 levels decline with age (Wiltshire and Couper 2004). As discussed in chapter one, tHcy concentrations become elevated when there are deficiencies of the B-vitamin cofactors essential in homocysteine metabolism. The tHcy level is not linearly related to the concentration of these cofactors. Homocysteine metabolism pathways are not affected until a certain level of vitamin
deficiency is reached. Therefore these pathways demonstrate a threshold phenomenon (Jacques, Bostom et al. 1996). As children have higher levels of these vitamin cofactors (folate and vitamin B12) than adults (Wiltshire and Couper 2004), children therefore have greater protection against B-vitamin deficiencies. As it is proposed that one mechanism for AED induced tHcy elevation is due to the induction of hepatic enzymes by the AED, resulting in decreased B-vitamins (Maxwell, Hunter et al. 1972; Reynolds 1975; Luoma, Sotaniemi et al. 1980), children with their higher levels of B-vitamins are protected. Consequently compared to adults, a greater AED effect, or enzyme induction, would be needed in children to reduce the vitamin cofactors enough to result in detectable tHcy elevations.

There is only one study that looks at homocysteine in adolescence. Verotti and colleagues investigated the effects of AED therapies on tHcy levels in individuals aged 14 – 18 years (Verrotti, Pascarella et al. 2000). During and after puberty, however, tHcy concentrations elevate to levels comparable to adults (Boushey, Beresford et al. 1995; Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Selhub 1999). Studies on adolescents are therefore more comparable to adult studies than studies of children.

Five studies have investigated the effects of AED therapy on tHcy in children with epilepsy. Similar results to ours have been reported in two other childhood studies that were unable to show a difference in tHcy levels in children with epilepsy (Huemer, Ausserer et al. 2005; Kurul, Unalp et al. 2007). One prospective study of 52 children with epilepsy demonstrated that tHcy levels increased after twenty weeks on either CBZ or VPA monotherapy (Attilakos, Papakonstantinou et al. 2006). As there was no control group it cannot be concluded that these increases were “abnormal” elevations of tHcy.
(i.e. elevated compared to normal) only that the tHcy increased compared to a baseline level. Indeed the levels reported in this study are comparable to the levels we observed (mean concentration of 7.7 ± 2.1 µmol/L in VPA and 7.6 ± 1.7 µmol/L in CBZ treatment in the Attilakos study versus our median values of 6.55 (5.30 – 8.65) µmol/L in VPA and 6.50 (4.70 – 9.00) µmol/L in CBZ) (Attilakos, Papakonstantinou et al. 2006). Two large studies in children have reported elevated tHcy on VPA and CBZ monotherapy (Vilaseca, Monros et al. 2000; Karabiber, Sonmezgoz et al. 2003). The first study by Vilaseca and colleagues studied 136 children with epilepsy and compared levels of tHcy, folate, vitamin B6 and vitamin B12 to historical reference values from children undergoing surgery (Vilaseca, Monros et al. 2000). The tHcy levels of the reference children however were not stated to be fasting which may over or underestimate results (Vilaseca, Moyano et al. 1997). The mean folate level in their reference population (17.4 nmol/L) was also much lower than in either our controls (23.95 nmol/L) or our children with epilepsy (25.62 nmol/L). This relative deficiency in folate in the Vilaseca population would suggest their children with epilepsy have less protection against AED induced vitamin reduction and so it is not surprising that they found elevated tHcy when this study did not (Vilaseca, Monros et al. 2000). This is also likely to be the case in the study by Karabiber and colleagues who found mean control folate levels of 20.34 nmol/L (Karabiber, Sonmezgoz et al. 2003). The Karabiber study concluded that tHcy was elevated from controls based on statistical methodology that assumes normal distribution of the data. This is unlikely to be the case as tHcy is typically skewed, demonstrated by their very wide standard deviations (tHcy level in the CBZ treated group was 16.0 ± 13.1 mmol/L) (Karabiber, Sonmezgoz et al. 2003). This inappropriate statistical analysis makes comparison of the results to other studies, such this one, difficult.
The hypothesis that tHcy becomes elevated when the B-vitamin cofactor levels decrease is supported by our findings of similar folate and PLP levels and no elevation of tHcy between children with and without epilepsy. In addition we were able to demonstrate a significant negative correlation between serum folate and tHcy. In children, reductions of serum folate and PLP are most often found in CBZ treated children (Kishi, Fujita et al. 1997; Attilakos, Papakonstantinou et al. 2006). In this study we had a small number of children treated with CBZ (7) and no children on other enzyme inducing AEDs. In the CBZ and polytherapy groups we found a trend for lower PLP however there was a trend for elevated levels in children treated with VPA. It is possible with larger numbers of children on CBZ we may have been able to show an affect of the AEDs on tHcy levels.

Unlike the other vitamin cofactors of homocysteine, we found elevated vitamin B12 concentrations compared to controls. Further analysis showed that this elevation in vitamin B12 levels was due to the effect of VPA. The mechanism for elevated vitamin B12 is not understood but it has been postulated by May and Sunder to be associated with increased irreversible plasma binding of the vitamin B12 in patients on VPA (May and Sunder 1993). Further research however is needed on the effects of VPA treatment on vitamin B12 concentrations. We also demonstrated a negative correlation between tHcy and vitamin B12, although statistical significance was not achieved. Elevated vitamin B12 levels with VPA have also been found in other child and adult studies (Vilaseca, Monros et al. 2000; Apeland, Mansoor et al. 2003; Attilakos, Papakonstantinou et al. 2006). Elevated vitamin B12 concentrations may partly explain our finding of normal tHcy levels in children with epilepsy on VPA. There are two pathways in the metabolism of homocysteine (see chapter two) (Mayer, Jacobsen et al. 1996; Refsum, Ueland
et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Fowler 2005). A deficiency of the vitamin cofactors in either pathway result in elevated homocysteine. Vitamin B12 and folate are important cofactors in the remethylation pathway (Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Fowler 2005). If the other homocysteine metabolic pathway, the transsulfuration pathway, is compromised by lack of its vitamin cofactor (PLP) and there is sufficient vitamin B12 or folate concentrations the overall metabolism of homocysteine would not be compromised and homocysteine levels would remain normal (Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; Selhub 1999; Fowler 2005).

Another factor that contributes to elevated tHcy levels observed in adults which is not likely seen in children is elevation due to renal disease. Renal dysfunction is known to significantly elevate tHcy levels via reduced homocysteine clearance (Mayer, Jacobsen et al. 1996; Refsum, Ueland et al. 1998; Welch and Loscalzo 1998; McCully 2007). As renal disease is commonly caused by atherosclerosis, these tHcy elevations in adults maybe secondary to atherosclerosis rather than causative.

Previous studies of tHcy in epilepsy have largely reported mean tHcy concentrations rather than the median levels as we have. tHcy levels are typically skewed to the right, exaggerating any difference noted between epilepsy subjects and controls if parametric statistical tests are used (i.e. students t-test or ANOVA). Mean tHcy values in the current study were higher than controls particularly in CBZ monotherapy (7.56 µmol/L) and polytherapy (7.56 µmol/L) treated children, compared with controls (6.81 µmol/L) and VPA monotherapy (6.83 µmol/L) was observed. Analysis of tHcy
levels in this way however is not appropriate, as tHcy levels are not normally distributed. The statistical analysis of the data in this study is a strength of this study allowing accurate analysis and inference.

As diet is such an important contributor to vitamin cofactor levels, one needs to be cautious in comparing studies performed in different populations as their dietary intake may be different. None of the published studies report on the dietary intake of these vitamins in their populations. Although they have control groups that are likely to have comparable dietary intake, it is the whole population’s nutritional status that is important. This is because the threshold affect of vitamin deficiency in the homocysteine metabolism pathways means that if a population (epilepsy patients and controls) has lower levels of these vitamins the epilepsy patients in this population are more likely to show an affect of the AEDs on tHcy than a population with better nutritional status.

Previous research has largely investigated the effect of AEDs that are enzyme inducers (CBZ and PHT) and VPA therapy. There has been little study of the newer AED therapies, such as LTG, Levetiracetam, Topiramate and Clobazam. My study included 7 subjects (23%) treated with these newer AEDs in monotherapy. In these AEDs we found no statistically significant effects on glucose, tHcy or its cofactors. As the number of children on these AEDs was small we cannot make any definitive statements. The affects of polytherapy on tHcy levels have been contradictory in previous research in adults with elevations in tHcy levels found by Hamed and colleagues (Hamed, Hamed et al. 2007) but no differences found by Apeland and colleagues (Apeland, Mansoor et al. 2003). The polytherapy group will always be a difficult group to compare from study to study as the proportions of patients
on various AEDs and the combinations of AEDs in this group will vary considerably from study to study. There were no differences in tHcy and its vitamin cofactors between children on polytherapy or controls in my study, again however this study had too few children (n = 4) in the polytherapy group to make any definitive statements.

In conclusion, I hypothesised that tHcy levels would be elevated in epilepsy subjects treated with AEDs compared to controls. I further hypothesised that this elevation in tHcy would be associated with deficiencies in homocysteine’s B-vitamin cofactors. I was unable to demonstrate significant differences in tHcy concentrations between controls and children with epilepsy or in any of the AED subgroups. Furthermore, the only cofactor difference apparent was elevations of vitamin B12 in VPA treated children.

**Glucose**

Reduced glucose concentrations in subjects treated with VPA monotherapy observed in this study confirm earlier studies by Pylvanen and colleagues and Aydin and colleagues (Aydin, Serdaroglu et al. 2005; Pylvanen, Pakarinen et al. 2006). Although we did not measure insulin levels in our study, it is likely that the decreased glucose is due to raised insulin levels which have been shown in subjects on VPA treatment in previous research (Isojarvi, Rattya et al. 1998; Verrotti, Basciani et al. 2002; Pylvanen, Knip et al. 2003; Aydin, Serdaroglu et al. 2005; Pylvanen, Pakarinen et al. 2006). Hyperinsulinemia is an independent risk factor for cardiovascular disease (Verrotti, Basciani et al. 2002), and it may be that raised insulin levels may contribute to the elevated cardiovascular and cerebrovascular disease risk in patients with epilepsy on VPA therapy. Studies investigating insulin concentrations in other AED treatments, such as CBZ and LTG, found no
differences in insulin levels in regards to controls (Isojarvi, Rattya et al. 1998; Pylvanen, Knip et al. 2003). The significant negative correlation between glucose and serum vitamin B12 in this study is likely to be due to the effects of VPA therapy on these two plasma variables.

*Lipids and Lipoproteins*

To better understand the cardiovascular risk in children with epilepsy, and because of their known effect on vascular structure and function, lipid and lipoprotein concentrations were investigated. Literature on the effects of AEDs on lipids and lipoproteins is controversial with both abnormal (Isojarvi, Pakarinen et al. 1993; Verrotti, Basciani et al. 1998; Hamed, Hamed et al. 2007; Tomoum, Awadallah et al. 2008) and normal (Apeland, Mansoor et al. 2001; Erdemir, Çullu et al. 2008) results reported in adults and children with epilepsy. Differences in lipid and lipoprotein levels between various studies may be due to differences in the AED treatments included in the study. In this study, statistically elevated total cholesterol, total cholesterol/ HDL cholesterol ratio, free triglycerides and apolipoprotein B concentrations were evident in CBZ treated children compared with the control group. This effect has previously been observed (Isojarvi, Pakarinen et al. 1993; Verrotti, Basciani et al. 1998; Bramswig, Kerksiek et al. 2002; Tomoum, Awadallah et al. 2008). Comparable lipids and lipoproteins observed in VPA treated children in this study are also in keeping with previous literature (Verrotti, Basciani et al. 1998; Apeland, Mansoor et al. 2001; Hamed, Hamed et al. 2007; Erdemir, Çullu et al. 2008; Tomoum, Awadallah et al. 2008; Verrotti, Scardapane et al. 2008). Not surprisingly, VPA, the first line AED for most epileptic seizures (Engel and Pedley 2008 p.1678), was the most frequent AED in this study used in 40% (12) of the children. The lipid and lipoprotein concentrations
observed in the whole group of epilepsy subjects may therefore be dominated by effects from the VPA treated population rather than other AED therapies.

As well as the differences observed between CBZ and VPA therapy, total cholesterol levels in CBZ treated children were elevated in comparison with subjects treated with other AED therapies and polytherapy. The effects of newer AED therapies, such as LTG, on lipid levels are largely unknown. One intervention study by Isojarvi and colleagues observed normalised HDL and free triglyceride levels after substitution of VPA for LTG (Isojarvi, Rattya et al. 1998). The low total cholesterol concentrations in subjects treated with other AED therapies may result in a lowered risk of atherosclerosis, although minimal research is available regarding vascular effects of newer AED treatments on the vasculature or on lipids. The small sizes of each treatment group in this study limit the comparisons of other AEDs in other lipid and lipoprotein variables. The reduced total cholesterol levels in children on polytherapy compared with CBZ treated subjects should be interpreted with caution as only four children with epilepsy were included in the polytherapy treatments group, and two of these had VPA as one of their AEDs. It is therefore likely that these two subjects had total cholesterol levels representing that of VPA therapy. Other newer AEDs, including LTG that are not thought to effect lipid levels, were also present in this treatment group.

In summary, no differences in lipid levels were apparent between children with epilepsy treated with AED therapies and controls. After analysis of separate AED treatments abnormal lipid levels were demonstrated in CBZ treated children indicating a greater atherosclerotic risk. I had hypothesised that abnormal lipid levels would be apparent in children treated with AED therapies. Therefore my hypothesis is accepted for CBZ therapy but not the other AEDs.
5.3.2 Vascular Function and Structure

Measurements of vascular function and structure using FMD and IMT are valuable predictors for the development of atherosclerosis and subsequently cardiovascular disease (Celermajer, Sorensen et al. 1992; Bots, Hoes et al. 1999; Jarvisalo, Jartti et al. 2001; Raitakari, Juonala et al. 2003; Widlansky, Gokce et al. 2003; Skilton and Celermajer 2006; Yeboah, Crouse et al. 2007). In this study we were unable to detect any differences in vascular function or structure between the two groups. There are several possible explanations which may contribute to this:

- It may be that there is no effect of AEDs on vascular function and structure.
- It may be that an effect was unable to be detected due to the small population size of our study.
- It may be that the atherosclerotic effect is only limited to certain AED therapies such as CBZ and as our population was dominated by VPA therapy we did not see an effect.
- It may be that detectable atherosclerosis takes longer to develop than the year of therapy these children were on.
- It may be that as in our subset of children we did not find any of the atherosclerotic risk factors such as elevated tHcy because we have studied an uninformative group of children.

The effects of AED treatments on endothelial function in epilepsy patients have received very little attention to date. One study by Gerstner and colleagues suggests that AED treatment in children with epilepsy enhanced the degeneration of vascular endothelium. They used microscopic techniques to
assess red blood cell velocity and capillary diameter and density, and did not look at endothelial function directly (Gerstner, Woelfing et al. 2006). This study, therefore, provides the first information on the extent of endothelial cell damage in children with epilepsy treated with AED therapies. The children with epilepsy demonstrated a trend for reduced endothelial function, although statistical significance was not achieved. A possible contributing factor to this result may be the young age group of the study population as atherosclerosis is a slowly progressive disease that develops with age (Berenson, Srinivasan et al. 1998). Consequently, endothelial dysfunction, to a detectable level using FMD measurement, may not have had sufficient time to develop. Reduced FMD in children is demonstrated by recent studies in patients with type I diabetes mellitus (Wiltshire, Gent et al. 2002). The risk of cardiovascular and cerebrovascular disease in patients with type I diabetes mellitus is four to twenty times greater than the general population (Roper, Bilous et al. 2002; Skrivarhaug, Bangstad et al. 2006). This is significantly higher than the up to five-fold maximum risk found amongst epilepsy populations (Hauser, Annegers et al. 1980; Cockerell, Johnson et al. 1994; Nilsson, Tomson et al. 1997). It is possible that the abnormal FMD measurement seen in type I diabetes mellitus children may be due to a faster progressing atherosclerotic development which can therefore be detected at a younger age.

There is little data available on the effect of AEDs on IMT, a measure of lipid deposition in the arterial intima (Skilton and Celermajer 2006). The cIMT results observed in this study are consistent with Tomoum and colleagues who reported no significant differences in left or right carotid arteries between children and adolescents on AED therapies compared with controls (Tomoum, Awadallah et al. 2008). In contradiction however, Eridemir and colleagues found significantly elevated cIMT in VPA treated children
compared with controls (Erdemir, Çullu et al. 2008). This study was significantly larger than the current study and the one conducted by Tomoum and colleagues allowing more statistical power for the detection of subtle changes in cIMT. The difference between the research studies may also be due to differences in duration of AED treatments and type of AED therapy. Erdemir and colleagues only investigated the effects of VPA treatment (Erdemir, Çullu et al. 2008) compared to Toumoum and colleagues and the current study that included other AED therapies (Tomoum, Awadallah et al. 2008). Hamed and colleagues observed abnormal cIMT measurements in adults but these individuals had been on AEDs for longer than our children which may account for advancement of atherosclerosis. It is of interest that this study also found increased cIMT in a group of adults with epilepsy who were on no AEDs. It is not clear, however, from the report whether these patients may have been on AEDs in the past (Hamed, Hamed et al. 2007).

The abdominal aorta, a common site of atherosclerosis preceding development in the carotid arteries (Berenson, Srinivasan et al. 1998), is a useful marker for the earliest detection of atherosclerosis development in children. No differences in aIMT measurements in this study were apparent between epilepsy and control groups. We did find significant correlations between mean aIMT and age, weight, BMI, HDL cholesterol, total cholesterol/HDL cholesterol and red cell folate. These correlations, between aIMT and known risk factors for atherosclerosis validate the measurements taken in this study.

The lack of any functional or structural vascular abnormalities in this study is consistent with the fact we were unable to show any abnormalities in tHcy, its cofactors or lipids. Literature suggests that increased atherosclerotic risk observed in patients with epilepsy results from abnormalities in tHcy and
lipid plasma concentrations. With normal levels of these risk factors, as in this study, one would not expect to have an effect on vascular function or structure, measured by FMD and IMT. The observed elevation of some lipid and lipoprotein levels in CBZ treated patients in this study would lead one to expect abnormal endothelial function and IMT in these children which this study did not find. This may be due to the slow progression of atherosclerosis. The mean duration of treatment in this study is only 2.8 years. As atherosclerosis is a gradual disease (Berenson, Srinivasan et al. 1998), the atherosclerotic changes caused by elevations in lipids observed in this study may not have had time to produce detectable changes in endothelial function and IMT measurements. Furthermore, the small sample sizes in this study means that there is less statistical power in determining differences between AED treatment groups. However the fact that CBZ therapy was associated with lipid abnormalities and lipid abnormalities in turn were associated with increase aIMT provides some evidence that CBZ therapy could be associated with the earliest stages of atherosclerosis and warrants further investigation.

In my hypothesis, I predicted significantly reduced endothelial function along with elevated cIMT and aIMT in epilepsy children and adolescents treated with AEDs compared with matched controls. The incomplete data available on vascular function and structure in this study however limits analysis of the results therefore my hypothesis can neither be supported nor rejected.
5.4 Implications of the Study

5.4.1 In Patients with Epilepsy

Epilepsy is a common disorder occurring in approximately 1-2% of the population (Menkes and Sankar 2000 p.919; Wallace and Farrell 2004 p.23). As epilepsy can require long-term therapy with AEDs it is important to understand the side effects of these drug treatments (Menkes and Sankar 2000; Wallace and Farrell 2004). Literature postulates that the increased cardiovascular risk factor for atherosclerosis amongst epilepsy populations results from elevations in tHcy and lipid levels secondary to AED treatments. Lipid and lipoprotein levels in this study demonstrated that epilepsy patients treated with CBZ monotherapy have a greater risk of abnormalities compared to controls or the other AEDs. As early vascular disease, especially in children, is potentially reversible (Celermajer, Sorensen et al. 1992; Widlansky, Gokce et al. 2003), intervention in at risk patients, such as children treated with CBZ therapy, could potentially help prevent the development of cardiovascular and cerebrovascular disease later in life. Previous studies have shown that interventions with B-vitamins are effective in reducing tHcy and its cofactor concentrations, therefore potentially reducing cardiovascular risk. It has therefore been recommended that all children on AEDS take vitamin supplemnetations such as folate (Apeland, Mansoor et al. 2002; Huemer, Ausserer et al. 2005). As we were unable to determine any differences in vascular structure, vascular function and biochemical cardiovascular risk factors between the epilepsy and control groups the recommendation of vitamin supplementation to all children on AEDs is most likely premature.
Our study is reassuring in that we were unable to detect any early atherosclerotic changes after one year and up to ten years of AED therapy implying that it either does not occur or have not yet had time to develop. In a New Zealand population of children we were not able to demonstrate any increase in cardiovascular risk factors for children on monotherapy with the newer AEDs or VPA. Although we did find abnormal lipids in children on CBZ monotherapy these children had not as yet developed early atherosclerotic changes.

5.4.2 In Other Disorders

The investigation of the affect of AED treatment on atherosclerotic risk factors in children with epilepsy will have significant implications for other paediatric specialties which use AEDs as therapy such as psychiatry who, commonly used AED therapies as mood stabilizers. Mortality from cardiovascular disease in patients with bipolar disease has been recorded as one of the leading causes of deaths in this group with an increased risk of cardiovascular disease up to three times that of the general population (Sharma and Markar 1994; Osby, Brandt et al. 2001; Angst, Stassen et al. 2002; Laursen, Munk-Olsen et al. 2007). This increased cardiovascular risk may be in part due to higher levels of other cardiovascular risk factors, such as smoking, or the direct effects of medication on weight gain (Newcomer 2006). Weight gain is most pronounced in VPA treated patients (Newcomer 2006). The effect of AEDs on cardiovascular disease in these populations however has not been investigated. Lithium and antidepressants, other treatments of bipolar disease have been shown to reduce cardiovascular risk (Angst, Stassen et al. 2002). It is possible that AED therapies may affect cardiovascular disease in psychiatric patients as well as patients with epilepsy.
5.5 Future Research

The aim of this research was to assess the effects of AED therapies on cardiovascular risk factors in children with epilepsy and look for changes associated with early atherosclerosis. This study was novel in that it is the only study to investigate vascular function and aortic and carotid vessel structure in children with epilepsy on AEDs. We were unable to demonstrate abnormal vascular function and structure in children with epilepsy. This may be that atherosclerosis develops slowly and is not yet apparent in children. Future research on the effects of AED therapies on early vascular disease should therefore be conducted in adolescent and adult populations.

5.5.1 Analytical Studies in Adolescents and Adults

Literature suggests that adults with epilepsy have an increased atherosclerotic risk, caused by abnormal tHcy and lipid concentrations. It would therefore be of value to assess endothelial function in a similar way as I have in this project in an adult population with elevated tHcy and lipids. In an adolescent and adult population it would be possible to compare vascular structure, vascular function and biochemical risk factors in different age groups (i.e. adolescents (14 -18 years), adults (19 – 40 and 41-64 years) and older adults (65+ years). This would allow an assessment as to when the endothelial vascular function and structure deteriorates.

Further research should assess the impact of the new AEDs on tHcy and lipids. Treatment groups for each AED however should be large enough to enable conclusions regarding the impact of each of the AEDs. If it becomes apparent that the newer AEDs do not seem to have an effect on tHcy and
lipids then individuals on these AEDs could be used as controls for the other treatment groups. This would eliminate the potential confounding factor of epilepsy and make it clearer that the changes in these cardiovascular risk factors are due to the AED.

Diet has a significant impact on atherosclerosis and B-vitamin cofactors involved in homocysteine metabolism. In future research it would be valuable to have a nutritional assessment of each participant via a quantitative food frequency questionnaire as has been done in children with diabetes (Wiltshire and Couper 2004). The measurement of insulin, as well as glucose levels, would also be important to help determine the effects of AED therapies, particularly VPA, on hyperinsulinaemia.

Another potential study would be a large case-control study which compared vascular structure and function in a cohort of epilepsy patients with elevated tHcy with patients with epilepsy with lower tHcy levels (i.e. compare patients with tHcy in the highest and lowest quartiles) and a cohort of epilepsy patients with abnormal lipid levels with a group of patients with epilepsy with normal lipids. These cohorts would be useful in strengthening the causative effect of tHcy and lipids with vascular structure and function in patients with epilepsy.

5.5.2 Intervention Studies

If early vascular changes in structure and function are found in the studies described above randomised control trials of vitamin enrichment by either diet or supplementation could be performed. Randomised control trials are beneficial in assessing the efficacy of intervention therapies while
avoiding biases in the intervention exposure (Rothman and Greenland 1998 pp.69-70). As literature suggests elevated tHcy levels are from deficiencies in B-vitamins, a trial of this type would be beneficial in investigating potential methods for reductions in cardiovascular risk in epilepsy patients who require long term AED therapy. It would also be beneficial to investigate the effects of dietary change alone in patients with epilepsy. Changes in diet may be more cost-effective than long-term vitamin supplementation and would also avoid the addition of further, and possibly unnecessary, tablets to be taken by each patient treated with AED therapies. Vitamin supplementation does not address abnormal lipid levels observed in patients treated with AED therapies. Dietary changes may be beneficial in reducing both tHcy and lipid levels.

5.6 Conclusion

In conclusion, this study investigated early cardiovascular disease in children with epilepsy treated with AED therapies. The key finding of this study was that AED treatment did not effectively alter vascular structure and function in the children with epilepsy. This was likely due to the tHcy and lipid levels in the children with epilepsy being similar to the control group, which was not what I had expected. Abnormal lipids were only demonstrated in CBZ treated children. Children on VPA showed elevation of vitamin B12. The lack of elevated tHcy levels observed in this study is likely due to the high folate and PLP status in children from this population as demonstrated by the control group, as well as raised vitamin B12 levels in VPA treated children. It is possible that tHcy may have been elevated in specific AEDs as shown in other adult and childhood populations but that this study did not have large enough numbers of children on these therapies to show this.
Previous researchers have recommended that all children with epilepsy on AED therapy should be given B-vitamin supplementation (Rothman and Greenland 1998; Apeland, Mansoor et al. 2002; Huemer, Ausserer et al. 2005). Our data would not support this recommendation in our population. Our population of children with epilepsy did not show elevations in tHcy therefore supplementing them with B-vitamins would be unnecessary. It is apparent that the dietary intake of B-vitamins and subsequent vitamin status has a significant impact on the development of raised tHcy in children on AEDs. This has been demonstrated by the finding of elevated tHcy in previous studies with poorer levels of these vitamins in their control populations than ours. It is important therefore for clinicians to consider the vitamin status of their population when deciding about the need for vitamin supplementation. It may be that subgroups of children within our population with poor vitamin cofactor intake are at higher risk for elevated tHcy and subsequently cardiovascular disease. Measuring tHcy levels in these children with subsequent supplementation may be warranted. Rather than vitamin supplementation in all children, recommendations of diets high in B-vitamin by paediatricians and neurologists would be of benefit. Recommendations of diets high in vitamins may additionally impact the abnormal lipid and lipoprotein levels observed in CBZ treated children as diets of food high in B-vitamins, such as fruits, vegetables and fortified cereals may encourage a reduction of foods with a high fat content. Vitamin supplementation in adolescents and adults treated with AED may also be beneficial as elevated tHcy levels are shown in adults with epilepsy.
References


affective disorder, schizoaffective disorder, and schizophrenia.[see comment]." Journal of Clinical Psychiatry 68(6): 899-907.


Appendices

Appendix A: Ethical Approval

6 December 2007

Dr Esko Wiltshire
Wellington School of Medicine & Health Sciences
Department of Paediatrics
Wellington School of Medicine and Health Science
PO Box 7343
Wellington South

Dear Esko

Early Vascular Disease In Children With Epilepsy Receiving Anticonvulsants
Dr Esko Wiltshire, Dr Lynette Sadleir
Capital & Coast DHB
CEN/07/10/071

The above study has been given ethical approval by the Central Regional Ethics Committee. A list of members of this committee is attached.

Approved Document

- Participant Information Sheet One, Version 2 dated 29 October 2007
- Participant Information Sheet two, Version 2 dated 29 October 2007
- Participant Information Sheet three, Version 1 dated 29 October 2007
- Participant Information Sheet two, Version 1 dated 29 October 2007
- Consent Form to Participate in Research, Version 2, dated October 2007
- Child Consent / Assent Form to Participate in Research, Version 2 Dated October 2007

Certification
The Committee is satisfied that this study is not being conducted principally for the benefit of the manufacturer or distributor of the medicine or item in respect of which the trial is being carried out.

Accreditation
The Committee involved in the approval of this study is accredited by the Health Research Council and is constituted and operates in accordance with the Operational Standard for Ethics Committees, April 2006.

Progress Reports
The study is approved until 1 November 2010. The Committee will review the approved application annually and notify the Principal Investigator if it withdraws approval. It is the Principal Investigator’s responsibility to forward a progress report covering all sites prior to ethical review of the project in 28 November 2008. The report form is available on http://www.newhealth.govt.nz/ethicscommittees. Please note that failure to provide a progress report may result in the withdrawal of ethical approval. A final report is also required at the conclusion of the study.

Requirements for SAE Reporting
The Principal Investigator will inform the Committee as soon as possible of the following:
- Any related study in another country that has stopped due to serious or unexpected adverse events
- withdrawal from the market for any reason
- all serious adverse events occurring during the study in New Zealand which result in the investigator breaking the blinding code at the time of the SAE or which result in hospitalisation or death.
- all serious adverse events occurring during the study worldwide which are considered related to the study medicine. Where there is a data safety monitoring board in place, serious adverse events occurring outside New Zealand may be reported quarterly.

Administered by the Ministry of Health
Approved by the Health Research Council
http://www.newhealth.govt.nz/ethicscommittees
All SAE reports must be signed by the Principal Investigator and include a comment on whether he/she considers there are any ethical issues relating to this study continuing due to this adverse event. It is assumed by signing the report, the Principal Investigator has undertaken to ensure that all New Zealand Investigators are made aware of the event.

Amendments
All amendments to the study must be advised to the Committee prior to their implementation, except in the case where immediate implementation is required for reasons of safety. In such cases the Committee must be notified as soon as possible of the change.

Please quote the above ethics committee reference number in all correspondence.

The Principal Investigator is responsible for advising any other study sites of approvals and all other correspondence with the Ethics Committee.

It should be noted that Ethics Committee approval does not imply any resource commitment or administrative facilitation by any healthcare provider within whose facility the research is to be carried out. Where applicable, authority for this must be obtained separately from the appropriate manager within the organisation.

Yours sincerely

Jiska van Bruggen
Central Regional Ethics Committee Administrator

Email: jiska_van_bruggen@moh.govt.nz
Dr Lynette G Sadleir  
Paediatrics and Child Health  
Wellington

Tēnā koe Dr Sadleir

**Title:** Early vascular disease in children with epilepsy receiving anticonvulsants

The Ngāi Tahu Research Consultation Committee (NTRCC) met on Tuesday, September 26 2006 to discuss your research proposition.

The NTRCC considers the research to be of importance to Māori health.

The Committee suggests that researchers collect ethnicity data based on the Census question on ethnicity.

The Committee noted that the researchers have undertaken to enable involvement of whānau and would like to commend the researchers for the work that has gone into the cultural aspects of the proposition and suggest researchers look at the Ōtago District Health Board’s Best Practice Tikanga document regarding patient engagement with regard to the tapu nature of any treatment and especially the collection, storage and disposal of blood and tissue samples.

The Committee suggest dissemination of the research findings to relevant Māori health organisations.

The Committee would also value a copy of the research findings.

Please contact me if you would like an electronic copy of this letter.

Nāhaku noa, nā

Mark Brunton  
Kaitakawaenga Rangahau Māori  
Facilitator Research Māori  
Research Division  
Te Whare Wānanga o Ōtāgo  
Ph: +64 3 479 8738  
email: mark.brunton@otago.ac.nz  
Web: www.otago.ac.nz

The Ngāi Tahu Research Consultation Committee has membership from:

*Te Rūnanga o Otākou Incorporated*  
*Kāti Haipara Rūnaka ki Puketeraki*  
*Te Rūnanga o Moeraki*
Appendix B: Information Sheets

Wellington School of Medicine and Health Sciences

Early Vascular Disease in Children with Epilepsy Receiving Anticonvulsants

PARTICIPANT INFORMATION SHEET 1
(Parental Information)

Your child is invited to participate in a study to help us learn more about the long term effects of anticonvulsant drugs on health, being conducted by Dr Lynette Sadleir and Dr Esko Wiltshire at the Wellington School of Medicine and Health Sciences, University of Otago. All participation in this research is entirely voluntary and you are free to withdraw from the study or to decline any particular question or test, at any time. You can take as much time as you need to decide whether or not to take part.

1) What is the study for and why is it being done?

People who take anticonvulsant therapy (epilepsy drugs) for a long time have been shown to have an increased risk of heart disease in later life. Early changes in blood vessel function are related in the long term to how healthy the blood vessels are in adult life. Previous studies have shown children with epilepsy receiving anticonvulsant epileptic drugs (AED’s) have high levels of homocysteine, a naturally occurring compound in the blood. High levels of homocysteine are linked to disease in the blood vessels.

So we can get a good picture of the effects of anticonvulsant drugs on blood vessel health we will need to look at a group of people who take these drugs as well as a group of people without epilepsy who do not take them.

We wish to look at the blood vessels in both children with epilepsy who are taking anticonvulsant medicine as well as in a control group of children to see whether there are any differences between the two groups.

We would also like to conduct blood tests to measure homocysteine and various other blood compounds that can indicate how healthy blood vessels are now and also how healthy they may be as your child grows older.
Other doctors have also looked carefully at the study and agree that it is an important study to carry out.

2) **What would I be asked to do if my child took part in this study?**

You and your child will be asked to visit the hospital for the study. We will ask you to come in first thing in the morning before breakfast as the tests must be conducted when the stomach is completely empty. We will need you to stay 25-30 minutes for the ultrasound study and blood test, followed by whatever time is required for your child’s breakfast (supplied by us at the hospital following the tests).

The way that we determine how the blood vessel is working is to use a technique called flow-mediated dilatation via an ultrasound test to examine an artery in your child’s arm and this works as follows:

1. An ultrasound picture is taken of the artery in the upper part of the arm to measure how wide it is and the blood flow in it.
2. A blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel would normally get larger (dilate). The vessel and blood flow is measured again with ultrasound, to check this difference. If the blood vessel is not working properly it will not dilate as much.
3. The ultrasound measurement is repeated after a period of 15 minutes rest, to allow the vessel to return to its initial size.
4. The ultrasound measurement is made a final time after administration of a medicine called Glyceryl trinitrate (GTN). This is a safe medicine, which is used in people with angina. It makes the blood vessel dilate (expand) to its maximal degree. The change in blood vessel size in the first part of the procedure is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

Carotid intima media thickness and abdominal aortic intima media thickness (the 2 different blood vessels we are testing) will be measured by taking ultrasound readings of these blood vessels in the neck and abdominal (tummy) regions.

We will also conduct a blood test to look at the levels of homocysteine and various other compounds associated with blood vessel disease. We can do this using local anaesthetic cream to numb the skin if your child would like. Your child’s blood sample when finished with will be disposed of by incineration (burning).

3) **Are there any risks of side effects associated with Flow Mediated Dilatation or any of the other tests conducted?**

Flow Mediated Dilatation is very safe and has been used in a large number of children and adults, including adults and young people with diabetes. The first part of the test with the cuff on the arm can be uncomfortable, although your child will probably not be too bothered by it. The medication, GTN, is very safe and only works for only a very short period of time (10-15 minutes). It may cause brief headaches in some people, although this is very unlikely, and they get better quickly with panadol if that does happen. GTN can also cause lower blood pressure, which
we will be keeping a close check on, light-headiness, facial flushing and a fast heart rate, again all of these effects are very unlikely, especially in children. The other ultrasound tests have no side-effects. The blood test may be slightly painful, but we can help stop this by using anaesthetic (numbing) cream.

4) Are there any costs associated with the study?

There are no costs to you associated with being involved in the study. We will pay you back for any transport costs you need to attend the appointments.

5) What happens if anything goes wrong?

In the very unlikely event of your child experiencing any adverse effects from participating in the trial, you would be fully covered by ACC.

6) What will be done with this information?

When the study is completed we should have results from a group of people who take anticonvulsant medication for their epilepsy, and from a group of people who do not suffer from epilepsy and do not therefore take anticonvulsant medication. By looking at and comparing the results from these two groups we will be able to see whether or not there are any differences in either blood vessel function or in certain blood compound levels between the two groups.

The ultrasound tests and blood test results will be stored securely and will not be used for any other study without your permission. We will also write articles about the study and publish these, or talk about the study at conferences, so that other people will be helped by the information. Results will also be reported to relevant community members, for example this may be via local iwi or through the New Zealand Epilepsy Society. All of the information will remain confidential and no information which could identify you will be used in any reports from the study.

7) Does my child have to take part in the study?

No, not at all. You should only take part in this part of the study if you want to be involved. If you choose not to take part it will not affect your child’s usual care or treatment in any way.

8) Can my child withdraw from the study at any time?

Yes, you can choose to leave the study at any time.

9) Will the study benefit my child in any way?

For patients with epilepsy we can’t be certain that there will be any benefit from taking part. However if we do find differences in the way blood vessels work in people with epilepsy, it may help us to work out ways of reducing the risk of blood vessel problems in the future.

We will let you know the results of your child’s ultrasound studies once all the studies have been done and the overall study results. There will be a delay in getting this information to you.
10) Do you have permission to do the study?

We have permission from the Central Regional Ethics Committee to do this study.

11) What if I have other questions about the study?

Please contact Dr Esko Wiltshire, Paediatrician, at any time. He can be paged through Wellington Hospital on (04) 3855999, pager 6912, or extension 6912. Or contact Dr Lynette Sadleir on 04 385 5999 ex 6147. Ngaire Keenan can also be contacted on 04 918 6138. If you have any questions or concerns about your rights as a participant in this study you may wish to contact the Health and Disability Advocate, Telephone: Mid and lower North Island 0800 42 36 38 (0800 4 ADNET) or the Central Regional Ethics Committee, Phone 04-496-2405
Wellington School of Medicine and Health Sciences

Early Vascular Disease in Children with Epilepsy Receiving Anticonvulsants

PARTICIPANT INFORMATION SHEET 3
(Parental Information; control group)

Your child is invited to participate in a study to help us learn more about the long term effects of anticonvulsant drugs on health, being conducted by Dr Lynette Sadleir and Dr Esko Wiltshire at the Wellington School of Medicine and Health Sciences, University of Otago. All participation in this research is entirely voluntary and you are free to withdraw from the study or to decline any particular question or test, at any time. You can take as much time as you need to decide whether or not to take part.

1) What is the study for and why is it being done?

People who take anticonvulsant therapy (epilepsy drugs) for a long time have been shown to have increased risk of heart disease in later life. Early changes in blood vessel function are related in the long term to how healthy the blood vessels are in adult life. Previous studies have shown children with epilepsy receiving anticonvulsant epileptic drugs (AED’s) have high levels of homocysteine, a naturally occurring compound in the blood. High levels of homocysteine are linked to disease in the blood vessels.

So we can get a good picture of the effects of anticonvulsant drugs on blood vessel health we will need to look at a group of people who take these drugs as well as a group of people without epilepsy who do not take them.

We wish to look at the blood vessels in both children with epilepsy who are taking anticonvulsant medicine as well as in a control group of children to see whether there are any differences between the two groups. Your child fits into the control group of children, so even though he/she does not have epilepsy you can see that his/her participation is very important!

We would also like to conduct blood tests to measure homocysteine and various other blood compounds that can indicate how healthy blood vessels are now and also how healthy they may be as your child grows older.
Other doctors have also looked carefully at the study and agree that it is an important study to carry out.

2) **What would I be asked to do if my child took part in this study?**

You and your child will be asked to visit the hospital for the study. We will ask you to come in first thing in the morning before breakfast as the tests must be conducted when the stomach is completely empty. We will need you to stay 25-30 minutes for the ultrasound study and blood test, followed by whatever time is required for your child’s breakfast (supplied by us at the hospital following the tests).

The way that we determine how the blood vessel is working is to use a technique called flow-mediated dilatation via an ultrasound test to examine an artery in your child’s arm and this works as follows:

1. An ultrasound picture is taken of the artery in the upper part of the arm to measure how wide it is and the blood flow in it.
2. A blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel would normally get larger (dilate). The vessel and blood flow is measured again with ultrasound, to check this difference. If the blood vessel is not working properly it will not dilate as much.
3. The ultrasound measurement is repeated after a period of 15 minutes rest, to allow the vessel to return to its initial size.
4. The ultrasound measurement is made a final time after administration of a medicine called Glyceryl trinitrate (GTN). This is a safe medicine, which is used in people with angina. It makes the blood vessel dilate (expand) to its maximal degree. The change in blood vessel size in the first part of the procedure is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

Carotid intima media thickness and abdominal aortic intima media thickness (the 2 different blood vessels we are testing) will be measured by taking ultrasound readings of these blood vessels in the neck and abdominal (tummy) regions.

We will also conduct a blood test to look at the levels of homocysteine and various other compounds associated with blood vessel disease. We can do this using local anaesthetic cream to numb the skin if your child would like. Your child’s blood sample when finished with will be disposed of by incineration.

3) **Are there any risks of side effects associated with Flow Mediated Dilatation or any of the other tests conducted?**

Flow Mediated Dilatation is very safe and has been used in a large number of children and adults, including adults and young people with diabetes. The first part of the test with the cuff on the arm can be uncomfortable, although your child/dependent will probably not be too bothered by it. The medication, GTN, is very safe and only works for only a very short period of time (10-15 minutes). It may cause brief headaches in some people, although this is very unlikely and they
get better quickly with panadol if that does happen. GTN can also cause lower blood pressure, which we will be keeping a close check on, light-headiness, facial flushing and a fast heart rate, again all of these effects are very unlikely, especially in children. The other ultrasound tests have no side-effects. The blood test may be slightly painful, but we can help stop this by using anaesthetic (numbing) cream.

4) Are there any costs associated with the study?

There are no costs to you associated with being involved in the study. We will pay you back for any transport costs you need to attend the appointments.

5) What happens if anything goes wrong?

In the very unlikely event of your child experiencing any adverse effects from participating in the trial, you would be fully covered by ACC.

6) What will be done with this information?

When the study is completed we should have results from a group of people who take anticonvulsant medication for their epilepsy, and from a group of people who do not suffer from epilepsy and do not therefore take anticonvulsant medication. By looking at and comparing the results from these two groups we will be able to see whether or not there are any differences in either blood vessel function or in certain blood compound levels between the two groups.

The ultrasound tests and blood test results will be stored securely and will not be used for any other study without your permission. We will also write articles about the study and publish these, or talk about the study at conferences, so that other people will be helped by the information. Results will also be reported to relevant community members, for example this may be via local iwi or through the New Zealand Epilepsy Society. All of the information will remain confidential and no information which could identify you will be used in any reports from the study.

7) Does my child have to take part in the study?

No, not at all. You should only take part in this part of the study if you want to be involved. If you choose not to take part it will not affect your child’s usual care or treatment in any way.

8) Can my child withdraw from the study at any time?

Yes, you can choose to leave the study at any time.

9) Will the study benefit my child in any way?

For patients with epilepsy we can’t be certain that there will be any benefit from taking part. However if we do find differences in the way blood vessels work in people with epilepsy, it may help us to work out ways of reducing the risk of blood vessel problems in the future.
As your child does not suffer from epilepsy there are no direct benefits to you for taking part, however the information that we hope to gain from the study has the potential to improve the health of many people in the future. We will let you know the results of your child’s ultrasound studies once all the studies have been done and the overall study results. There will be a delay in getting this information to you.

10) **Do you have permission to do the study?**

We have permission from the Central Regional Ethics Committee to do this study.

11) **What if I have other questions about the study?**

Please contact Dr Esko Wiltshire, Paediatrician, at any time. He can be paged through Wellington Hospital on (04) 3855999, pager 6912, or extension 6912. Or contact Dr Lynette Sadleir on 04 385 5999 ex 6147. Ngaire Keenan can also be contacted on 04 918 6138. If you have any questions or concerns about your rights as a participant in this study you may wish to contact the Health and Disability Advocate, Telephone: Mid and lower North Island 0800 42 36 38 (0800 4 ADNET) or the Central Regional Ethics Committee, Phone 04-496-2405
Wellington School of Medicine and Health Sciences

Early Vascular Disease in Children with Epilepsy Receiving Anticonvulsants

PARTICIPANT INFORMATION SHEET 2
(child information)

You are invited to participate in a study to help us learn more about the long term effects of anticonvulsant drugs on health, being conducted by Dr Lynette Sadleir and Dr Esko Wiltshire at the Wellington School of Medicine and Health Sciences, University of Otago. All participation in this research is entirely voluntary and you are free to withdraw from the study or to decline any particular question or test, at any time. You can take as much time as you need to decide whether or not to take part.

1) What is the study for and why is it being done?

People who take anticonvulsant therapy (epilepsy drugs) for a long time have been shown to have an increased risk of heart disease in later life. Early changes in blood vessel function are related in the long term to how healthy the blood vessels are in adult life. Previous studies have shown children with epilepsy receiving anticonvulsant epileptic drugs (AED’s) have high levels of homocysteine, a naturally occurring compound in the blood. High levels of homocysteine are linked to disease in the blood vessels.

So we can get a good picture of the effects of anticonvulsant drugs on blood vessel health we will need to look at a group of people who take these drugs as well as a group of people without epilepsy who do not take them.

We wish to look at the blood vessels in both children with epilepsy who are taking anticonvulsant medicine as well as in a control group of children to see whether there are any differences between the two groups.

We would also like to conduct blood tests to measure homocysteine and various other blood compounds that can indicate how healthy blood vessels are now and also how healthy they may be as you grow older.

Other doctors have also looked carefully at the study and agree that it is an important study to carry out.
2) **What would I be asked to do if I took part in this study?**

You will be asked to visit the hospital for the study. We will ask you to come in first thing in the morning before breakfast as the tests must be conducted when the stomach is completely empty. We will need you to stay 25-30 minutes for the ultrasound study and blood test, followed by whatever time is required for your breakfast (supplied by us at the hospital following the tests).

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1. An ultrasound picture is taken of the artery in the upper part of the arm to measure how wide it is and the blood flow in it.
2. A blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel would normally get larger (dilate). The vessel and blood flow is measured again with ultrasound, to check this difference. If the blood vessel is not working properly it will not dilate as much.
3. The ultrasound measurement is repeated after a period of 15 minutes rest, to allow the vessel to return to its initial size.
4. The ultrasound measurement is made a final time after administration of a medicine called Glyceryl trinitrate (GTN). This is a safe medicine, which is used in people with angina. It makes the blood vessel dilate (expand) to its maximal degree. The change in blood vessel size in the first part of the procedure is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

Carotid intima media thickness and abdominal aortic intima media thickness (the 2 different blood vessels we are testing) will be measured by taking ultrasound readings of these blood vessels in your neck and abdominal (tummy) regions.

We will also conduct a blood test to look at the levels of homocysteine and various other compounds associated with blood vessel disease. We can do this using local anaesthetic cream to numb the skin if you would like. Your blood sample when finished with will be disposed of by incineration. (burning)

3) **Are there any risks of side effects associated with Flow Mediated Dilatation or any of the other tests conducted?**

Flow Mediated Dilatation is very safe and has been used in a large number of children and adults, including adults and young people with diabetes. The first part of the test with the cuff on the arm can be uncomfortable, although you will probably not be too bothered by it. The medication, GTN, is very safe and only works for only a very short period of time (10-15 minutes). It may cause brief headaches in some people, although this is very unlikely and they get better quickly with panadol if that does happen. GTN can also cause lower blood pressure, which we will be keeping a close check on, light-headiness, facial flushing and a fast heart rate, again all of these effects are very unlikely, especially in children. The other ultrasound tests
have no side-effects. The blood test may be slightly painful, but we can help stop this by using anaesthetic (numbing) cream.

4) Are there any costs associated with the study?

There are no costs to you associated with being involved in the study. We will pay you back for any transport costs you need to attend the appointments.

5) What happens if anything goes wrong?

In the very unlikely event of you experiencing any adverse effects from participating in the trial, you would be fully covered by ACC.

6) What will be done with this information?

When the study is completed we should have results from a group of people who take anticonvulsant medication for their epilepsy, and from a group of people who do not suffer from epilepsy and do not therefore take anticonvulsant medication. By looking at and comparing the results we will be able to see whether or not there are any differences in either blood vessel function or in certain blood compound levels between the two groups.

The ultrasound tests and blood test results will be stored securely and will not be used for any other study without your permission. We will also write articles about the study and publish these, or talk about the study at conferences, so that other people will be helped by the information. Results will also be reported to relevant community members, for example this may be via local iwi or through the New Zealand Epilepsy Society. All of the information will remain confidential and no information which could identify you will be used in any reports from the study.

7) Do I have to take part in the study?

No, not at all. You should only take part in this part of the study if you want to be involved. If you choose not to take part it will not affect your usual care or treatment in any way.

8) Can I withdraw from the study at any time?

Yes, you can choose to leave the study at any time.

9) Will the study benefit me in any way?

For patients with epilepsy we can’t be certain that there will be any benefit from taking part. However if we do find differences in the way blood vessels work in people with epilepsy, it may help us to work out ways of reducing the risk of blood vessel problems in the future.

We will let you know the results of your ultrasound studies once all the studies have been done and the overall study results. There will be a delay in getting this information to you.
10) **Do you have permission to do the study?**

We have permission from the Central Regional Ethics Committee to do this study.

11) **What if I have other questions about the study?**

Please contact Dr Esko Wiltshire, Paediatrician, at any time. He can be paged through Wellington Hospital on (04) 3855999, pager 6912, or extension 6912. Or contact Dr Lynette Sadleir on 04 385 5999 ex 6147. Ngaire Keenan can also be contacted on 04 918 6138. If you have any questions or concerns about your rights as a participant in this study you may wish to contact the Health and Disability Advocate, Telephone: Mid and lower North Island 0800 42 36 38 (0800 4 ADNET) or the Central Regional Ethics Committee, Phone 04-496 2405.
Wellington School of Medicine and Health Sciences

Early Vascular Disease in Children with Epilepsy Receiving Anticonvulsants

PARTICIPANT INFORMATION SHEET 4
(Child Information; control group)

You are invited to participate in a study to help us learn more about the long term effects of anticonvulsant drugs on health, being conducted by Dr Lynette Sadleir and Dr Esko Wiltshire at the Wellington School of Medicine and Health Sciences, University of Otago. All participation in this research is entirely voluntary and you are free to withdraw from the study or to decline any particular question or test, at any time. You can take as much time as you need to decide whether or not to take part.

1) What is the study for and why is it being done?

People who take anticonvulsant therapy (epilepsy drugs) for a long time have been shown to have an increased risk of heart disease in later life. Early changes in blood vessel function are related to how healthy the blood vessels are in adult life. Previous studies have shown children with epilepsy receiving anticonvulsant epileptic drugs (AED’s) have high levels of homocysteine, a naturally occurring compound in the blood. High levels of homocysteine are linked to disease in the blood vessels.

So we can get a good picture of the effects of anticonvulsant drugs on blood vessel health we will need to look at a group of people who take these drugs as well as a group of people without epilepsy who do not take them.

We wish to look at the blood vessels in both children with epilepsy who are taking anticonvulsant medicine as well as in a control group of children to see whether there are any differences between the two groups. You fit into the control group of children, so even though you do not have epilepsy you can see that your participation is very important!

We would also like to conduct blood tests to measure homocysteine and various other blood compounds that can indicate how healthy blood vessels are now and also how healthy they may be as you grow older.
Other doctors have also looked carefully at the study and agree that it is an important study to carry out.

2) **What would I be asked to do if I took part in this study?**

You will be asked to visit the hospital for the study. We will ask you to come in first thing in the morning before breakfast as the tests must be conducted when the stomach is completely empty. We will need you to stay 25-30 minutes for the ultrasound study and blood test, followed by whatever time is required for your breakfast (supplied by us at the hospital following the tests).

The way that we determine how the blood vessel is working is to use a technique called flow-mediated dilatation via an ultrasound test to examine an artery in your arm and this works as follows:

1. An ultrasound picture is taken of the artery in the upper part of the arm to measure how wide it is and the blood flow in it.
2. A blood pressure cuff is blown up around the arm for 4 minutes. When the cuff is let down the blood vessel would normally get larger (dilate). The vessel and blood flow is measured again with ultrasound, to check this difference. If the blood vessel is not working properly it will not dilate as much.
3. The ultrasound measurement is repeated after a period of 15 minutes rest, to allow the vessel to return to its initial size.
4. The ultrasound measurement is made a final time after administration of a medicine called Glyceryl trinitrate (GTN). This is a safe medicine, which is used in people with angina. It makes the blood vessel dilate (expand) to its maximal degree. The change in blood vessel size in the first part of the procedure is then compared with the change with GTN and this gives the best measure of how well the lining of the blood vessel is working.

Carotid intima media thickness and abdominal aortic intima media thickness (the 2 different blood vessels we are testing) will be measured by taking ultrasound readings of these blood vessels in your neck and abdominal (tummy) regions.

We will also conduct a blood test to look at the levels of homocysteine and various other compounds associated with blood vessel disease. We can do this using local anaesthetic cream to numb the skin if you would like. Your blood sample when finished with will be disposed of by incineration. (burning)

3) **Are there any risks of side effects associated with Flow Mediated Dilatation or any of the other tests conducted?**

Flow Mediated Dilatation is very safe and has been used in a large number of children and adults, including adults and young people with diabetes. The first part of the test with the cuff on the arm can be uncomfortable, although you will probably not be too bothered by it. The medication, GTN, is very safe and only works for only a very short period of time (10-15 minutes). It may cause brief headaches in some people, although this is very unlikely and they get better quickly with panadol if that does happen. GTN can also cause lower blood pressure, which we will be
keeping a close check on, light-headiness, facial flushing and a fast heart rate, again all of these effects are very unlikely, especially in children. The other ultrasound tests have no side-effects. The blood test may be slightly painful, but we can help stop this by using anaesthetic (numbing) cream.

4) Are there any costs associated with the study?

There are no costs to you associated with being involved in the study. We will pay you back for any transport costs you need to attend the appointments.

5) What happens if anything goes wrong?

In the very unlikely event of you experiencing any adverse effects from participating in the trial, you would be fully covered by ACC.

6) What will be done with this information?

When the study is completed we should have results from a group of people who take anticonvulsant medication for their epilepsy, and from a group of people who do not suffer from epilepsy and do not therefore take anticonvulsant medication. By looking at and comparing the results we will be able to see whether or not there are any differences in either blood vessel function or in certain blood compound levels between the two groups.

The ultrasound tests and blood test results will be stored securely and will not be used for any other study without your permission. We will also write articles about the study and publish these, or talk about the study at conferences, so that other people will be helped by the information. Results will also be reported to relevant community members, for example this may be via local iwi or through the New Zealand Epilepsy Society. All of the information will remain confidential and no information which could identify you will be used in any reports from the study.

7) Do I have to take part in the study?

No, not at all. You should only take part in this part of the study if you want to be involved. If you choose not to take part it will not affect your usual care or treatment in any way.

8) Can I withdraw from the study at any time?

Yes, you can choose to leave the study at any time.

9) Will the study benefit me in any way?

For patients with epilepsy we can’t be certain that there will be any benefit from taking part. However if we do find differences in the way blood vessels work in people with epilepsy, it may help us to work out ways of reducing the risk of blood vessel problems in the future.
As you do not suffer from epilepsy there are no direct benefits to you for taking part, however the information that we hope to gain from the study has the potential to improve the health of many people in the future. We will let you know the results of your ultrasound studies once all the studies have been done and the overall study results. There will be a delay in getting this information to you.

10) **Do you have permission to do the study?**

We have permission from the Central Regional Ethics Committee to do this study.

11) **What if I have other questions about the study?**

Please contact Dr Esko Wiltshire, Paediatrician, at any time. He can be paged through Wellington Hospital on (04) 3855999, pager 6912, or extension 6912. Or contact Dr Lynette Sadleir on 04 385 5999 ex 6147. Ngaire Keenan can also be contacted on 04 918 6138. If you have any questions or concerns about your rights as a participant in this study you may wish to contact the Health and Disability Advocate, Telephone: Mid and lower North Island 0800 42 36 38 (0800 4 ADNET) or the Central Regional Ethics Committee, Phone 04-496 2405.
**Appendix C: Consent Form**

Wellington School of Medicine and Health Sciences

*Child Consent/Assent Form to Participate in Research*

*‘Early Vascular Disease in Children with Epilepsy Receiving Anticonvulsants’*

**REQUEST FOR INTERPRETER**
*(to be included on all consent forms)*

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<th>Language</th>
<th>English Description</th>
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<th>No</th>
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<td>I wish to have an interpreter.</td>
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<td></td>
</tr>
<tr>
<td>Maori</td>
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<td>Ae</td>
<td>Kao</td>
</tr>
<tr>
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<td>Ioe</td>
<td>Leai</td>
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<tr>
<td>Tongan</td>
<td>Oku ou fiema’u ha fakatunnela.</td>
<td>Io</td>
<td>Ikai</td>
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<td>Cook Island</td>
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<td>Ae</td>
<td>Kare</td>
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<tr>
<td>Niuean</td>
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<td>E</td>
<td>Nakai</td>
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<td>Other</td>
<td>Other languages to be added following consultation with relevant communities.</td>
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1. I have read and I understand the information sheet dated 4th September 2007 entitled ‘Early Vascular Disease In Children With Epilepsy Receiving Anticonvulsants’ I have had the opportunity to discuss this study with my parents and the researcher. I am happy to take part.

2. I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time and this will in no way affect my future health care or continuing health care.

3. I have had this project explained to me by ________________________________.

4. I understand that my participation in this study is private and that nothing that could identify me will be used in any reports on this study.

5. I understand that the investigation will be stopped if it should appear harmful to me.
6. I understand what happens if anything goes wrong (that is the compensation provisions for this study).

7. I have had time to consider if I want to take part.

8. I know whom to contact if I have any side effects to the study.

9. I know whom to contact if I have any questions about the study.

10. I consent to storage of ultrasound images for further use in this research project ……….YES/NO

11. I consent to storage of ultrasound images for use in future research projects that have received ethical approval from the Central Ethics Committee …………….YES/NO

13. I would like the researcher to discuss the outcomes of the study with me …………….YES/NO

14. I understand that my GP will be informed of my participation in this study/the results of my participation in this study, if I wish……………………………………………..YES/NO

I ____________________________________________________________________________ (full name) hereby consent to take part in this study.

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<th>Date</th>
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<tbody>
<tr>
<td>Signature</td>
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<tr>
<td>Full names of Researchers</td>
<td></td>
</tr>
<tr>
<td>Contact Phone Number for researchers</td>
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<tr>
<td>Project explained by</td>
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<tr>
<td>Project role</td>
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<tr>
<td>Researcher Signature</td>
<td></td>
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<tr>
<td>Date</td>
<td></td>
</tr>
</tbody>
</table>

For more information about ethical issues or other concerns regarding this research

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