

Effects of cold and clothing on metabolism in female cyclists

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ABSTRACT

Research investigating substrate metabolism during exercise with female participants is limited, especially regarding effects of cold stress. The aims of this study were to determine whether exercising in cold ambient temperature affects substrate metabolism in females and whether the addition of a thermal layer alters the metabolic response in the cold. Trained female cyclists ($n = 11$, age 26 ± 7 y, height 1.69 ± 0.08 m, body mass 63.9 ± 7.2 kg, $\sum 8$ skinfolds 111.6 ± 22.5 mm, body fat $20 \pm 3\%$, $\dot{V}O_{2\max}$ 48 ± 8 ml.kg⁻¹.min⁻¹, mean \pm SD) performed three trials in pseudo-random order; two at 5 °C (Cold and Clothing (C5 °C)) and one at 15 °C (Temperate), 93 and 79% relative humidity (RH), resp. Exercise comprised warm-up (30 min $\sim 30\%$ $\dot{V}O_{2\max}$) in 17.8 °C (43% RH), 75 min cycling at $\sim 74\%$ $\dot{V}O_{2\max}$, and a 4 km time trial in the test environment. The same attire was worn in each trial, with the addition of an extra thermal layer in the second 5 °C trial (Clothing; C5 °C), all with a fan at 3.9 m.s⁻¹. Total carbohydrate (CHO) and fat oxidation (indirect calorimetry every 15 min) were calculated and corrected for protein oxidation (from sweat and urine urea). CHO oxidation was partitioned using [¹³C₆]glucose tracer methodology, measuring enrichment in breath (CO₂) and blood (glucose). Heart rate (HR), skin temperature (\bar{T}_{sk}) (nine sites), core temperature (T_c) (rectal), and subjective measures of thermal sensation, discomfort and rating of perceived exertion (RPE) were recorded every 15 min. Protein oxidation was not different between conditions ($p < 0.05$). Neither total CHO, fat oxidation, muscle glycogen or liver-derived glucose oxidation were different ($p > 0.05$) between Cold and Temperate. Reduced fat oxidation in Clothing versus Cold (5 °C: 0.33 ± 0.15 g.min⁻¹; C5 °C: 0.25 ± 0.21 g.min⁻¹, mean \pm SD) was observed ($p = .031$), and a trend of increased CHO oxidation in Cold (5 °C: 1.96 ± 0.44 g.min⁻¹; C5 °C: 2.19 ± 0.48 g.min⁻¹; $p = .058$). T_c increased across exercise ($p < .001$) but was not different between Cold and either Temperate or Clothing. \bar{T}_{sk} was ~ 6 °C and ~ 1 °C lower in Cold compared to Temperate ($p < .001$) and Clothing (p

< .05). Thermal sensation was lower and discomfort was higher in Cold than Temperate (both $p < .001$). Only thermal sensation was modified by Clothing ($p < .001$). There were no differences in time trial performance between Cold and Temperate ($p = .23$), and Cold and Clothing ($p = .60$). In conclusion, there is no large difference in metabolism between moderately intensive cycling at 5 °C vs. 15 °C (with substantial facing air velocity) in women. The women in this study may be resilient to temperature variation possibly due to body composition and or sex hormone influences. Interestingly, the addition of clothing tends to stimulate CHO metabolism in the cold, decreases fat metabolism, and may reflect heat stress rather than a lack of cooling.

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LIST OF ABBREVIATIONS

15 °C	-	Temperate condition
5 °C	-	Cold condition
BSA	-	Body surface area
C5 °C	-	Clothing condition
CHO	-	Carbohydrate
CO ₂	-	Carbon dioxide
CVS	-	Cardiovascular system
FFA	-	Free fatty acids
F _{total}	-	Total free fatty acid oxidation
G _{muscle}	-	Muscle glycogen oxidation
G _{plasma}	-	Blood borne glucose oxidation
G _{total}	-	Total glucose oxidation
HR	-	Heart rate
IL-6	-	Interleukin-6
IMCL	-	Intramyocellular lipid content
LBM	-	Lean body mass
MG	-	Muscle glycogen
OCP	-	Oral contraceptive pill
R _a	-	Rate of appearance of glucose
R _d	-	Rate of disappearance of glucose
R _{exp}	-	¹³ C isotope abundance of breath CO ₂
R _{glu}	-	¹³ C isotope abundance of plasma glucose
RER	-	Respiratory exchange ratio
RH	-	Relative humidity
RPE	-	Rating of Perceived Exertion

RQ	-	Respiratory quotient
TE	-	Total energy
T_a	-	Ambient temperature (°C)
T_c	-	Core (rectal) temperature (°C)
T_m	-	Muscle temperature (°C)
\bar{T}_{sk}	-	Mean skin temperature (°C)
U_{SG}	-	Urine specific gravity
$\dot{V} CO_2$	-	Volume of carbon dioxide expired (L/min)
$\dot{V} E$	-	Volume of expired air
$\dot{V} O_2$	-	Volume of oxygen consumed (L/min)
$\dot{V} O_{2 \max}$	-	Maximal volume of oxygen consumed (L/min)

1.0 INTRODUCTION

Environmental temperature can affect substrate metabolism during exercise. In particular, the effects of high ambient temperature have been extensively studied and results are consistent. In contrast, far less research has been conducted on the effects of low ambient temperature on substrate metabolism in the cold and results are equivocal. Research on energy metabolism during exercise has been confounded by varying methodologies including lack of specification of attire and any convection cooling, as well as intensity of exercise and technique of measuring metabolism. Additionally, the majority of research detailing substrate metabolism during exercise has been conducted with men. Therefore, our study was designed to address these shortcomings and evaluate substrate metabolism in women in the cold, with and without a thermal layer of clothing.

Both sexes perform sport at various levels, yet the majority of exercise physiology research continues to use male participants, hence the female population seems to be at a disadvantage with regard to knowledge of their exercise metabolism. Hormonal differences across the menstrual cycle in female participants may influence responses to exercise, so most researchers have opted for male participants. However, several studies have illustrated metabolic differences due to sex (Hardy & Du Bois, 1940; for a review see Tarnopolsky, 2008), thereby indicating a need for more in-depth research to understand factors that may influence metabolism in both sexes. More studies with female participants would increase the knowledge base which could allow the exercise demands of female athletes to be met with appropriate training and nutritional strategies.

Sportswomen and sportsmen typically train nearly all year round especially in sports such as triathlon, running and cycling (Doubt, 1991). In New Zealand, as in many parts of the world, winter air temperatures are often below 10 °C. Exercise in hot as well as cold temperatures can alter substrate utilisation; in particular carbohydrate (CHO) usage may be

increased in cold environmental conditions (Febbraio, Snow, Stathis, Hargreaves, & Carey, 1996b; Layden, Patterson, & Nimmo, 2002; Patterson, Reid, Gray, & Nimmo, 2008). There is, however, a paucity of research into CHO metabolism in exercise in cold temperatures, and results are equivocal (decreased CHO utilisation - Fink, Costill, & Van Handel, 1975; Dolny & Lemon, 1988; increased CHO utilisation - Febbraio et al., 1996b; Layden et al., 2002; Patterson et al., 2008). Furthermore, there are no published studies of the effect of cold environmental conditions on CHO metabolism during exercise in females.

Similarly, effects of clothing on metabolism during exercise have not been extensively investigated. Cyclists normally wear little during competition yet tend to train with more layers when it is cold. However, when training in cool conditions there may be increased use of muscle and liver glycogen, and therefore a greater stimulus for adaptation, as a result of increased glycogen depletion.

The purpose of this study was to investigate the effects of low ambient temperature on substrate metabolism, in particular on carbohydrate metabolism. A second purpose was to investigate the effect of clothing on substrate metabolism when exercising in cold temperatures (5 °C). It was hypothesised that CHO metabolism would be increased in the cold compared to the temperate environment, and that the addition of clothing would be similar to a temperate environment hence utilisation of CHO would be increased in the cold compared to the clothing condition.

2.0 LITERATURE REVIEW

2.1 *Substrate partitioning and exercise*

The amount of carbohydrate (CHO) oxidised during exercise depends on many factors including duration and intensity of exercise, feeding prior to exercise, and reserves of CHO in the body (Brooks & Mercier, 1994). However, if exercise was to be supported by CHO alone then exercise could not last longer than ~2 h due to limited glycogen reserves. This is especially evident if exercise intensity is higher than ~60% of the maximal rate of oxygen consumption ($\dot{V}O_{2\max}$) (Bergström, Hermansen, Hultman, & Saltin, 1967; Coyle & Coggan, 1984). Liver glycogenolysis contributes more to total CHO oxidation as muscle glycogen depletes which increases with the duration or intensity of exercise. However the liver gluconeogenic pathways cannot fully compensate for the depletion (Ahlborg & Felig, 1982; Coyle & Coggan, 1984). Therefore, the source of CHO and the relative contribution of fat and CHO to total energy expenditure is influenced by the intensity and duration of exercise.

Training status influences substrate metabolism. Increases in training status increase the relative contribution of fat oxidation and therefore CHO oxidation decreases during exercise in the fasted state (Christensen & Hansen, 1939 cited in van Loon, Jeukendrup, Saris, & Wagenmakers, 1999; Horton, Grunwald, Lavelly, & Donahoo, 2006). The training-induced increase in fat oxidation was shown to be present during moderate-intensity (55% $\dot{V}O_{2\max}$) exercise even when easily metabolised exogenous glucose is available (van Loon et al., 1999). Therefore, CHO oxidation is altered through mechanisms that relate to the relative intensity and duration of exercise. Carbohydrate is not a continuing source of energy over long periods of exercise as its proportional contribution to energy supply decreases over time and increases in response to increasing exercise intensity. Therefore,

CHO is the primary energy source for high intensity exercise, i.e. in most competition situations < 2 h duration, therefore it is important to understand what mechanisms alter CHO oxidation and how it can be measured.

Measuring carbohydrate utilisation

Several methods have been used to measure substrate utilisation during exercise. The most common method is determination of the respiratory exchange ratio (RER) through indirect calorimetry to represent the respiratory quotient (RQ) at the cellular level (Krogh & Lindhard, 1920 cited in Hultman, 1967). This method has been used to the greatest extent during exercise as it is non-invasive, reliable and provides a good estimate of CHO and fat utilisation when exercise is fueled by aerobic metabolism.

The muscle biopsy technique (Bergström, 1962) combined with biochemical analyses is also used in many studies as it provides measurement of muscle glycogen concentration at the sampling times, and therefore muscle glycogen baseline availability and extent of use across exercise, as well as fibre-type differences. However, measurement via muscle biopsy is invasive, poses an inherent risk of injury and infection and there are various errors associated with the measurement. First, it only represents a single muscle and a small sample of that muscle, taken to be representative of the whole. Second, each measurement is depth dependent, and subsequently multiple measures over time are limited. Therefore, an alternate method to allow calculation of muscle glycogen, such as the stable isotope, technique is useful in exercise science.

Within the last ten years, a surge of studies has emerged using various stable isotope techniques. Two of the most common involve either a [6,6-²H]glucose tracer which involves ~2.5 h of infusion prior to exercise, or a [¹³C₆]glucose tracer ingested ~30 min prior to exercise. The hydrogen (6,6-²H) tracer is designed to measure whole body glucose kinetics via the rate of appearance (R_a) and rate of disappearance (R_d) of glucose. However, this

method uses slightly different assumptions for calculating estimated muscle glycogen utilization compared to the [$^{13}\text{C}_6$]glucose method, as explained below.

The [$^{13}\text{C}_6$]glucose stable isotope technique is an accurate and less invasive method for measuring CHO oxidation over time and partitioning it into muscle and liver glycogen oxidation (Burelle, Peronnet, Charpentier et al., 1999). Previous methods have used corn-based glucose solutions to naturally label the glucose, with small amounts of highly enriched glucose to ensure the enrichment is high enough. This method also works when investigating CHO feeding studies. The current method involves participants ingesting small (< 1 g) amounts of highly enriched [$^{13}\text{C}_6$]glucose (99% enriched) at least 30 min prior to exercise to saturate the bicarbonate pool so that results during steady state exercise are reliable (Coggan, 1999). Therefore, the enrichment of [$^{13}\text{C}_6$]glucose in the blood and the ratio of $^{13}\text{C}/^{12}\text{C}$ in CO_2 in the breath, measured throughout exercise, can be measured via mass spectrometry, which is assumed to be representative to that of the liver.

Harvey et al. (2007) validated this stable isotope method with the muscle biopsy technique to show that although the isotope technique measures total muscle glycogen use and the biopsy technique measures a change in concentration in one musculature, the two are highly correlated ($r = 0.81$) in trained male cyclists during steady state cycling at both 48 and 78% $\dot{V}\text{O}_{2\text{max}}$. However, Devries, Hamadeh, Phillips and Tarnopolsky (2006) also used both a stable isotope tracer and muscle biopsies with female cyclists during 90 min cycling at 65% $\dot{V}\text{O}_{2\text{max}}$ and found very little correlation ($r = 0.1$) between methodologies. This is possibly due to the different stable isotope tracers used between the two studies as Harvey et al. (2007) used a carbon tracer whereas the Devries et al. (2006) study used a hydrogen tracer. As mentioned previously, the two methods differ in the calculations inherent in estimating muscle glycogen.

The differences in popular stable isotope methodologies was investigated by Jeukendrup, Raben, Gijssen et al. (1999). The glucose kinetics during 120 min of cycling at

50% $\dot{V}O_{2\max}$ was compared using a [$^{13}\text{C}_6$]glucose tracer and a [6,6- ^2H]glucose tracer, concurrently. The primary investigation of this study was to compare no CHO (i.e. water), low-glucose, and high-glucose during exercise. In addition, each participant completed each trial twice, once without isotope infusion to correct the infusion trials for background $^{13}\text{CO}_2$ enrichment (Jeukendrup et al., 1999).

The study of Jeukendrup et al. (1999) provides two important pieces of information when comparing studies using slightly different stable isotope methodologies. Firstly, the trials without tracer infusion did not significantly change background $^{13}\text{CO}_2$ enrichment. This means that the tracer technique is accurate in that the high enrichment allows accurate measurements so use of any stores of natural [$^{13}\text{C}_6$]glucose are negligible. Secondly, the [$^{13}\text{C}_6$]glucose tracer slightly underestimated the R_a compared to the [6,6- ^2H]glucose tracer, however this was not significant in any of the three trials (Jeukendrup et al., 1999). Importantly, data collected from the [$^{13}\text{C}_6$]glucose tracer was only used from 60 min onwards, as that was when $^{13}\text{CO}_2/^{12}\text{CO}_2$ breath ratios reached a plateau, allowing accurate calculations of partitioning from 60 min. With these three points in mind, the stable isotope technique using [$^{13}\text{C}_6$]glucose is able to provide accurate measurements of muscle glycogen (MG) and liver-derived glucose across time and as a total, without the limitations of muscle biopsy measures.

Using stable isotopes to examine substrate utilisation during exercise in the cold would allow the amount of glycogen utilised when cold stressed during exercise to be calculated and therefore partition where the dominant source of CHO is from. Furthermore, as there appear to be some discrepancies regarding differences in metabolism due to sex, this method is appropriate to investigate whether exercise in cold ambient temperatures (T_a) does increase CHO oxidation during exercise in females as previous studies with male studies have suggested, and to investigate whether the partitioning of the CHO oxidised is different between cold and temperate conditions.

Sex

It is unclear whether there is a clear sex difference in metabolism, since sex differences that have been noted due to differences in body composition or training status can be negated as training status increases and with it often the difference in body fat (Romijn, Coyle, Sidossis, Rosenblatt, & Wolfe, 2000). Both of these effects can reduce female sex hormones and, therefore, the sex difference could be attributed to reduced female sex hormone production as well as the reduced body fat per se. Romijn and colleagues (2000) found that after correcting for absolute lean body mass between sexes, no difference in substrate metabolism was observed at 65 and 85% of $\dot{V}O_{2\max}$, hence, as both groups were highly trained, training status may have overridden the hormonal effects. Irrespective of temperature, Tarnopolsky (2008) concluded that some of the observable sex difference in metabolism during endurance exercise could be attributed to the increased 17β -oestradiol in women as this reproductive hormone upregulates fat metabolism during endurance exercise. These conclusions were based on a lower RER observed in women exercising at 65% $\dot{V}O_{2\max}$ compared to men at the same relative intensity (Tarnopolsky, Macdougall, Atkinson, Tarnopolsky, & Sutton, 1990; Phillips, Atkinson, Tarnopolsky, & Macdougall, 1993) which indicated more fat being metabolised.

In a review of exercise in the cold Nimmo (2004) describes details of females in the cold under the heading “Special Populations” (p. 907). However, the only special detail about females is the hormonal milieu alluded to earlier hence resulting in the lack of data from females amongst the current literature. It could be speculated that physiological changes related to metabolism (i.e. the regulation of fat and CHO utilisation) during exercise in the cold could differ between sexes due to a greater percentage body fat in females and in combination with, or because of, different sex hormones. Matching males and females for body mass and composition, age, acclimation to thermal environment and $\dot{V}O_2$ negates thermoregulation differences of sex, but results cannot always be applied for female athletes

in training and competition (Kaciuba-Uscilko & Grucza, 2001) and even then this does not take into consideration the more usual hormonal sex differences. A more practical option would be to match males and females with heterogeneous characteristics then determine the regulatory responses during exercise in the alternate environments (Kaciuba-Uscilko & Grucza, 2001), however it could be argued that this is not necessarily more insightful or realistic.

Kaciuba-Uscilko and Grucza (2001) also alluded to the lack of information given in some studies that use female participants with regard to which menstrual cycle phase was tested, as this could add to variability in results. In addition, training status, diet, and hormonal status of female participants are all known to affect substrate metabolism (Tarnopolsky et al., 1990) therefore they must be controlled across studies so an accurate comparison of the relative changes can be made. It is time consuming when testing is done in a particular phase of the menstrual cycle, but it is feasible and necessary to control variability to be able to give more robust results. Although many studies have been conducted to investigate substrate metabolism in females, none have evaluated the effects of environmental temperature. Stevens, Graham, and Wilson (1987) conducted the only study that was designed to investigate metabolism of females exercising in the cold but it is based solely on indirect calorimetry and therefore contributions from various source of carbohydrate could not be partitioned. To date, substrate metabolism during exercise in the cold has only been evaluated in male participants and since some difference in metabolism have been noted between the sexes, it may be that the response to exercise in the cold may differ for females and therefore should be investigated.

2.2 Factors influencing metabolism during exercise in the cold

As far back as the 1970s studies were conducted to investigate mechanisms for coping with cold environments whilst exercising (Fink et al., 1975). Exercise intensity and duration both affect metabolism in relatively temperate environments and these metabolic differences

are increased during exercise in cold temperatures (Layden et al., 2002). Convection and clothing are also important methodological factors to consider across studies to evaluate differences in observed results (Saunders, Dugas, Tucker, Lambert, & Noakes, 2005; Jett, Adams, & Stamford, 2006). Therefore, it is pertinent that these factors are considered when comparing studies of exercise in the cold.

Exercise intensity and duration

Early studies of cold environments were focused mainly on the metabolic changes associated with shivering in extreme environments (Vallerand & Jacobs, 1989). Shivering begins in the torso muscles spreading to the limbs, recruiting more musculature and increasing oxygen consumption (Castellani, Young, Ducharme et al., 2006), hence more CHO and fat are oxidised (Burton & Edholm, 1955 cited in Jacobs, Romet, & Kerriganbrown, 1985), especially if the exercise intensity is quite low. The duration of exercise influences the substrate utilised, with proportionally more fat oxidised as the duration increases when exogenous CHO is not available, i.e. as per in exercise (Coyle, Coggan, Hemmert, & Ivy, 1986). Therefore, these studies can be compared accurately when duration and intensity of exercise are considered to evaluate substrate utilisation.

The amount of time spent in the cold prior to exercise can also alter metabolism, therefore making it difficult to compare studies. However, it provides more information than if all studies had used the same amount of cold exposure before exercise. For example, Timmons, Araujo and Thomas (1985) had cyclists sit in an environment of -10 °C or 22 °C for 30 min prior to exercise and observed a significant decrease in RER at -10 °C. Therefore, this could be related to being cold stressed before the exercise began, rather than a result of exercising cold stress. Similarly, Hurley and Haymes (1982) had participants sit in 10 °C for 60 min prior to exercise and found a significant reduction in the RER for the 60 min exercise protocol (at 50 W in 10 °C) compared to sitting in a 'neutral environment' of

25 °C for 30 min before the same exercise. This clearly illustrates the effect the cold thermal environment has prior to exercise, as does the intensity of the exercise.

Many investigators seem to have chosen an exercise intensity of around 60-70% $\dot{V}O_{2\max}$ but the duration of exercise in these studies varied from 40 to 90 min (Timmons et al., 1985; Dolny & Lemon, 1988; Sink, Thomas, Araujo, & Hill, 1989; Febbraio, Murton, Selig et al., 1996a; Febbraio et al., 1996b; Galloway & Maughan, 1997; Layden et al., 2002). The variation in exercise duration, as alluded to earlier, could explain some of the metabolic differences observed. Exercise intensity $\sim 65\% \dot{V}O_{2\max}$ has been proposed by Brooks and Mercier (1994) as the ‘crossover’ above which CHO utilisation predominates over that of fat, depending on endurance training status i.e. greater if more trained.

Therefore the majority of research is conducted at an intensity (i.e. $\sim 60-65\% \dot{V}O_{2\max}$), which is near the “crossover” so that a change in T_a could elicit a change in substrate utilisation. Hence, it seems more important to investigate whether there is indeed a change in CHO metabolism when the intensity is such that CHO metabolism would need to be predominant for exercise to be able to be sustained. Similarly, as the duration increases, so does the mobilisation of fat as a fuel source, but it is unknown whether this phenomena is also observed when exercising in cold environmental conditions.

Research across sexes with regard to fat metabolism has led to the suggestion that female athletes are better suited to endurance events such as marathons and ultramarathons, due to superior lipid oxidation of females over their male counterparts (Tarnopolsky et al., 1990; Brooks & Mercier, 1994; Tarnopolsky, 2008). The mechanism behind increased lipid oxidation in females is not fully understood but increased quantities of fatty acid translocase and associated transporters, lower levels of testosterone, and increased intramyocellular lipid (IMCL) content and use during exercise, are all factors that contribute to differences between sexes (Tarnopolsky, 2008). However, as previously discussed, the differences between sexes is not clear cut, albeit in the majority of studies, increased fat oxidation has

been observed in females, although the relative effect that body composition may have has not been thoroughly examined.

The intensity and duration of exercise are very important considerations when determining whether environmental temperature has an effect on substrate metabolism. Similarly, the environment prior to exercise may have some, if not more, effect on substrate utilisation than the exercise itself, especially if the exercise intensity is low. Studies using female participants have indicated an upregulation of fat metabolism in endurance exercise, making it more pertinent to investigate whether this upregulation of fat metabolism still occurs when exercising in low T_a .

Facing air velocity

A cyclist is almost always creating convection therefore it is appropriate to try to replicate this in the lab, or application of results could be misleading. Unfortunately though, description of air velocity, and thus convective heat transfer potential, is disregarded in published research in which temperature is being monitored and or controlled (e.g. Febbraio et al., 1996a) or is insufficient to replicate the velocity of cycling (e.g. Galloway & Maughan, 1997; Layden et al., 2002). Thus, the important but mostly neglected role of air velocity limits the validity of findings from most lab-based studies for exercise in the field.

Saunders et al. (2005) investigated the change in heat storage by altering the air velocity via fans. They found that heat storage was reduced when cycling with a wind speed of $33 \text{ km}\cdot\text{h}^{-1}$ compared to $10 \text{ km}\cdot\text{h}^{-1}$. Similarly, wind speeds of $0.3 \text{ m}\cdot\text{s}^{-1}$ and $3.0 \text{ m}\cdot\text{s}^{-1}$ were compared in temperatures of $24 \text{ }^\circ\text{C}$ and $35 \text{ }^\circ\text{C}$ to also illustrate increased strain via core temperature (T_c) and mean skin temperature (\bar{T}_{sk}) (Adams, Mack, Langhans, & Nadel, 1992) and therefore the importance of appropriate facing air velocity. Although this exercise testing was conducted in the heat, it can still be relevant to exercise in the cold as the convection cooling would increase the external validity by reducing heat storage and thereby enhancing any effect on metabolism. In cold T_a , the extra convective cooling due to wind

($1.0 \text{ m}\cdot\text{s}^{-1}$ and $5.0 \text{ m}\cdot\text{s}^{-1}$) at a low temperature ($-10 \text{ }^\circ\text{C}$) has been found to affect thermoregulation but not metabolic rate when walking at $2.8 \text{ m}\cdot\text{s}^{-1}$ (Makinen, Gavhed, Holmer, & Rintamaki, 2001). However, the temperature used by Makinen et al. (2001) is considerably low, when wind chill is taken into consideration the temperature is even lower (Osczevski & Bluestein, 2005), therefore it may be an effect of the temperature being so low that the low fan speeds had no effect on metabolic rate. However, it still stands that the use of a fan in lab-based testing would make the results realistic and applicable, especially in this instance as many cyclists would be cycling at speeds approximating $30 \text{ km}\cdot\text{h}^{-1}$ in training.

Clothing

The issue of clothing in the cold has been reviewed by Jett, Adams, and Stamford (2006), who note that there is a particular lack of information provided in the reports of many studies on clothing worn. The hands, feet and face provide the best sites for thermoregulation in offloading heat in hot conditions due to their ability to vasodilate (Cotter & Taylor, 2005). Conversely, it could be that hands and feet vasoconstrict to a greater extent in the cold to save heat, and the head may be where the majority of the heat is lost. If this is the case, whether the participants wore gloves, shoes and socks, or even a helmet is an important factor not only when studies are compared, but also when assessing the practical application of results. The knowledge of clothing items worn in studies that are published can also become especially important when trying to relate results of studies in the cold to competition situations, where exercising in very little clothing may be the norm.

Conversely, training attire may comprise of extra layers when environmental temperatures are low (below $10 \text{ }^\circ\text{C}$), although thermal clothing items are not normally worn due to the decreased ability to dissipate heat as sweat (Pandolf, Sawka, & Gonzalez, 1988; Gavin, 2003). However, textile and garment design are constantly changing and thus it could be expected that garments should be specifically designed with such parameters in mind. Furthermore, asking participants to exercise in cold T_a wearing clothing appropriate to

compare to temperate environments, but most likely inappropriate for cold environments makes data collected inappropriate to apply to practical situations.

The textile being examined in this study is a silk, merino blend is not specifically designed for wearing during exercise. The clothing would be expected to increase \bar{T}_{sk} by virtue of reducing radiative, convective and evaporative heat transfer, which could ultimately also cause the T_c to increase to a greater extent than without clothing. For example, plasma osmolarity would become higher while plasma volume would diminish to a greater extent – both of which can raise T_c – and more cardiac output would be required to perfuse the skin.

As discussed further on, substrate metabolism during exercise of a moderate intensity in cold ambient conditions is altered, such that more CHO is oxidised than in a warmer environment. These studies have exhibited higher T_c and or lower \bar{T}_{sk} . (Febbraio et al., 1996b; Layden et al., 2002). Therefore, if the additional clothing increases \bar{T}_{sk} and T_c it would be expected that CHO oxidation might be higher with the additional clothing.

Body adiposity also plays a large role in insulating the body (Kaciuba-Uscilko & Grucza, 2001). Women have a higher body fat percentage compared to (leaner) males. The extra amount of body fat serves as additional insulation, in which females require less intensive exercise to maintain body temperature. Therefore an alteration in substrate metabolism has been observed (Tarnopolsky et al., 1990). Consequentially, investigating whether thermal clothing has any effect on substrate metabolism in females has its merits as the results could be used to make recommendations for women to manipulate substrate usage. Thereby, maximising power output over a given distance and/or period of time, thus enhance performance. Additionally, outside of the competitive realm, understanding metabolic demands in different environmental conditions may contribute to appropriate strategies, nutritionally and behaviourally, to reduce exposure risks associated with exercising in the cold.

2.3 *Metabolic changes in the cold*

Most of the evidence indicates that CHO utilisation is reduced with exercise in the cold as decreased RER has been observed (Fink et al., 1975; Hurley & Haymes, 1982; Timmons et al., 1985; Dolny & Lemon, 1988). However, the results of several recent studies contradict these findings reporting increased CHO utilisation via RER measures at $\sim 65 \dot{V}O_{2\max}$ (Febbraio et al., 1996b; Layden et al., 2002). The low intensity of Hurley & Haymes (1982) and large temperature difference of Fink et al. (1975) and Timmons et al. (1985) could explain some of the differences compared to results of Febbraio et al. (1996), Layden et al. (2002) and Patterson et al. (2008). The intensity of exercise of $\sim 65\% \dot{V}O_{2\max}$, presumably near the “crossover” intensity (Brooks & Mercier, 1994) in research by Febbraio et al. (1996b) and Layden et al. (2002) could be one of the main explanations for the contradictory findings. However, the participants in Patterson et al. (2008) exercised at $70\% \dot{V}O_{2\max}$ to find a similar difference in RER towards increased fat oxidation in the colder temperature.

Only one study has been conducted to investigate the effects of metabolism whilst exercising in cold temperatures in females. Stevens, Graham, and Wilson (1987) compared the cardiovascular and metabolic responses of males to those of females, but did not report specific metabolic data. Both sexes completed the same incremental cycling protocol at the same absolute intensities (i.e. higher $\% \dot{V}O_{2\max}$ for females). Therefore, metabolic differences would be expected, irrespective of temperature, due to the higher ratio of fat mass to free fat mass, and also the oxygen carrying capacity of females being $\sim 10\%$ lower even when composition is matched (Tarnopolsky et al., 1990). However, the only differences between sexes were observed when exercising in 5°C . The temperature had no effect on $\dot{V}O_2$ in the women between conditions (5°C vs. 21°C). However men had significantly increased $\dot{V}O_2$ relative to those in women at two of the four measurement intensities (50 and 150 W) whilst exercising in 5°C .

The data reported by Steven et al. (1987) are hard to interpret, as they are in other studies, as the relative intensity of exercise was not matched between sexes. Stevens et al. (1987) attributed the difference in ventilation to shivering or non-shivering thermogenesis. Also, the women had approximately 9% greater body fat than the men, which may have provided greater insulation and therefore the effect of the cold causing shivering could have been minimised in the women. This notion is supported by both the higher T_c of males compared to females regardless of condition and the higher T_c in 5 °C compared to 20 °C regardless of sex (Stevens et al., 1987). Although a difference in ventilation was reported, it is unknown whether a difference in fat and CHO utilisation occurred. Similarly, the women were exercising at a different relative intensity and therefore the substrate partitioning difference that could have been observed is more likely to be attributed to the intensity rather than the environmental conditions.

In another study comparing men, eumenorrhic women, and amenorrhic women, responses to exercise were compared in 5 °C and 22 °C and at rest (Graham, Viswanathan, Van Dijk, Bonen, & George, 1989). They found no metabolic differences that could not be attributed to body fatness or body surface area. They found that men responded more quickly to the cold temperature with an earlier $\dot{V}O_2$ response, but no differences between eumenorrhic women and amenorrhic women were observed, indicating that men are initially more sensitive to temperature changes (Graham et al., 1989), agreeing with research indicating that the metabolic response to cold is linearly dependent on the lack of adiposity (Keatinge, 1960). It also indicates that the hormonal response may not be responsible as the hormonal milieu would be expected to be different in eumenorrhic than in amenorrhic women.

Earlier studies of ventilatory data of exercise metabolism in the cold have shown a significant decrease in RER, indicating higher fat use than in warmer conditions (Hurley & Haymes, 1982; Timmons et al., 1985; Dolny & Lemon, 1988). However, all of these studies

compared the data from the cold temperatures ranging from $-10\text{ }^{\circ}\text{C}$ to $+10\text{ }^{\circ}\text{C}$ to that of $\sim +20\text{ }^{\circ}\text{C}$ to $+25\text{ }^{\circ}\text{C}$ which could be considered warm in relation to performing exercise. Therefore, the effect of cold per se may not be as pronounced or may be quite different if compared with a more temperate environment. More recently, Febbraio and colleagues (1996b) observed a significant increase in RER in exercise at $65\% \dot{V}O_{2\text{max}}$ in $3\text{ }^{\circ}\text{C}$ compared to $20\text{ }^{\circ}\text{C}$. There was no mention of air velocity, and exercise duration was 40 min. Muscle glycogen utilisation decreased in the cold environment (Febbraio et al., 1996b). As adrenaline concentrations decrease with exercise in the cold, it was assumed that total CHO utilisation would also be reduced and therefore the RER would decrease. However, a higher RER was observed in the cold which could be explained by increased liver glycogenolysis (in the face of lower muscle glycogen use). Febbraio et al. (1996b) also offered shivering in the inactive muscle as an explanation, but this was not visually observed. As the muscle glycogen concentration was not compromised due to intensity and duration, the increased liver glycogenolysis could be related to increased energy needs for warmth.

An increase in fat utilisation while cycling in $9\text{ }^{\circ}\text{C}$ versus a $41\text{ }^{\circ}\text{C}$ environment was observed in a series of experiments by Fink et al. (1975). Six males completed three 15-min exercise bouts at an intensity of $\sim 70\text{-}85\% \dot{V}O_{2\text{max}}$, with 10 min rest periods in between. Fat metabolism, measured as change in muscle triglyceride concentration, was significantly greater in the colder condition, whereas $\dot{V}O_2$ and blood lactate were elevated to a greater extent in the warmer condition. Rectal temperature was maintained at normal levels throughout the cold trial, and increased nearly $2\text{ }^{\circ}\text{C}$ in the hot condition even though exercise intensity averaged a relatively low 138 W. No mention was made of fans or other means of heat loss from the subjects apart from evaporation, so the significance of this study to the cycling population is limited. The observed T_c rise may have been attenuated with the use of a fan due to a lower \bar{T}_{sk} and therefore a bigger gradient of heat dissipation through sweating. With a lower T_c , fat metabolism may not have been as downregulated and

therefore the difference may have been less. That is to say that a difference of 32 °C between conditions is quite extreme; so whether convection would make any difference is unknown.

In accordance with the findings of Febbraio et al. (1996b), Layden et al. (2002) observed increased RER and, thus, whole-body CHO oxidation, during 90 min of exercise at 64% $\dot{V}O_{2\max}$ in -10 °C and 0 °C compared with exercise in +10 °C and +20 °C. No reference was made to air velocity; however clothing was detailed as they used mittens and earmuffs in the two cold conditions. Following on from the Layden et al. (2002) study, Patterson, Reid, Gray, and Nimmo (2008) investigated the possible role of interleukin-6 (IL-6) in the altered metabolism illustrated during exercise in the cold. Males exercised at 70% $\dot{V}O_{2\max}$ for an hour in environmental temperatures of 0 °C and +20 °C, with very low wind speed ($0.36\text{ m}\cdot\text{s}^{-1}$). Circulating IL-6 concentrations were found not to be responsible for the change in metabolism, but more importantly, a significant change in substrate utilisation was observed using indirect calorimetry. Supporting the results of Layden et al. (2002), the trained males showed a reduced fat and increased carbohydrate utilisation for a mere 5 min of the 1 h exercise block.

Increased fat utilisation as the environmental temperature is reduced has been observed in male participants (Hurley & Haymes, 1982; Timmons et al., 1985; Dolny & Lemon, 1988). Notably, these studies did not report the use of facing air velocity. They also used high temperatures as a comparative, assuming these were temperatures for optimal performance (i.e. +25, +20, and +22 °C respectively), yet these may be heat stressful. Hence the warmer temperatures, combined with the presumed lack of convection, could be responsible for the observed difference in CHO utilisation between conditions, more so than if a less heat stressful environment had been used. Conversely, increases in CHO metabolism in cold environments have also been observed (+3 and 0 °C) (Febbraio et al., 1996b; Layden et al., 2002; Patterson et al., 2008). All six of these studies were conducted

using male participants, and, therefore, whether the same effects would be exhibited in female participants is as yet unknown. Moreover, whether an upregulation or downregulation of CHO utilisation would occur is unknown.

2.4 *Other hormonal influences*

Changes in catecholamines could underlie observed shifts in substrate metabolism with cold exposure. The increase of circulating levels of catecholamine hormones adrenaline and noradrenaline during prolonged exercise act to progressively increase use of CHO and endogenous fuels at intensities above $\sim 60\% \dot{V}O_{2\max}$ (Borer, 2003). An elevation in noradrenaline concentration during exercise in a cold environment has been reported (Anderson & Hickey, 1994) along with a decreased response (Febbraio et al., 1996b). Similarly, adrenaline responses have shown both an increase (Vallerand, Jacobs, & Kavanagh, 1989) and decrease (Graham, Sathasivam, & Macnaughton, 1991) as a result of cold exposure during rest and exercise, respectively. In relation to metabolism, adrenaline is known to act on the liver in response to increased blood glucose, at the muscle in response to increased blood lactate, and at the adipose tissue in response to increased FFAs (Zouhal, Jacob, Delamarche, & Gratas-Delamarche, 2008). Therefore, an additional measure of catecholamine response could be an important factor when explaining any change in substrate metabolism that might occur with changes in environmental temperatures.

It is well known that insulin plays a role in metabolism, as it is upregulated after feeding for glucose transport into cells (Horton & Terjung, 1988; Maughan, Gleeson, & Greenhaff, 1997). Insulin concentration is depressed during exercise in order for glucose and plasma free fatty acids (FFA) to be available in the bloodstream for energy utilisation (Holloszy & Kohrt, 1996). Increased catecholamines inhibit the release of insulin with sympathetic nervous system activation in exercise (Maughan et al., 1997). The response of catecholamines and insulin relate to different types of strain on the body, i.e. blood glucose and exercise intensity for insulin, and blood flow and cardiovascular system (CVS) strain for

catecholamines, and therefore, if changes in catecholamines and metabolism are evident insulin may give more insight into the predominant mechanisms.

2.5 *Summary*

The published research on substrate metabolism of women exercising presents results that are often different to men. As many countries regularly experience temperatures below 10 °C, it is of practical interest to know how substrate metabolism is affected as this may be of benefit for training and nutritional recommendations, and safety in relation to continued substrate availability during prolonged cold exposure during exercise (e.g., channel swimming, skiing or sled racing). Control of air velocity and participants' clothing during exercise are important details often not included in publications of modified environmental temperature affecting metabolic responses to exercise. Importantly, results of such studies need to be compared to those of a more temperate environment for exercise (i.e. ~15 °C) rather than warm where a plethora of literature exists and an upregulation of CHO metabolism, and to elucidate the effects of cold per se. Hence, comparing with a more 'thermoneutral' environment can give accurate measures of differences in substrate metabolism. Accordingly, the use of the stable isotope method similar to that of Burelle et al. (1999) to determine liver and muscle glycogen utilisation can provide additional information concerning possible alterations in the source of carbohydrate oxidised. In summary, the literature of exercise in cold thermal environments, especially with regard to the changes in CHO metabolism and the source of CHO oxidised, in females is unknown. Hence, the purpose of the current study was to investigate the effects of low ambient temperature on carbohydrate metabolism via the hypothesis that CHO metabolism would be increased in the cold compared to the temperate environment. Secondly, the study was designed to investigate the modulating effect of clothing on substrate metabolism when exercising in cold temperatures (5 °C), hypothesising that the addition of clothing would be more like a temperate environment and therefore reduce CHO utilisation compared to Cold.

3.0 METHODS

3.1 Study design

Each participant completed three trials during the follicular phase of hormonally controlled menstrual cycle randomised for first participant, then sequenced such that a balanced and fully repeated measures design was completed. Testing was employed during the autumn-winter months of the southern hemisphere (March-August; average daily maximum outside temperature of 13.4-5.3 °C, respectively; National Institute of Water and Atmospheric Research (NIWA), 2008). Two trials were conducted in an environmental temperature of 5 °C, one in normal cycling attire and one with the addition of thermal clothing. The third trial was conducted at 15 °C in normal cycling attire, deemed temperate. Henceforth, the trials will be referred to as 'Cold' (5 °C), 'Clothing' (C5 °C) and 'Temperate' (15 °C), respectively. Exercise consisted of a warm-up of 30 min at 30% $\dot{V}O_{2\max}$, before 75 min at 70% $\dot{V}O_{2\max}$, followed by a 4 km time trial. Venous blood and breath were sampled at rest and during exercise (see Figure 3.2). Indirect calorimetry and the appearance of ^{13}C from similarly labeled glucose in blood and expired breath were used to partition the substrate oxidation, including the source of glucose oxidised.

3.2 Power Analysis

The number of participants chosen was 10 based upon power analyses of earlier work on exercise metabolism in similar temperatures. The first determination was of minimum expected change in oxidation of total CHO from the data of Galloway and Maughan (1997); where a difference of $\sim 0.3 \text{ g}\cdot\text{min}^{-1}$, a standard deviation of $\sim 0.3 \text{ g}\cdot\text{min}^{-1}$ and statistical power of 0.8 gave a sample size of 10 (actual power = 0.803). The second determination was based on the minimum expected change in RER, again from the data of Galloway and Maughan (1997); where a difference of $\sim 0.02 \text{ g}\cdot\text{min}^{-1}$, a standard deviation of $\sim 0.02 \text{ g}\cdot\text{min}^{-1}$, and

statistical power of 0.8 also gave a sample size of 10 (actual power = 0.833). However, as the present study involved three trials (but only pair wise comparisons for the two separate questions, of ambient temperature and clothing) and an expected a drop out rate of ~12-20%, 11 volunteers were recruited.

3.3 Participants

Eleven healthy, trained female cyclists and triathletes aged 18-43 y that were taking oral contraceptive pills (OCP) were recruited to participate in this study. All women were on monophasic OCP for at least three months prior to entering the study and reported regular menstrual cycles. Each participant was informed of testing procedures and risks before giving written voluntary informed consent. The procedures and practices of this project were approved by the University of Otago Ethics Committee (ethics application 162/08).

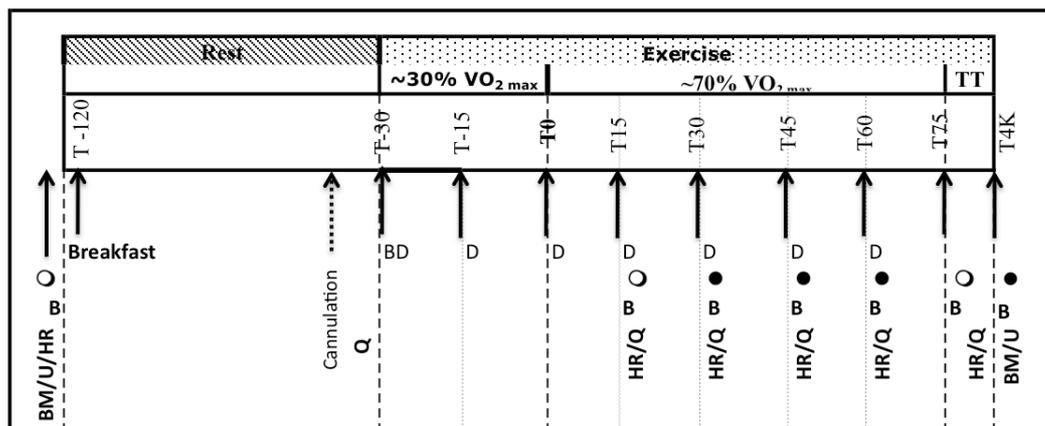


Figure 3.3. Timeline of experimental session. BD = bolus labeled water (0.04 g CHO); D = labeled water (0.0133 g); ○ = 12 mL blood sample; ● = 9 mL blood sample; B = breath sample/ $\dot{V}O_2/\dot{V}CO_2/\dot{V}E$; BM = body mass; U = urine specific gravity; HR = heart rate; Q = questionnaires (RPE/TD/TS).

3.4 Preliminary testing

Height, mass, and an estimate of body composition using bioimpedance analysis (InBody 230 Body Composition Analyser, Biospace Co Ltd, Seoul, Korea) were recorded at baseline. All participants underwent an incremental cycling test to exhaustion to ensure that they met the criteria for being categorised as 'trained', specifically defined as a maximal

oxygen uptake ($\dot{V} O_{2 \max}$) $> 40 \text{ ml O}_2 \cdot \text{kg}^{-1} \text{ body mass} \cdot \text{min}^{-1}$ and training $> 1.0 \text{ h}$ at least three times per week. The incremental cycling test to exhaustion, as described by Kuipers, Keizer, Brouns, and Saris (1987), was performed using open circuit spirometry (Cosmed Cardio Pulmonary Exercise Testing, CosmedSrl, Rome, Italy). For each individual, 70% of the $\dot{V} O_{2 \max}$ was calculated and the data were interpolated to find the corresponding workload (Watts), which was then set in experimental trials. Participants were asked to self-monitor menstrual cycle by recording the name, brand and cycle of the pill. Bioimpedance analysis was also conducted at the participants' third trial (i.e. at least three months after the initial assessment). Participants' phase of the menstrual cycle was not controlled for the preliminary testing.

3.5 *Experimental testing*

Participants performed all three trials, each separated by at least 1 month, and maximally 2 months, on days 3 – 11 of the menstrual cycle, where day 1 is first day of active pills. All testing sessions were conducted in the morning. Blood plasma concentrations of progesterone and oestrogen were taken to confirm menstrual cycle phase.

3.6 *Standardisation*

The participants were required to keep a training and dietary log for two days prior to each of the three experimental trials. This was replicated for the remaining trials. Two days prior to testing participants were asked to complete ~90 min of moderate intensity to deplete muscle glycogen stores. Exercise was limited to 2 h during the 48-24 h prior to testing and no exercise was to be conducted during the 24 h preceding testing.

Participants were asked to refrain from consuming foodstuffs containing cane sugar and corn for at least two days prior to testing, due to the high natural enrichment of ^{13}C , which was later verified with the food diaries. This depletion and selective CHO repletion

minimised the natural abundance of ^{13}C in endogenous glycogen stores, thereby also minimising ^{13}C background levels in breath $^{13}\text{CO}_2$ and plasma glucose (Burelle *et al.*, 1999).

For all trials, participants wore their own short-sleeved cycle top, cycle shorts, sports bra, socks, shoes, short-fingered gloves and helmet. Participants wore the same set of clothing in each of the three trials. In the Clothing condition, participants additionally wore a ‘thermal’ layer (Silkspun, Silkbody, Dunedin, New Zealand) consisting of a long sleeved top (style SB101) worn under the cycle top to be as close to the skin as possible, and long pants (style SB113) worn over the cycle shorts for comfort reasons. The garments were a single jersey knit structure composed of $158.9\text{ g}\cdot\text{m}^2$ fabric, made up of 72% silk, 13% wool, and 15% cotton. Fabric thickness was 0.74 mm, “dry” thermal resistance was $0.01789\text{ m}^2\text{K}\cdot\text{W}^{-1}$, and “wet” thermal resistance was $0.00305\text{ m}^2\text{K}\cdot\text{W}^{-1}$ (van Amber, 2009).

3.7 *Experimental procedures*

Participants arrived at the laboratory between 6:45 – 7:00 am having fasted for at least 10 h. A urine sample was taken and baseline body mass was then recorded to a resolution of 0.02 kg with calibrated, electronic scales (Wedderburn scales LTD, Dunedin) with participants wearing only a sports bra and cycle shorts. Thereafter, a heart rate monitor (Polar S810i Heart Rate monitor, Polar Electro Inc., Port Washington, NY) was fitted and participants rested in a reclining chair for at least 10 min. During the last 5 min, resting breath measurements were collected.

Participants then consumed a standardised breakfast (Sustagen™ Sport powder chocolate flavour, Nestlé; 45 g mixed with 330 ml trim milk; providing 1385 kJ energy, 46.8 g CHO, 23.8 g protein, 4.5 g fat). Participants were asked to consume the meal within ten minutes. Consequentially, time of completed consumption was recorded and this then set the timing of the trial as T-120. Participants were allowed to study or read until T-60 after which core and skin thermistors were attached and a flexible cannula (22 gauge Teflon intravenous

cannula) was inserted into an antecubital vein of the left forearm and a resting blood sample was drawn (T-45 – 30).

Protocol

Participants began a 30-min warm up (T-30) at 30% $\dot{V}O_{2\max}$ in the ante-room (adjoining the environmental chamber, 17.8 ± 2.2 °C and $43 \pm 5\%$ RH) at 60-75 W on a Monark 828E cycle ergometer (Monark Exercise AB, Vansbro, Sweden) wearing only their cycle top, sports bra, shorts and socks. At the cessation of the warm-up participants were given the option of voiding, with the volume measured to the nearest 1 mL. Shoes, helmet, gloves and glasses were donned, as was the extra layer of thermal clothing in the Clothing trial. The participant then entered the environmental chamber and sat on an electromagnetically-braked cycle ergometer (Veletron Dynafit Pro, RacerMate® Inc, Seattle, USA) prior to starting the exercise (approximately 2 min).

The participant then began exercising at $\sim 70\%$ $\dot{V}O_{2\max}$ for 75 min with convection provided via a large standing fan at $3.9 \text{ m}\cdot\text{s}^{-1}$ (655 mm blade, Imasu, Japan). Once the 75 min had been completed, the participant stopped for 1 min to allow the change of program from the set intensity to the time trial program. The participant rated her perceived exertion (RPE), thermal discomfort, and thermal sensation every 15 min.

Participants then completed a 4 km time trial as quickly as possible (Version 1.5, RacerMate® Inc, Seattle, WA) with workload recorded at 1 Hz and averaged over the trial. Participants were informed only of distance (each 500m, then 200m and 100m before the finish). Total time was not revealed. Once the sampling had finished the participant exited the chamber and then voided her bladder and removed all apparatus before reweighing in only shorts and sports bra.

3.8 *Experimental techniques*

Respiratory measures

During the last 3 min of every 15-min of exercise, the face mask was attached to measure expired volume (\dot{V}_E), volume of oxygen consumed ($\dot{V}O_2$), and volume of carbon dioxide expired ($\dot{V}CO_2$) (Cosmed Cardio Pulmonary Exercise Testing (CPET), CosmedSrl, Rome, Italy). During the last 30 s, expirate was collected into an adjoining latex Douglas bag vacated before attachment. Duplicate breath samples were transferred in 1 mL aliquots into 10 mL exetainers for storage and subsequent analysis of ^{13}C from breath CO_2 . For a detailed description of the procedure of taking breath samples see Appendix H. The face mask was left on for the duration of the time trial for continuous measurement of the breath variables. At the completion of the time trial, the Douglas bag was attached and 30 s of breath was collected immediately following exercise cessation.

Skin folds

During the rest/study time in each participant's second trial, skin folds were measured at the eight sites. Measurements of skin fold thickness (mm) were taken from eight right-side sites: triceps, biceps, iliac crest, abdominal, supraspinale, subscapular, front thigh, and medial calf (Marfell-Jones, Olds, Stewart, & Carter, 2006). These were summated (Σ Skin folds).

Temperatures

Core temperature (T_c) was measured using a flexible, sterile and disposable thermistor (Mallinckrodt Medical Inc., St Louis, USA) which participants placed to ~10 cm. Following the rectal thermistor insertion, skin thermistors (Grant Instruments, Cambridge, England) were taped to nine right-sites; 1) forehead, above the eyebrow; 2) chest, midway between the nipple and the axilla; 3) bicep, over the belly of the muscle approximately half way between the elbow crease and acromion process; 4) scapula, medially from the inferior angle; 5)

forearm, posterolateral side approximately 2-3 cm below the line of the elbow joint; 6) 3rd finger, dorsal side over the middle phalanx; 7) thigh, anterior surface, midway down the femur; 8) posterior leg, at the widest part of the calf, and; 9) dorsum of the foot. Resting core and skin temperatures were recorded after cannula insertion, and were measured for the warm up at T-15. Temperatures were logged (Grant 1200 series Squirrel Data Logger, Grant Instruments Cambridge, England) at 15 s intervals throughout the 75 min and time-trial exercise.

Urea

To sample for urea concentration, a simple disposable sweat collector (Brisson, Boisvert, Peronnet *et al.*, 1991) was placed over the centre of the lower back prior to the warm-up phase. Urine and sweat samples (~1.5 mL ea) were collected at the cessation of the trial.

Stable isotope methodology

Each participant consumed 0.12 g of uniformly labeled [$^{13}\text{C}_6$]glucose (99atom%, Isotech) in 7 aliquots totaling 800 mL. An initial 200 mL bolus of ^{13}C labeled water (0.04 g U- ^{13}C glucose) was ingested at the beginning of the 30 min warm up (T-30). Thereafter, a 100 mL bolus of ^{13}C labeled water (0.013 g U- ^{13}C glucose) was consumed every 15 min (T-15, T0, T15, T30, T45, T60) immediately after the face mask had been removed. The U- ^{13}C D-glucose used was uniformly labeled on all 6-carbon atoms and had a guaranteed minimum purity of 99 atom %. This ensures a strong ^{13}C signal to be expressed in the breath, in excess of background isotope enrichment.

Hydration status

A urine sample was taken prior to recording body mass and following the time-trial. From these samples, urine specific gravity (U_{SG}) was taken as a measure of hydration status using a hand-held refractometer (Atago, Japan).

Blood collection

At rest, during the last two min of each 15-min block and within one min of the cessation of the time trial (T83), blood samples were drawn. Twelve mL was drawn at T15 and T75, and 9 mL was drawn at T30, T45, T60, and T83.

Resting blood and all subsequent samples were collected as follows: A small (~ 1 mL) waste sample was taken followed by a 12 mL blood sample, thereafter the catheter line was flushed with 1 mL saline. Seven mL of blood was put into a non-additive vacutainer, gently inverted five times then spun immediately for 10 min at 3000 rpm at 4 °C. For samples at rest (T-120), T15 and T75, 250 µL of serum was collected for insulin determination. The remaining serum (~2 mL) was collected in duplicate for ¹³C glucose isolation. Two mL of blood was put into a fluoride / oxalate vacutainer, gently inverted eight times upon which it was immediately spun and 250 µL of plasma was collected for plasma glucose concentration determination.

The extra blood drawn at rest, T15, and T75 (3 mL) was put into an EDTA vacutainer and gently inverted eight times upon which it was immediately spun and 250 µL of plasma was collected for catecholamine analyses. A 200 µL capillary tube (Scientific Glass Inc, Rockwood, USA) of blood was half-filled for measurement of blood lactate (1500 sport YSI lactate analyser). Furthermore, an extra 4 mL was collected when the cannula was first inserted, put into an EDTA vacutainer, and following the same process, duplicate samples of ~250 µL of plasma was collected for analyses of oestrogen and progesterone. All samples were frozen and stored in labeled microcentrifuge flip-top tubes at -80 °C until analysis.

Psychophysical measures

During breath collection stages (i.e. the last two min of each block) participants were asked to point to the number on a sheet placed in front of them that represented their rating of perceived exertion (RPE; 6: very very light – 20: very very hard), thermal sensation (1:

Unbearably Cold – 13: Unbearably Hot), and thermal discomfort (1: Comfortable – 10.0: Uncomfortable) (See Appendices G-J).

3.9 Analyses

Plasma glucose, sweat, and urine urea samples were analysed using an automated spectrophotometric analyser (Cobas c 111, Roche Instrument Centre, Switzerland). Plasma glucose concentration was determined using the hexokinase reaction (GLUC2, Roche Diagnostics GmbH, Indianapolis, USA). Sweat and urine samples were analysed for urea concentration using the glutamate dehydrogenase reaction (UREAL Urea/BUN, Roche Diagnostics GmbH, Indianapolis, USA). Plasma insulin levels were determined using an Electrochemiluminescence Immunoassay (ECLIA Insulin, Elecsys Insulin kit, Roche Diagnostics, Indianapolis, USA) in conjunction with Mr Ashley Duncan (Dept of Human Nutrition). The coefficient of variation (CV) for these measures was < 2.7%.

Catecholamine, progesterone and oestradiol samples were analysed by EndoLab laboratories (Christchurch, NZ) by HPLC-EP, enzyme-linked immunosorbent assay (ELISA) (Elder, Yeo, Lewis, & Clifford, 1987) and radioimmunoassay (spectria E2 sensitive, Orion Diagnostics, Finland). Coefficients of variation (averaged across concentrations, and as specified by the laboratory) were < 3.8%, < 8.0%, < 6.4%, and < 18% for noradrenaline, adrenaline, progesterone and oestradiol, respectively.

Serum from the non-additive vacutainers for ¹³C analyses was used to isolate the glucose using double-bed ion exchange chromatography (see Appendix I). The resulting supernatant was freeze-dried for analysis by isotope ratio mass spectrometry (IRMS) (Thermo Finnigan Trace GC Ultrac with GC combustion III interface, Waltham, USA). Breath samples were analysed in duplicate by gas chromatography continuous flow isotope ratio mass spectrometry (GC-IRMS; Thermo Finnigan Delta^{Plus} Advantage, Waltham, USA).

Calculations

$\dot{V} O_2$ ($L \cdot \text{min}^{-1}$) data is presented as the average over 2 min of steady state for each 15 min block. The time trial data, including $\dot{V} O_2$ and HR, is the average over the entire time that it took to complete the 4km distance. When RER values exceeded > 1.0 , further substrate oxidation partitioning analyses were not possible. This occurred at T75 for PP8 in Cold and Temperate trials, and for the majority of participants in the 4km time trial.

Total glucose and fatty acid oxidation were computed by indirect calorimetry (Cosmed CPET, CosmedSrl, Rome, Italy) and corrected for protein oxidation (Péronnet & Massicotte, 1991). Sweat loss was estimated as the change in body mass accounting for the ingestion of fluid during the exercise trial, urinary output, and corrected for respiratory exchange of CO_2 and O_2 and water vapour loss from the lungs. Urea in sweat was estimated by estimated sweat loss and its subsequent concentration. Similarly, urea in the urine was estimated by the volume collected and its subsequent concentration (Lemon & Mullin, 1980).

The amount of energy gained from oxidation of protein during exercise is substantial (Lemon & Mullin, 1980) and correcting indirect calorimetry for this can influence calculated proportions of fat and CHO (van Hamont, Harvey, Massicotte et al., 2005). The energy gain is calculated from measurements of urea content in the urine and sweat, neglecting the relatively small change in plasma urea (Burelle et al., 1999). The calculation also takes into account that 1 g of urea is equivalent to 2.9 g of protein oxidised, and that protein provides 19.695 kJ g^{-1} ($4.704 \text{ kcal} \cdot \text{g}^{-1}$) of energy (Livesey & Elia, 1988).

$$\text{Protein (kJ)} = \text{urea (mmol} \cdot \text{L}^{-1}) \times 60.045 \text{ (g} \cdot \text{mol}^{-1}) \times 2.9 \times 19.695 \text{ (kJ} \cdot \text{g}^{-1}) \quad (1)$$

Total glucose (G_{total}) and free fatty acid (F_{total}) oxidation are calculated from $\dot{V} O_2$ and $\dot{V} \text{CO}_2$ (Péronnet & Massicotte, 1991); corrected for the volume of O_2 and CO_2 that can be accounted for by protein oxidation ($1.010 \text{ L} \cdot \text{g}^{-1}$) and $0.843 \text{ L} \cdot \text{g}^{-1}$. The volume of O_2 and

CO₂ are determined from \dot{V}_E and their respective fractions in inhaled air and exhaled breath (Livesey & Elia, 1988);

$$G_{\text{total}} (\text{g}\cdot\text{min}^{-1}) = 4.5850 \dot{V} \text{CO}_2 - 3.2255 \dot{V} \text{O}_2 \quad (2)$$

and

$$F_{\text{total}} (\text{g}\cdot\text{min}^{-1}) = -1.7012 \dot{V} \text{CO}_2 + 1.6946 \dot{V} \text{O}_2 \quad (3)$$

with mass in g and volume in L·min⁻¹ at STPD. The energy gained from oxidation of glucose and fat is calculated on the basis that at 37 °C, one gram provides 16.1958 and 40.8054 kJ·g⁻¹ (3.8683 and 9.7460 kcal·g⁻¹) respectively (Péronnet & Massicotte, 1991).

The ¹³C isotope abundance of breath CO₂ (R_{exp}) and CO₂ released from the combustion of plasma glucose (R_{glu}) were determined by mass spectrometry, using the NBS-19 calibration standard.

In this case, there is virtually no exogenous glucose oxidation; therefore the rate of glucose oxidation is equivalent of the endogenous glucose oxidation.

$$G_{\text{endo}} (\text{g}\cdot\text{min}^{-1}) = G_{\text{total}} \quad (4)$$

Based on the ¹³C isotope abundances of plasma glucose (R_{glu}) and breath CO₂, the oxidation rate of blood borne glucose (G_{plasma}) (Derman, Hawley, Noakes, & Dennis, 1996) is computed as follows:

$$G_{\text{plasma}} (\text{g}\cdot\text{min}^{-1}) = \dot{V} \text{CO}_2 [(R_{\text{exp}} - R_{\text{ref}})/(R_{\text{glu}} - R_{\text{ref}})] \div k \quad (5)$$

Hepatic glucose flux derived from liver glycogen is calculated as the difference between plasma glucose oxidation (G_{plasma}, equation 5) and exogenous glucose, which in this case is effectively nil and therefore;

$$G_{\text{liver}} (\text{g}\cdot\text{min}^{-1}) = G_{\text{plasma}} \quad (6)$$

The oxidation of glucose and C₃ products derived from muscle glycogen, either directly or through the lactate shuttle (Brooks, 1986), is calculated as the difference in total glucose oxidation (G_{total}, equation 2) and plasma glucose oxidation (G_{plasma}, equation 5).

$$G_{\text{muscle}} (\text{g}\cdot\text{min}^{-1}) = G_{\text{total}} - G_{\text{plasma}} \quad (7)$$

To account for the delay in equilibrium of the $^{13}\text{CO}_2$ production in the expired breath and tissues, the computations were only made during the last 45 min of the exercise period allowing for a total of 60 min equilibration period during warm up and at the beginning of exercise, similar to other studies (Burelle et al., 1999; Harvey et al., 2007; Wallis, Yeo, Blannin, & Jeukendrup, 2007; Tremblay, Péronnet, Lavoie, & Massicotte, 2009).

Body surface area was calculated using Du Bois and Du Bois (1916, cited in Wang, Moss, & Thisted, 1992) where weight is in kg and height in cm:

$$\text{BSA} = 0.007184 \times \text{weight}^{0.425} \times \text{height}^{0.725}$$

Mean skin temperature (\bar{T}_{sk} , °C) was calculated from standard weightings, (ISO 9886, 2004) as:

$$\bar{T}_{\text{sk}} = 0.07 \cdot \text{forehead} + 0.175 \cdot \text{chest} + 0.07 \cdot \text{bicep} + 0.175 \cdot \text{scapula} + 0.07 \cdot \text{forearm} + 0.05 \cdot \text{finger} + 0.19 \cdot \text{thigh} + 0.15 \cdot \text{calf} + 0.05 \cdot \text{foot} \quad (8)$$

Data analysis

SPSS (Version 16.0, SPSS Inc, Chicago, IL) was used to conduct Linear Mixed Model analysis with factors of subject (maximum 11 subjects), and repeated measurement factors of time (maximum 5 levels) and condition (2 levels) for each measure. The levels of significant main effects for each factor were compared using the post-hoc Sidak comparison test. Paired t-tests with unequal variances for singular measures across conditions (resting, and 4km time trial values) were used. Cold and Temperate trials were compared, as were results from Cold and Clothing trials. Values presented in tables and figures are means (\pm SD) computed via SPSS and confidence limits were set at 95%.

Non-parametric measures of thermal scores and RPE were also analysed using mixed model analysis, with plots of residuals confirming normality across each measure.

Compound symmetry model was used for measures of thermal discomfort, thermal sensation, RPE and blood lactate. A first-order aggressive (AR1) model was used for measures of \bar{T}_{sk} , T_c change, blood glucose, RER, raw ^{13}C computations, catecholamine

concentrations (adrenaline and noradrenaline) and CHO, fat, muscle glycogen, and liver-derived glucose oxidation rates. Work rate ($\text{W}\cdot\text{m}^2$) and Σ Skin folds were used as covariates in mixed model computations. A log transform was applied to blood lactate concentration analyses so as to conform to the assumption of normality for the data. Significance was accepted at $p < .05$.

4.0 RESULTS

4.1 *Participant characteristics and compliance*

Ten participants completed all three trials, within the four-month maximum testing period. An additional participant (PP8) completed only two trials (Temperate and Cold) due to missing her second scheduled trial (sickness) and her fourth scheduled trial (injury). Furthermore, due to experimental error, PP10 completed her Clothing trial at a lower absolute intensity, therefore the relative workrate was also lower in that trial, and subsequently the data from this trial were not used in calculation of means and in statistical analyses. Therefore, the Clothing analyses were based on 9 of the 11 participants. As data from 9 participants were available for the Clothing condition, the mean of those 9 participants is presented as Cold (n=9) to distinguish between the two data sets.

RER values > 1.00 were removed from analysis (PP8 Cold T75, PP8 Clothing T75, and PP11 Clothing T75) as this indicates that the participants were working partially anaerobically, preventing accurate determination of CHO and fat oxidation rates. Due to mechanical error in measuring breath samples, partitioning results for PP3 Cold trial were unavailable and therefore, this participant's Cold trial was excluded from calculations of muscle glycogen and liver-derived glucose oxidation. As the average completion time of the 4 km time trial was ~ 7 min and therefore the time trial data points are plotted on figures at T83 to represent the time trial completion (taking into account the 1 min break between $\sim 74\% \dot{V}O_{2\max}$).

Diet records were checked to see if participants complied with limiting cane sugar and corn products. Although participants were instructed to avoid cane sugar and maize, as well as alcohol, one participant consumed maize on the evening prior to testing (PP3 Cold trial), and another participant consumed one glass of wine prior to each testing session (PP9).

Participants were questioned after testing to determine any changes in their training status over the course of the testing period. Six participants reported a decrease in training volume, one reported the same, and four reported an increase in training volume, over the time of the three trials. On average, participants were completing 7 ± 3 h cycling training per week prior to completion of their first trials, and 7 ± 4 h cycling per week prior to completion of their third trials.

Table 4.1 *Physical characteristics of participants (Mean \pm SD)*

	n=11	n=9
Age (y)	26 ± 7	26 ± 8
Body mass ^a (kg)	63.9 ± 7.2	63.9 ± 7.7
Height (cm)	169 ± 8	167 ± 8
BSA (m ²)	1.73 ± 0.14	1.72 ± 0.15
Body fat ^b (%)	20 ± 3	20 ± 3
LBM ^b (kg)	50.4 ± 6.0	50.1 ± 6.5
Sum of skinfolds ^c (mm)	111.6 ± 22.5	112.2 ± 23.0
$\dot{V}O_{2\max}$ (L·min ⁻¹)	3.09 ± 0.39	3.03 ± 0.41
$\dot{V}O_{2\max}$ (ml·kg ⁻¹ ·min ⁻¹)	48 ± 8	47 ± 7
$\dot{V}O_{2\max}$ (ml·kg LBM ⁻¹ ·min ⁻¹)	61 ± 7	61 ± 8

^a measured on day of $\dot{V}O_{2\max}$ pre-trial; ^b calculated from bioimpedence; LBM – lean body mass; ^c 8 sites

Six participants completed all three trials on the same day of their cycle. The ranges of days studied for each condition were: Temperate days 3 – 11, Cold days 3 – 11, and Clothing days 3 – 9. Oestradiol (pmol·L⁻¹) and progesterone (nmol·L⁻¹) concentrations (Table 4.2) confirmed the days of testing were similar between Temperate and Cold conditions, and Cold and Clothing conditions (oestradiol: $p = .16$, and $p = .23$, resp.; progesterone: $p = .85$, and $p = .99$, resp.).

Table 4.2. *Pre-exercise plasma sex hormones concentration (Mean ± SD)*

	Condition			
	Temperate (n=11)	Cold (n=11)	Cold (n=9)	Clothing (n=9)
Oestradiol (pmol·L ⁻¹)	45.6 ± 14.7	37.7 ± 17.0	35.7 ± 15.7	34.9 ± 14.0
Progesterone (nmol·L ⁻¹)	3.5 ± 1.4	3.6 ± 1.8	3.7 ± 1.6	3.4 ± 1.8

Participants were all on OC for >3 months prior to starting the study. Seven participants were taking the same combination OC. Each participant's OC is described in Table 4.3.

Table 4.3. *Monophasic oral contraceptive pill varieties used by participants*

Brand Name*	Ethinylloestradiol (mcg)*	Progesterone (mcg) and type*	Participant #
Levlen ED™, Monophene	30	150 – Levenogestrel	1,2,3,8 4,7,9
Marvelon™	30	150 - Desogestrel	5
Loette™	20	100 - Levenogestrel	6
Normin 28day™	35	500 - Norethisterone	10
Estelle™-35ED	35	2000– Cyproterone acetate	11

* information was obtained from (The Royal Women's Hospital, 2006)

4.2 *Environmental conditions and body mass changes*

Body mass was not different between Temperate and Cold, and Cold and Clothing conditions before exercise ($p = .68$, $p = .20$, resp.) and after exercise ($p = .40$, $p = .34$, resp.). Environmental conditions were equivalent between Cold and Clothing (Table 4.4, $p = .73$ temp, and $p = .70$ RH).

Table 4.4. *Initial environmental conditions and physiological variables (Mean ± SD)*

	Condition			
	Temperate (n=11)	Cold (n=11)	Cold (n=9)	Clothing (n=9)
Pre trial BM ^a (kg)	64.0 ± 7.0	63.9 ± 6.6	63.5 ± 7.6	63.9 ± 7.8
Post trial BM ^b (kg)	62.5 ± 7.2	63.1 ± 6.6	62.7 ± 7.6	63.0 ± 7.9
Environmental temp. (°C)	14.9 ± 0.2	5.2 ± 0.3	5.1 ± 0.2	5.1 ± 0.4
RH (%)	77 ± 6	92 ± 5	92 ± 3	93 ± 4

^a prior to experimentation; ^b corrected for breakfast weight (~380 g), volume consumed during exercise (800 mL) and urine excretion (~0.3-0.4 L); RH, relative humidity;

4.3 Exercise intensity and performance

The workload of 158 ± 19 W employed during the 75-min trials elicited an average absolute $\dot{V}O_2$ and relative intensity (Table 4.5) that was $0.05 \text{ L}\cdot\text{min}^{-1}$ and ~2% higher in the Cold condition (95%CI: $0.02 - 0.09 \text{ L}\cdot\text{min}^{-1}$, and 0.9 - 3%) than Temperate ($p < .001$), but not different to Clothing ($p = .62$). However, energy expended over the 75-min exercise period was similar between Temperate and Cold ($p = .28$), and Cold and Clothing ($p = .91$, Table 4.5).

Table 4.5. *Exercise intensity and energy expenditure (Mean ± SD) during ~74% $\dot{V}O_{2\text{max}}$ 75 min cycling in cold without (Cold) or with thermal clothing (Clothing) and in temperate (Temperate) conditions*

	Condition			
	Temperate (n=11)	Cold (n=11)	Cold (n=9)	Clothing (n=9)
$\dot{V}O_2$ ($\text{L}\cdot\text{min}^{-1}$) ^a	2.25 ± 0.26	2.30 ± 0.23	2.26 ± 0.24	2.26 ± 0.23
Intensity (% $\dot{V}O_{2\text{max}}$)	73 ± 2	75 ± 5	75 ± 5	75 ± 3
Energy expended (kJ)	3605 ± 420	3664 ± 374	3600 ± 379	3608 ± 355

^a not corrected for protein oxidation

4.4 Time trial performance

Time trial performance (Table 4.6) was not statistically different between Cold and Temperate, or between Cold and Clothing, when measured as time ($p = .23$ and $p = .60$,

resp.), work rate in Watts ($p = .37$ and $p = .42$, resp.) or work rate expressed as a percentage of $\dot{V}O_{2\max}$ ($p = .053$ and $p = .19$, resp.). HR was not different when comparing Cold to Temperate ($p = .45$) or to Clothing ($p = .84$). Subjective measures of rate of perceived exertion (RPE), thermal discomfort, and thermal sensation were all similar at the end of the time trial across conditions ($p > .05$) with the exception of participants rating their thermal sensation higher in the Temperate condition (Table 4.6, 95%CI: 1 – 3 points).

Table 4.6. *Intensity, physiological, and subjective responses (mean \pm SD) in the 4 km time trial cycle, in cold without (Cold) or with thermal clothing (Clothing) and in temperate (Temperate) conditions*

	Condition			
	Temperate (n=11)	Cold (n=11)	Cold (n=9)	Clothing (n=9)
Completion time (sec)	425 \pm 21	419 \pm 25	423 \pm 26	431 \pm 27
Intensity (W)	213 \pm 29	219 \pm 31	213 \pm 31	202 \pm 32
Intensity (% $\dot{V}O_{2\max}$)	87.8 \pm 5.5	91.2 \pm 6.0	91.5 \pm 5.2	89.2 \pm 6.2
HR (bpm)	179 \pm 11	180 \pm 11	179 \pm 12	179 \pm 11
RPE (scale 1-20)	18 \pm 1	18 \pm 1	18 \pm 1	18 \pm 1
TD (scale 1-10)	3 \pm 1	4 \pm 2	3 \pm 1	3 \pm 2
TS (scale 1-13)	9 \pm 2*	7 \pm 2	7 \pm 2	8 \pm 2

Time trial conducted after 75 min cycling at $\sim 74\%$ $\dot{V}O_{2\max}$; W, Watts; HR, heart rate; bpm, beats per min; RPE, rating of perceived exertion; TD, thermal discomfort; TS, thermal sensation, * significantly different to Cold (n=11) $p < 0.05$

4.5 Hydration status and body mass change

Pre-exercise hydration, measured by U_{SG} , did not differ between Cold and Temperate, and Cold and Clothing trials (15 °C: 1.020 \pm 0.009, 5 °C (n=11): 1.019 \pm 0.008; 5 °C (n=9): 1.018 \pm 0.008, C5 °C: 1.019 \pm 0.007; $p = .59$ and $p = .91$ resp.). As 800 mL of fluid was consumed during each testing session, participants were more hydrated after exercise ($p < 0.03$) in all conditions (15 °C: 1.015 \pm 0.008, 5 °C (n=11): 1.010 \pm 0.004; 5 °C (n=9): 1.010 \pm 0.004, C5 °C: 1.013 \pm 0.008; $p = .05$ and $p = .34$). Accordingly, no change in body mass was observed in any condition (Temperate: $p = .10$, Cold: $p = .30$, Clothing: $p = .28$).

4.6 Subjective measures

Thermal sensation was higher in Temperate than Cold (95%CI: 1.6 - 2.3; $p < .001$) and ratings did not change over time ($p = .72$, *Figure 4.1*). Clothing moderated thermal sensation so that ratings were higher (95%CI: 0.3 - 1.0) than for Cold ($p < .001$) but scores did not change over time ($p = .21$).

Thermal discomfort was lower ($p < .001$) in Temperate than Cold, but ratings were similar across the 75 min ($p = .68$, *Figure 4.2*). Thermal discomfort was not different in Clothing compared to Cold ($p = .68$).

RPE increased over time in all conditions (*Figure 4.3*), however the increase was greater in Temperate than in Cold ($p = .027$). Mean RPE was similar (< 1 unit) between Cold and Clothing ($p = .77$).

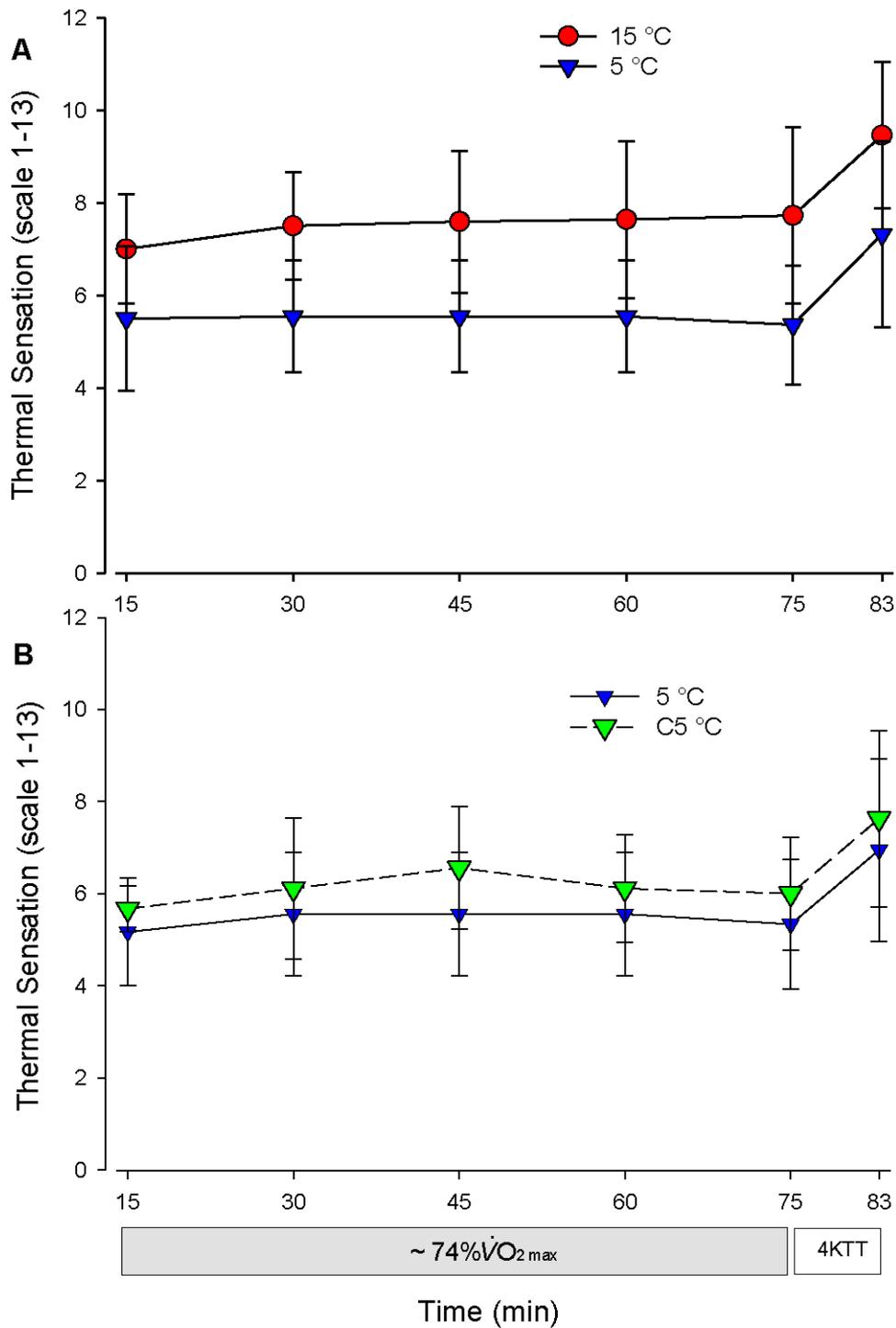


Figure 4.1. Mean (\pm SD) subjective responses of thermal sensation (scale 1 – 13, where 7 = neutral) during 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km time trial in the same environment.

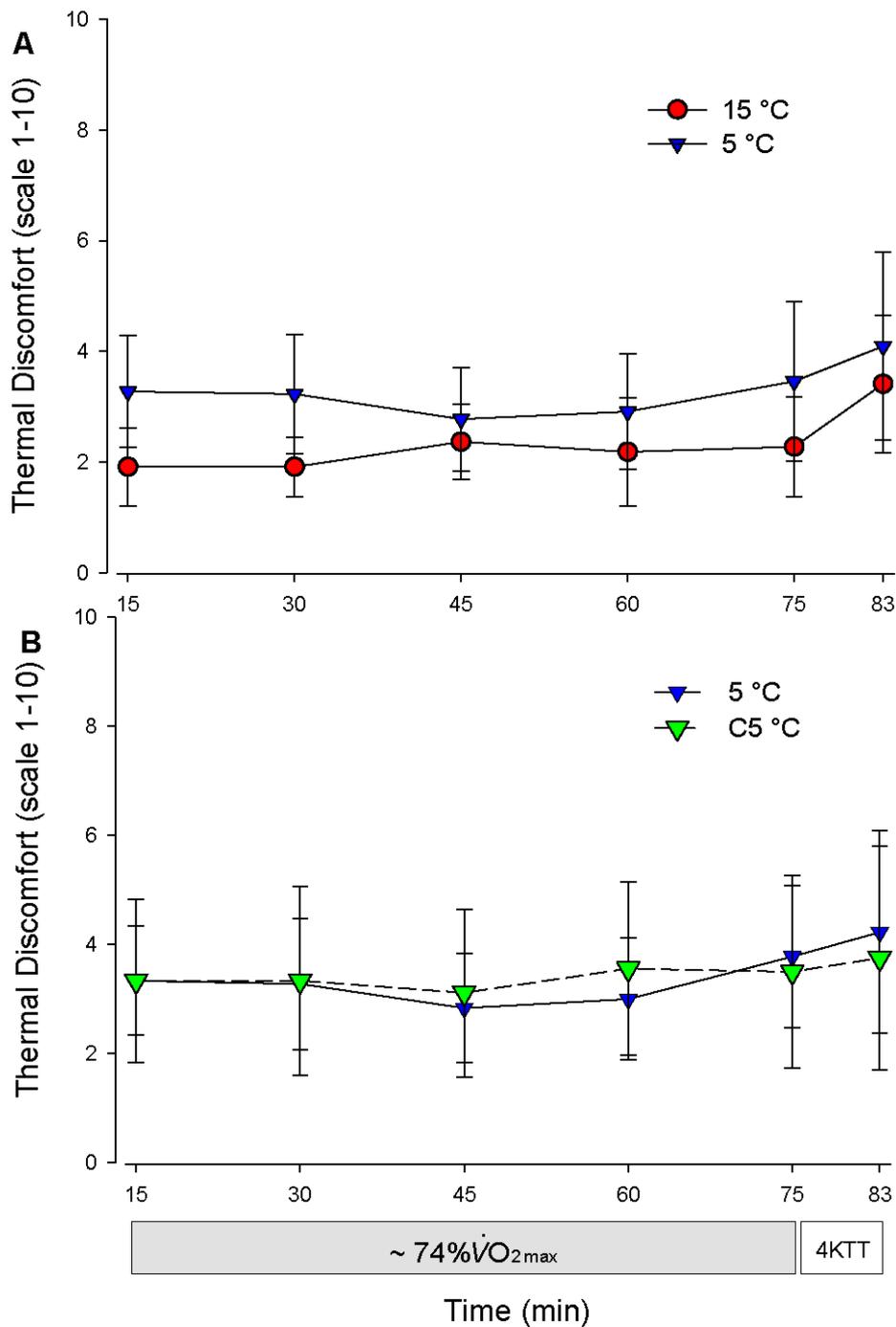


Figure 4.2. Mean (\pm SD) subjective responses of thermal discomfort (B; scale 1 – 10, where 1 = comfortable and 10 = very uncomfortable) during 75 min cycling at $\sim 74\% \dot{V}O_{2max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km time trial in the same environment.

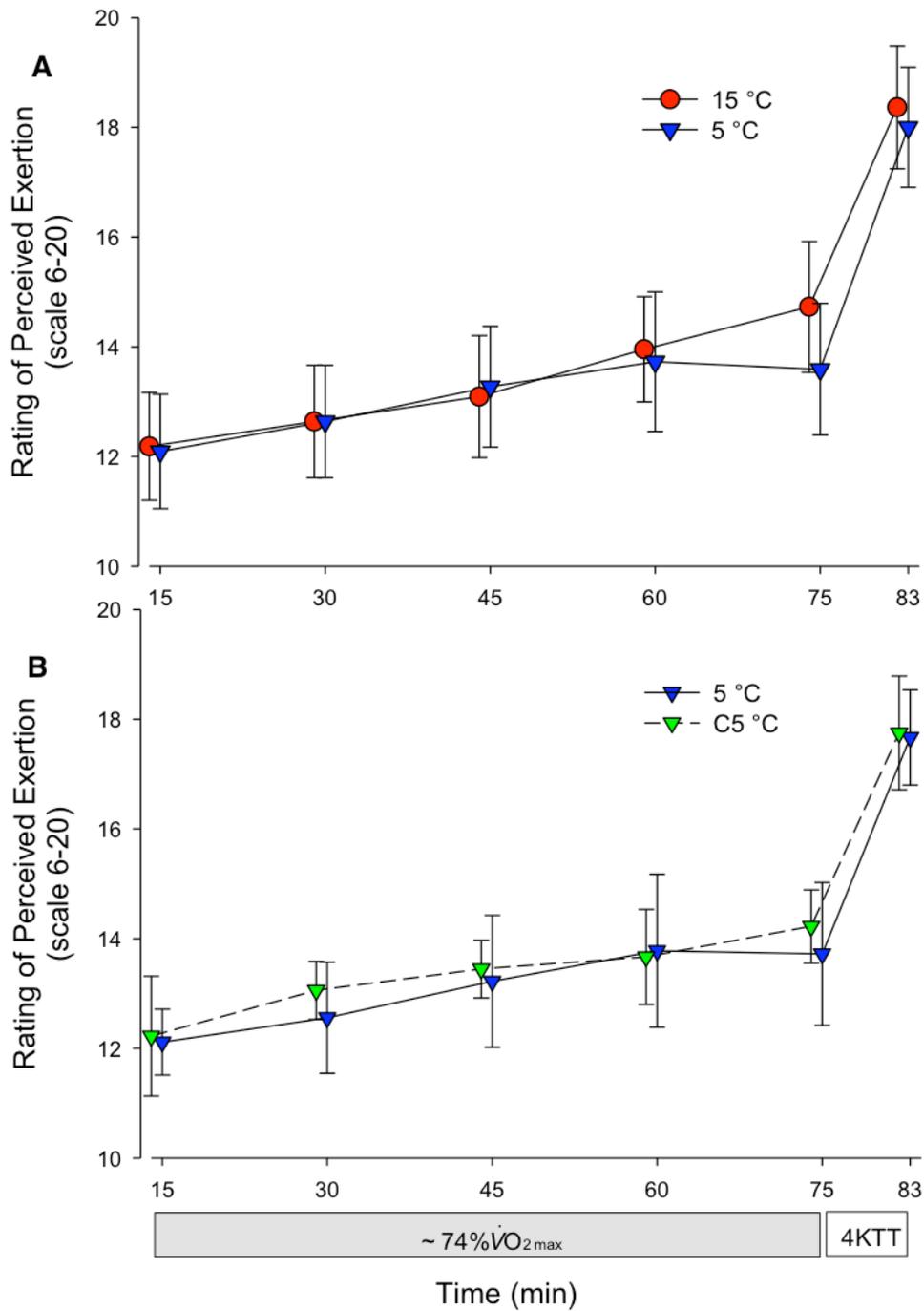


Figure 4.3. Mean (\pm SD) subjective responses rate of perceived exertion (Borg scale 6 - 20) during 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km time trial in the same environment.

4.7 Cardiovascular and thermal responses

Resting HR was similar between Temperate (57 ± 9 bpm) and Cold (57 ± 9 bpm) trials ($p = .84$). HR in Clothing was ~ 3 bpm higher (5 °C (n=9): 56 ± 10 bpm; C5 °C: 59 ± 9 bpm; 95% CI: 0.3-5.7 bpm, $p = .035$) than Cold at rest. HR increased during steady state exercise (*Figure 4.4*, $p < .001$) similarly across Temperate and Cold conditions ($p = .34$). Clothing also increased HR during steady state exercise ($p < .001$) however there was a trend for HR in the Clothing condition to be higher (95%CI: -0.2 - 3.7 bpm) than in Cold ($p = .08$).

Mean resting T_c was not different between Cold and Temperate ($p = .74$) or between Cold and Clothing ($p = .43$, *Figure 4.5*). All T_c data from PP2 was not included due to insufficient T_c recordings. Resting T_c varied across participants (*Figure 4.5*), therefore data are presented and analysed as the change from the resting value (ΔT_c , *Figure 4.6*).

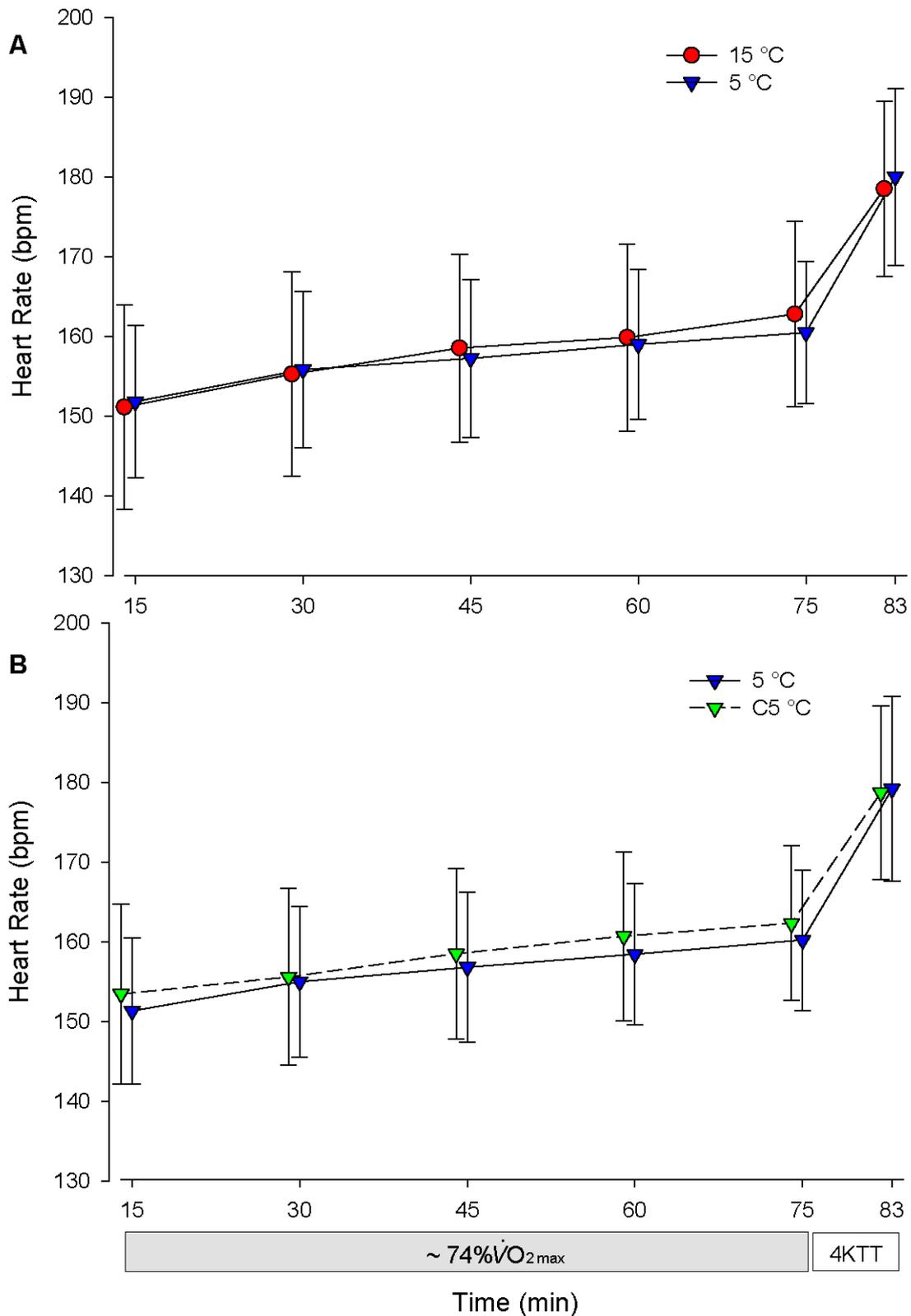


Figure 4.4. Mean (SD) heart rate (bpm) response to 75 min cycling at $\sim 74\% \dot{V}O_{2max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km time trial in the same environment.

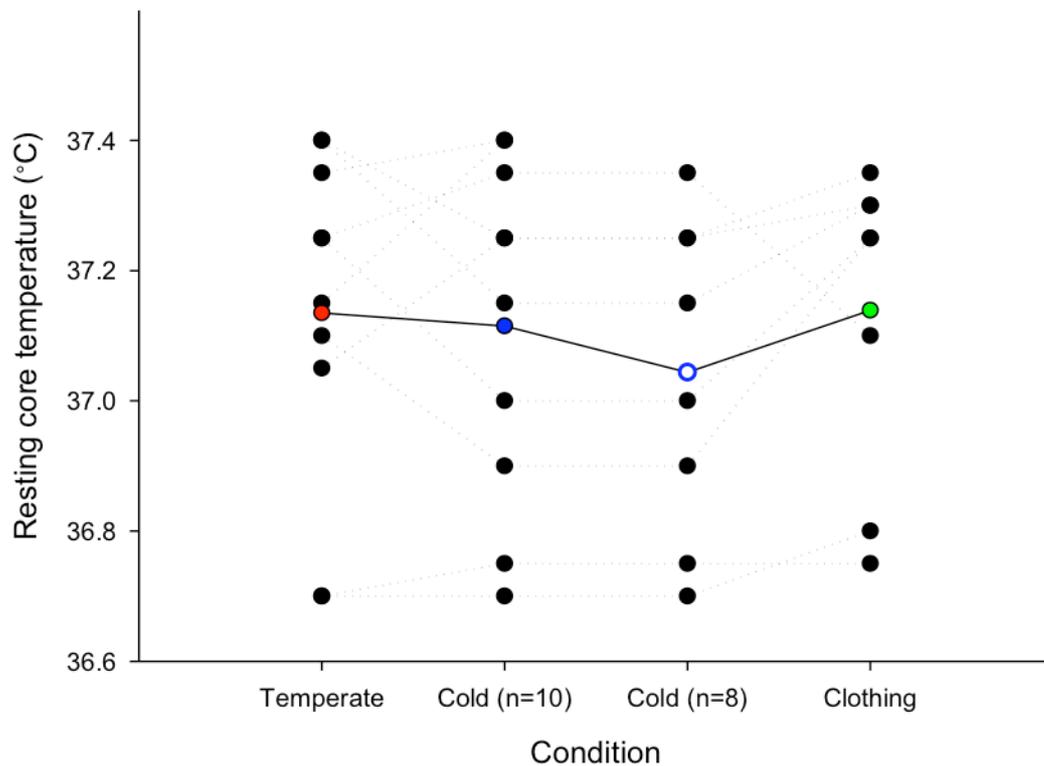


Figure 4.5. Core Temperatures (°C, rectal) measured at rest prior to starting warm up for each condition. Coloured points represent mean values for each condition. Mean \pm SD, Temperate (n=10): 37.14 \pm 0.26 °C, Cold (n=10): 37.12 \pm 0.26 °C, Cold (n=8): 37.04 \pm 0.24 °C, Clothing (n=8): 37.14 \pm 0.22 °C.

Mean ΔT_c increased progressively in exercise similarly ($p < .001$) in both Cold and Temperate, with no difference between the two conditions (Figure 4.6A, $p = .92$). Work rate ($W \cdot m^2$) was positively related to the extent of rise of ΔT_c . In Clothing the same progressive increase in ΔT_c during exercise was observed (Figure 4.6B, $p < .001$), and was lower than Cold, but not significantly so ($p = .12$).

Mean \bar{T}_{sk} declined progressively in exercise ($p < .001$), but more quickly in Cold than in Temperate ($p < .001$), such that it was ~ 4 °C cooler (95%CI: 3.4 - 4.4 °C) by 75 min of exercise (Figure 4.7A). Clothing attenuated the rate of reduction in mean \bar{T}_{sk} in the 5 °C environment (Figure 4.7B; $p = .005$), such that it was ~ 1 °C (95%CI: 0.8 - 1.4 °C) higher in Clothing by 75 min of exercise. Subcutaneous adiposity (Σ Skin folds) was significantly related to the reduction in \bar{T}_{sk} between Cold and Temperate ($p < .001$) and between Cold and Clothing ($p = .03$).

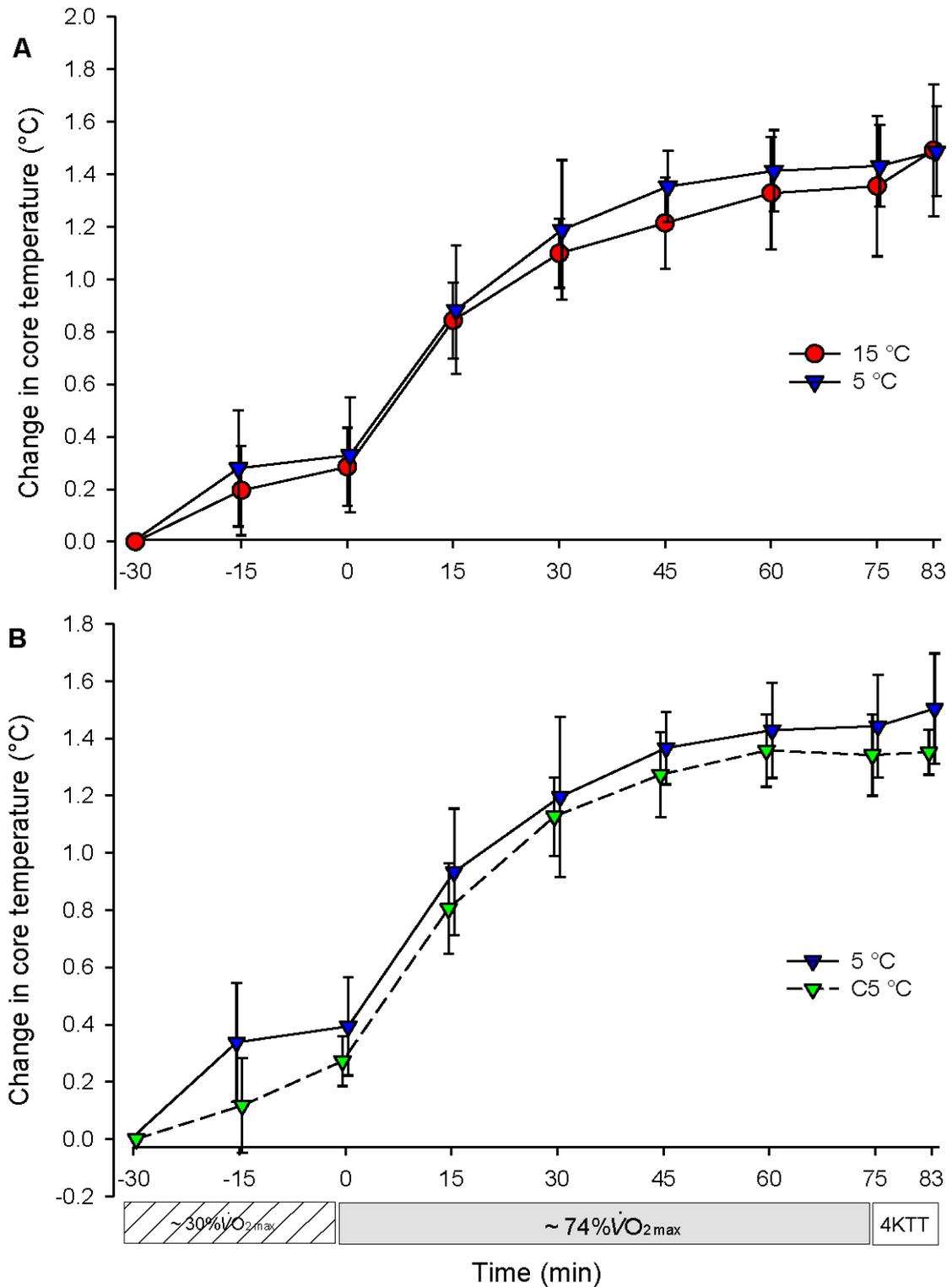


Figure 4.6. Mean (\pm SD) change in core temperature (rectal) from baseline (rest) during a 30 min warm up at $\sim 30\% \dot{V}O_{2\max}$ in 19°C , 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A) 5°C (RH 93%, Cold, $n=11$) and 15°C (RH 77%, Temperate, $n=11$) or (B) 5°C (RH 93%, Cold, $n=9$) and C5 $^{\circ}\text{C}$ (RH 93%, Clothing in cold, $n=9$), immediately followed by a 4 km time trial in the same environment.

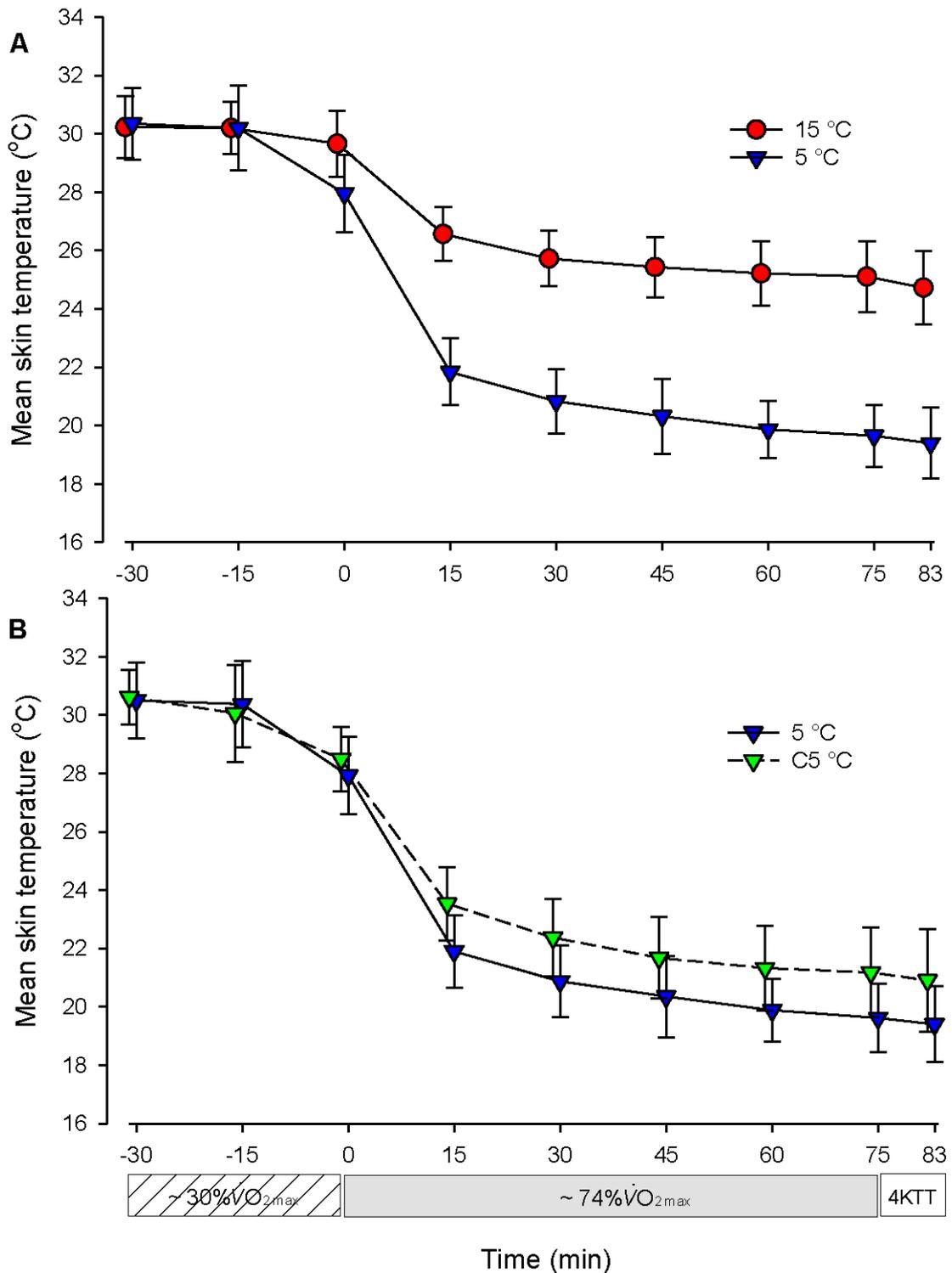


Figure 4.7. Mean (\pm SD) changes in mean skin temperature (\bar{T}_{sk} , nine sites) during a 30 min warm up at $\sim 30\% \dot{V}O_{2max}$ in 19°C, 75 min cycling at $\sim 74\% \dot{V}O_{2max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km time trial in the same environment.

4.8 Plasma metabolites, insulin, and catecholamines

Blood glucose concentration was similar in Temperate ($p = .56$) and Clothing ($p = .39$) compared to Cold. Blood glucose did not alter significantly across time in Temperate and Cold (*Figure 4.8A*, $p = .06$) and in Clothing and Cold (*Figure 4.8B*, $p = .09$). Furthermore, the interaction between condition and time was also not significant in Temperate and Cold ($p = .61$), or Clothing and Cold ($p = .34$).

Blood lactate concentrations were similar over the 75 min of steady state exercise (*Figure 4.9A*, $p = .41$), between conditions ($p = .16$) and across the 75 min exercise for Temperate and Cold conditions ($p = .74$). Clothing did not change blood lactate compared to Cold (*Figure 4.9B*, $p = .10$) but increased over time in both ($p = .04$), and this increase was the same across the 75 min for Cold and Clothing ($p = .92$).

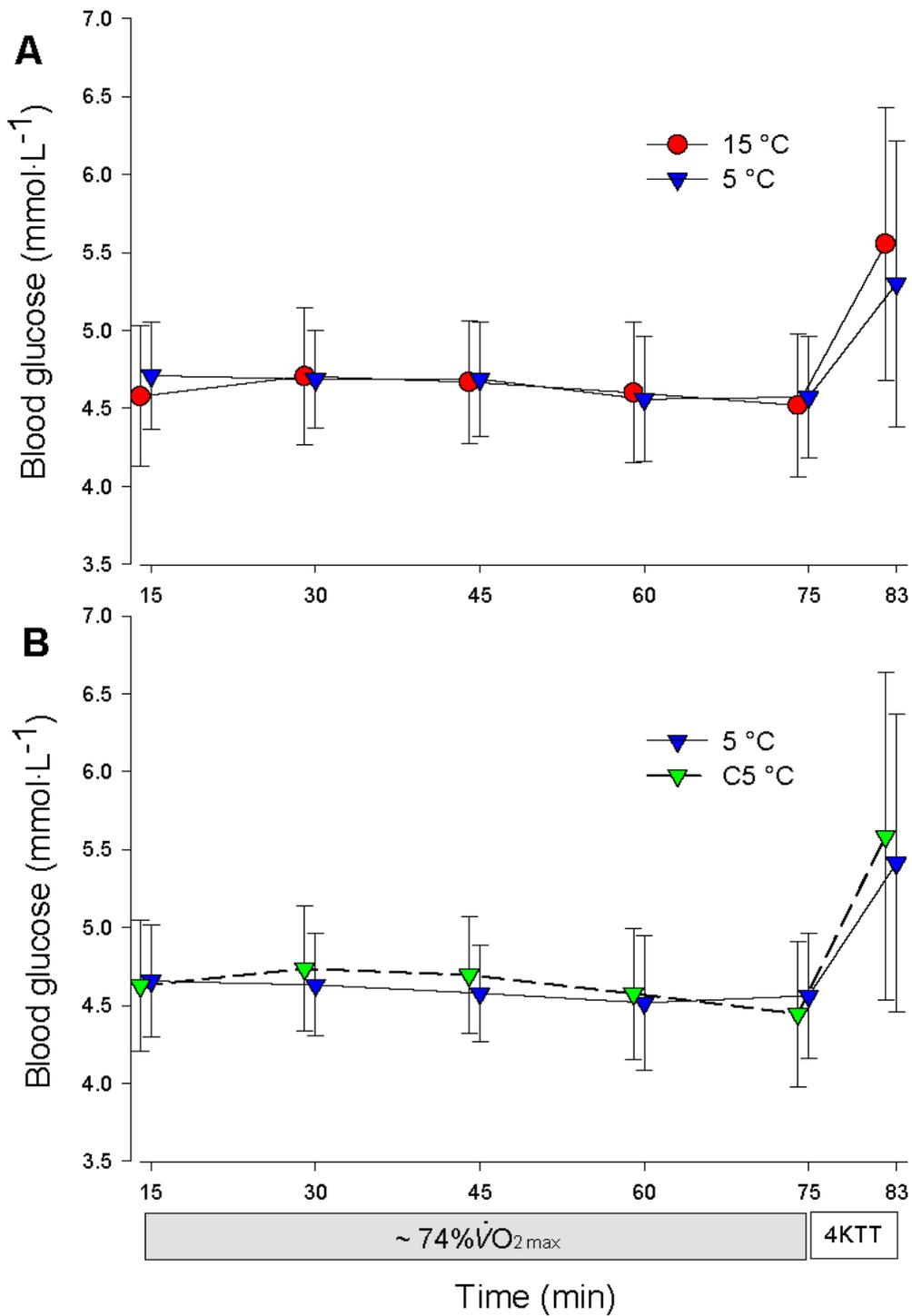


Figure 4.8. Mean (\pm SD) concentrations of blood glucose present during 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km time trial in the same environment.

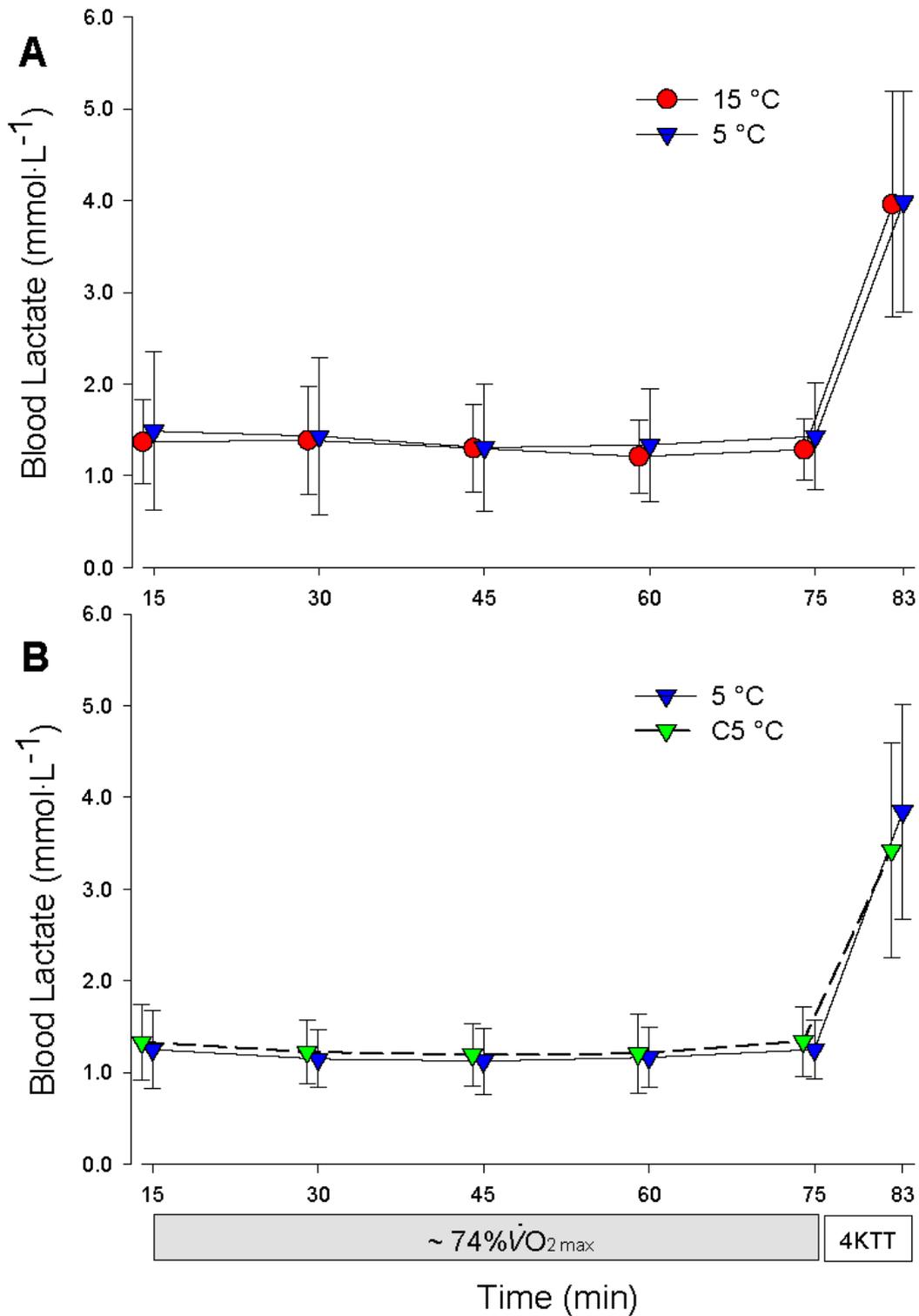


Figure 4.9. Mean (\pm SD) concentrations blood lactate (B), present during 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km Time trial in the same environment.

Insulin concentration measured at baseline was similar between Temperate and Cold ($p = .69$), and Clothing and Cold ($p = .86$). Measurements taken at T15 and T75 are reported as a change from baseline. Hence, the change in insulin concentration from baseline was not different at either time point $\Delta T15$ for Cold and Temperate ($p = .83$), and Cold and Clothing ($p = .97$), or $\Delta T75$ for Cold and Temperate ($p = .91$), and Cold and Clothing ($p = .42$) (Table 4.7).

Table 4.7. *Insulin concentration (mean \pm SD) measured at rest (baseline, T0), after 15 min (T15), after 75 min (T75) and as the change from baseline at T15 ($\Delta T15$), and T75 ($\Delta T75$) during 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either 5 °C (RH 93%, Cold, $n=11$), 15 °C (RH 77%, Temperate, $n=11$) or 5 °C (RH 93%, Clothing in cold, $n=9$)*

	Condition			
	Temperate ($n=11$)	Cold ($n=11$)	Cold ($n=9$)	Clothing ($n=9$)
T0 (mIU·ml ⁻¹)	9.36 \pm 3.87	9.93 \pm 4.81	9.53 \pm 4.54	9.97 \pm 4.80
T15 (mIU·ml ⁻¹)	4.59 \pm 2.30	4.90 \pm 2.54	3.97 \pm 1.64	4.94 \pm 3.33
T75 (mIU·ml ⁻¹)	2.01 \pm 2.38	3.02 \pm 2.96	2.91 \pm 3.34	1.68 \pm 1.77
$\Delta T15$ (mIU·ml ⁻¹)	-4.77 \pm 2.90	-5.03 \pm 3.97	-5.55 \pm 3.45	-5.03 \pm 2.99
$\Delta T75$ (mIU·ml ⁻¹)	-7.35 \pm 3.57	-7.19 \pm 5.47	-6.94 \pm 5.36	-7.54 \pm 4.30

Adrenaline was not different at rest in Cold compared to Temperate (Table 4.8; $p = .09$), or between Cold and Clothing ($p = .17$). Over time, adrenaline increased from baseline in Temperate and Cold ($p < .001$) and in Cold and Clothing ($p < .001$). The increase of adrenaline concentration across time was significantly greater in Temperate ($p = .015$) than Cold. There was, however, no difference in the change in adrenaline concentration between Clothing and Cold ($p = .72$) and no effect of condition was observed in Temperate ($p = .19$) or Clothing ($p = .50$) compared to Cold. Post hoc t-tests illustrate that the $\Delta T15$ there was a significantly greater adrenaline concentration in Cold ($p = .046$) and the $\Delta T75$ there was a trend for lower adrenaline concentration in Cold (95% CI: -1215 – 20 mmol·L⁻¹ $p = .057$), compared to Temperate.

Resting noradrenaline concentration was similar between conditions (Table 4.8, $p = .69$ for both comparisons). Noradrenaline concentration increased across time for Temperate and Cold ($p < .001$) and for Cold and Clothing ($p < .001$). No difference was observed between condition (Temperate vs. Cold, $p = .19$; Cold vs. Clothing, $p = .57$). The increase across time was not different for Temperate ($p = .68$) or Clothing ($p = .99$) when compared to Cold.

Table 4.8. Catecholamine concentrations (mean \pm SD) measured at rest (baseline, T0), after 15 min (T15), after 75 min (T75) and as the change from baseline at T15 (Δ T15), and T75 (Δ T75) during 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either 5 °C (RH 93%, Cold, $n=11$), 15 °C (RH 77%, Temperate, $n=11$) or 5 °C (RH 93%, Clothing in cold, $n=9$)

	Condition			
	Temperate (n=11)	Cold (n=11)	Cold (n=9)	Clothing (n=9)
Adrenaline				
T0 (pmol·L ⁻¹)	67 \pm 42	136 \pm 126	152 \pm 132	77 \pm 30
T15 (pmol·L ⁻¹)	476 \pm 121*	593 \pm 222	613 \pm 243	452 \pm 124
T75 (pmol·L ⁻¹)	1520 \pm 978	1015 \pm 499	1079 \pm 545	1451 \pm 1160
Noradrenaline				
T0 (pmol·L ⁻¹)	1744 \pm 705	1819 \pm 936	1873 \pm 1011	1751 \pm 482
T15 (pmol·L ⁻¹)	6576 \pm 1515	6896 \pm 1730	6856 \pm 1827	7170 \pm 2004
T75 (pmol·L ⁻¹)	11903 \pm 3833	10903 \pm 3835	11210 \pm 4247	11428 \pm 3720

*significantly different than Cold (n=11)

4.9 Metabolic responses

Resting RER was not different between Cold and Temperate ($p = .43$), and Cold and Clothing ($p = .84$). RER values were corrected for estimated protein oxidation during exercise (Figure 4.10) and were not different between Cold and Temperate ($p = .58$), and Cold and Clothing ($p = .53$) across time. Mean RER for the 4 km time trial was measured as an average over the entire time trial, and is plotted on Figure 4.10 at 83 min, as this was the average time of completion for the time trial. There was no difference in the RER for the time trial between Cold and Temperate ($p = .11$), and Cold and Clothing ($p = .75$).

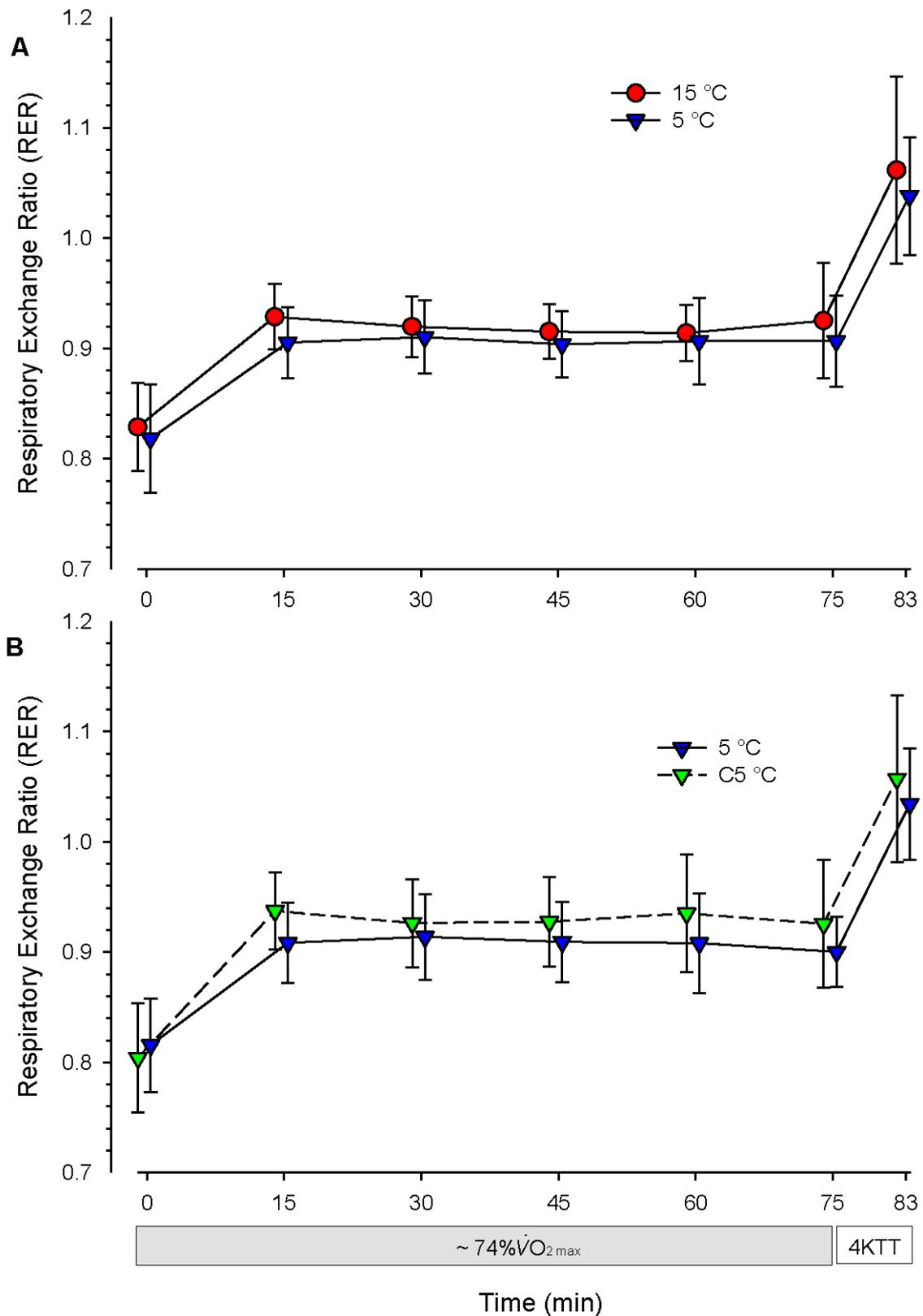


Figure 4.10. Mean (\pm SD) Respiratory Exchange Ratio (RER) measured at rest in 19°C, then during 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km time trial in the same environment.

4.10 Substrate Partitioning

The greatest amount of urea was excreted in the urine, with sweat contributing a minor portion (Table 4.9). Protein oxidation was similar between conditions (Table 4.10, Temperate and Cold, $p = .65$; Clothing and Cold, $p = .95$). Protein oxidation was ~ 10 g (5% of total energy) for all conditions for the 75 min exercise.

Table 4.9. Urea concentration, volume, and amount of urea in sweat and urine (Mean \pm SD) during 75 min of $\sim 74\%$ $\dot{V}O_{2\max}$ cycling in cold without (Cold) or with thermal clothing (Clothing) and in temperate (Temperate) conditions

	Conditions			
	Temperate (n=11)	Cold (n=11)	Cold (n=9)	Clothing (n=9)
Sweat				
Urea, mmol·L ⁻¹	7.3 \pm 2.3	7.9 \pm 2.8	7.4 \pm 2.5	7.8 \pm 2.2
Volume, L	0.70 \pm 0.24	0.47 \pm 0.15	0.48 \pm 0.17	0.63 \pm 0.26
Urea, g	0.31 \pm 0.15	0.22 \pm 0.08	0.20 \pm 0.07	0.27 \pm 0.10
Urine				
Urea, mmol·L ⁻¹	224 \pm 114	176 \pm 85	177 \pm 81	221 \pm 129
Volume, L	0.32 \pm 0.21	0.33 \pm 0.16	0.37 \pm 0.31	0.32 \pm 0.20
Urea, g	3.30 \pm 1.24	2.98 \pm 1.18	3.60 \pm 1.55	3.29 \pm 0.89

There was no difference in total CHO oxidation (g·min⁻¹) in Temperate versus Cold ($p = .65$), or Clothing versus Cold ($p = .20$). Total CHO oxidation over the 75 min steady state exercise was 160 \pm 19, 154 \pm 30, 148 \pm 30, and 165 \pm 33 g and therefore contributed 72 \pm 8, 68 \pm 11, 67 \pm 11, and 75 \pm 14 % to total energy (TE; *Figure 4.12* and *Figure 4.13*) for Temperate, Cold (n=11), Cold (n=9), and Clothing trials, respectively. Total CHO oxidation was not different in Temperate ($p = .49$), or Clothing ($p = .18$), compared to Cold.

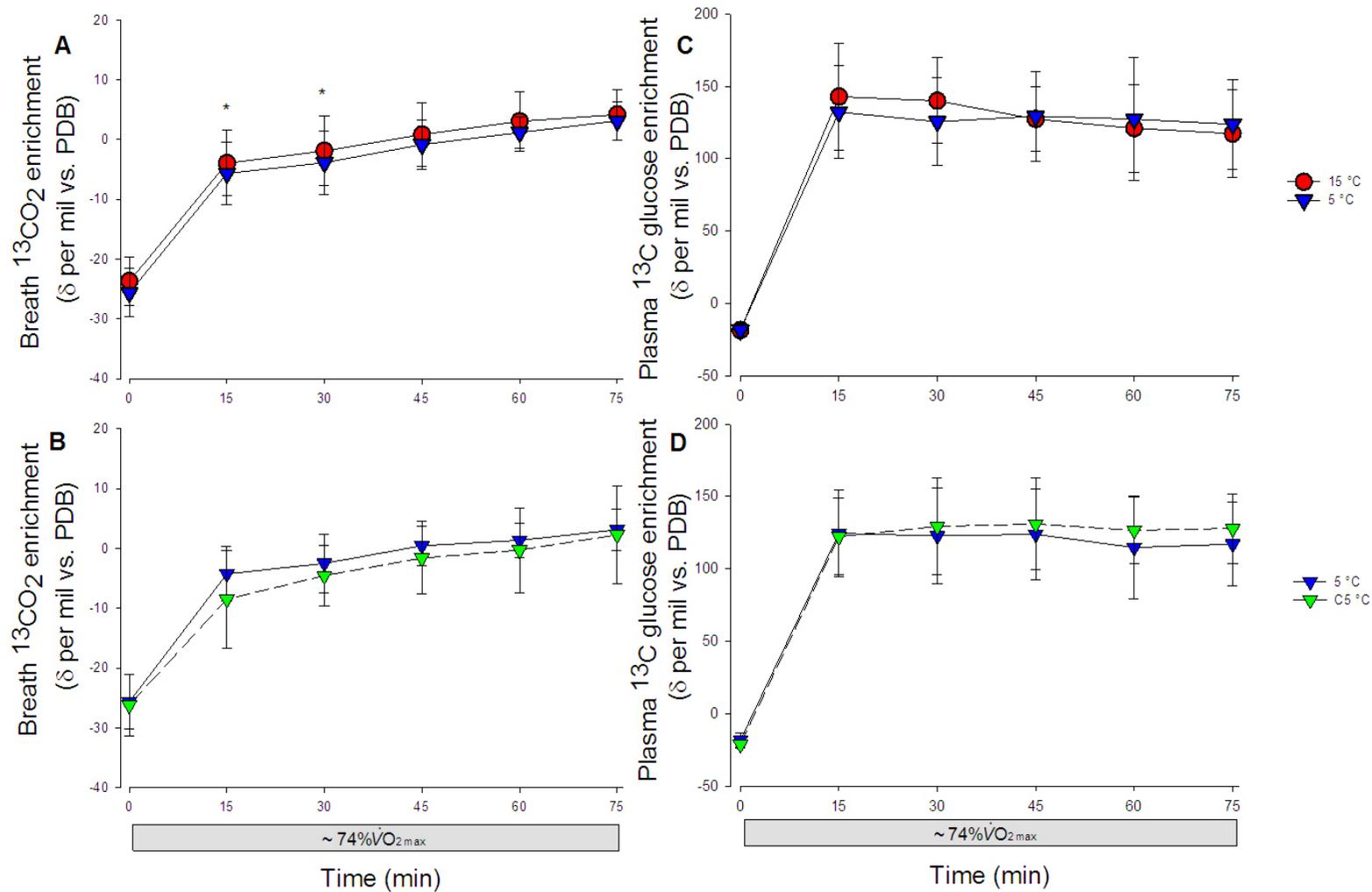


Figure 4.11. ^{13}C partitioning from the breath (A, B) and the plasma (C, D) measured at rest in 19°C , then during 75 min cycling at $\sim 74\% \dot{V}\text{O}_{2\text{max}}$ in either (A, C) 5°C (RH 93%, Cold, $n=11$) and 15°C (RH 77%, Temperate, $n=11$) or (B, D) 5°C (RH 93%, Cold, $n=9$) and $C5^\circ\text{C}$ (RH 93%, Clothing in cold, $n=9$), immediately followed by a 4 km Time trial in the same environment.

In this instance, breath [^{13}C]glucose enrichment reached a plateau from 45 min, and from 15 min in the plasma [^{13}C]glucose (*Figure 4.11*). Hence, as the calculations involve both measurements, the last 45-min of exercise is represented in the following partitioning.

No differences in CHO oxidation in the last 45-min exercise period were observed between Temperate and Cold across time (*Figure 4.14A*, $p = .84$). There was a trend for CHO oxidation to be greater in Clothing than in Cold (95%CI: $-0.122 - 7.031 \text{ kJ}\cdot\text{min}^{-1}$, *Figure 4.14B*, $p = .058$) but CHO oxidation did not change across the 45 min period ($p = .28$). As the total energy over the 75-min trial was slightly different between conditions, the relative contribution of substrates to energy is depicted in *Figure 4.12* and *Figure 4.13*. Muscle glycogen made up the majority of the CHO oxidation for the 75-min exercise, contributing 58, 54, 51, and 60 % to total energy utilisation in Temperate, Cold (n=11), Cold (n=9), and Clothing conditions respectively.

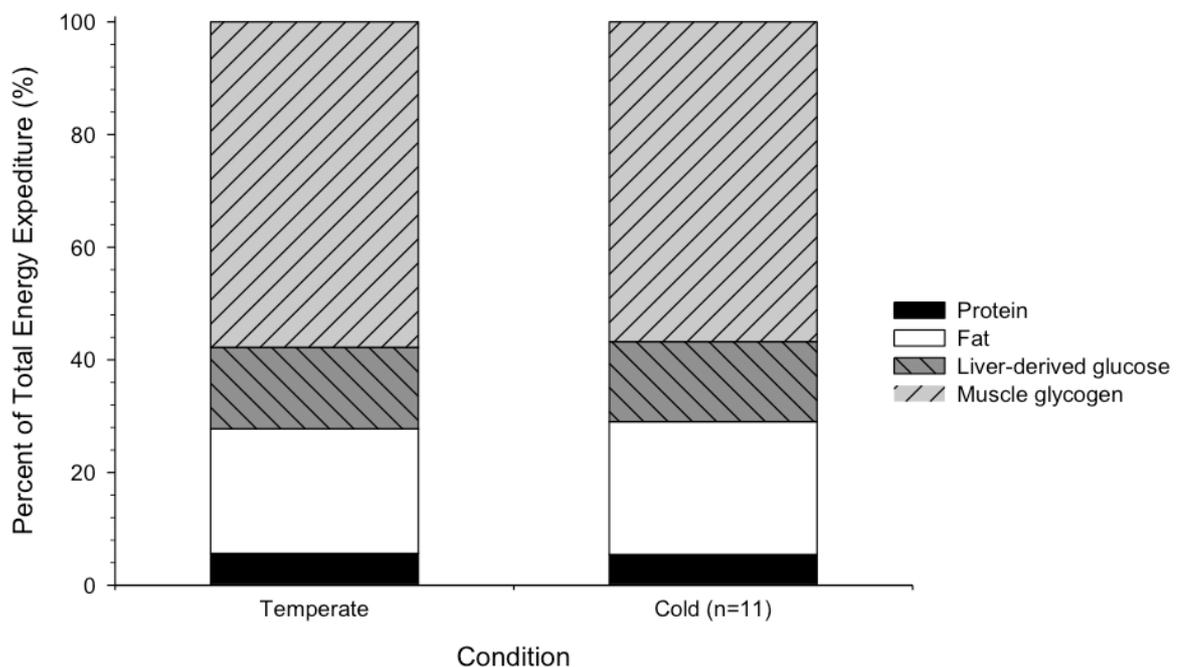


Figure 4.12. Mean relative contribution of substrates to total energy expenditure during the last 45-min of 75 min cycling at $\sim 74\% \dot{V}\text{O}_{2\text{max}}$ in $5\text{ }^{\circ}\text{C}$ (RH 93%, Cold, n=11) and $15\text{ }^{\circ}\text{C}$ (RH 79%, Temperate, n=11)

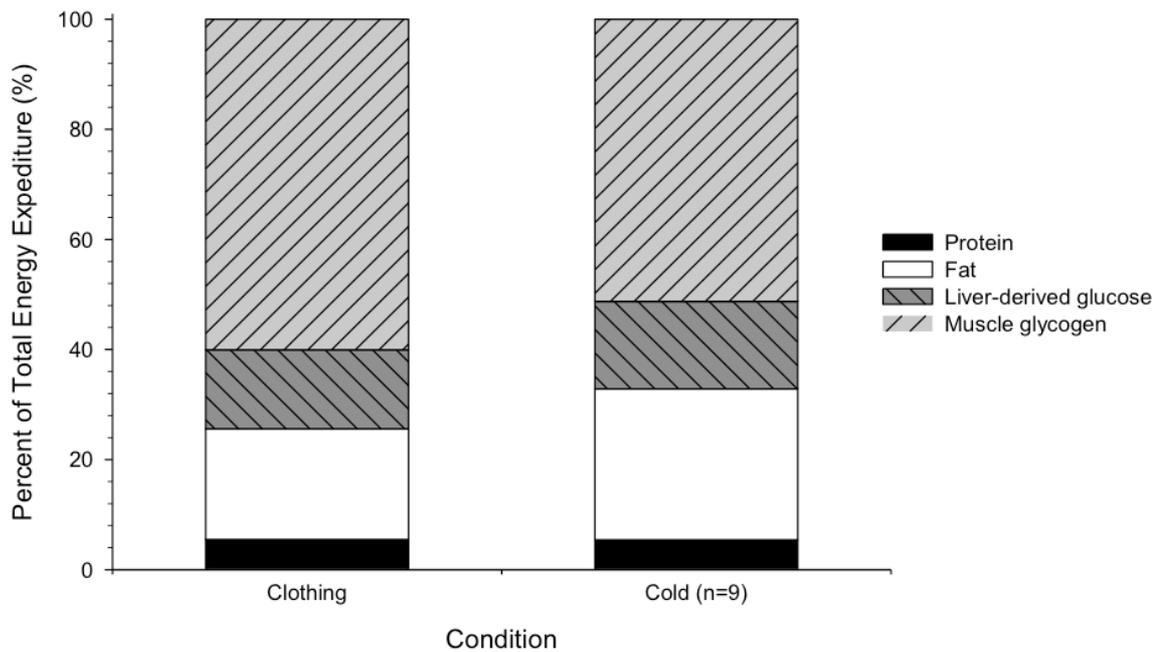


Figure 4.13. Mean relative contribution of substrates to total energy expenditure during the last 45-min of 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in $5\text{ }^{\circ}\text{C}$ (RH 93%, Cold, $n=9$) and $5\text{ }^{\circ}\text{C}$ (RH 93%, Clothing in cold, $n=9$)

Fat oxidation ($\text{g}\cdot\text{min}^{-1}$) was not different in Cold compared to Temperate ($p = .36$) and Clothing ($p = .18$) conditions. Total fat (g) over the 75 min steady state exercise was 20 ± 10 , 23 ± 10 , 24 ± 11 , and 18 ± 14 g accounting for 22 ± 9 , 26 ± 10 , 27 ± 11 , and $20 \pm 15\%$ of TE (*Figure 4.12* and *Figure 4.13*) for Temperate, Cold ($n=11$), Cold ($n=9$), and Clothing trials, respectively. Total fat oxidation (g) was not different in Temperate ($p = .26$), or Clothing ($p = .13$), compared to Cold.

No differences in fat oxidation ($\text{kJ}\cdot\text{min}^{-1}$) were observed across the last 45-min of exercise (*Figure 4.14C*) between Temperate and Cold conditions ($p = .94$). An effect of condition was observed between Cold and Clothing conditions where fat oxidation was higher in the Cold condition (95%CI: $0.30 - 6.18\text{ kJ}\cdot\text{min}^{-1}$, *Figure 4.14D*, $p = .031$) but no difference in fat oxidation across the 45 min within ($p = .10$) conditions was observed.

Calculated muscle glycogen oxidation ($\text{kJ}\cdot\text{min}^{-1}$ over 75-min: Table 4.10, % of TE: *Figure 4.12* and *Figure 4.13*) was similar in Temperate ($p = .64$), and Clothing ($p = .10$), compared to Cold. There was no change in muscle glycogen across time ($\text{kJ}\cdot\text{min}^{-1}$ over 45-min, *Figure 4.15A*) irrespective of temperature Cold or Temperate ($p = .75$). However, in the Clothing condition, muscle glycogen oxidation was higher than in the Cold (*Figure 4.15B*, $p = .017$), and did not change across 45-min ($p = .12$). Total muscle glycogen oxidation was 128, 120, 113, and 133 g in Temperate, Cold (n=11), Cold (n=9), and Clothing conditions respectively for the 75 min trial.

Liver-derived glucose oxidation ($\text{kJ}\cdot\text{min}^{-1}$ over 75 min: Table 4.10, % of TE: *Figure 4.12C* and *Figure 4.13D*) was not different in Temperate ($p = .96$), or in Clothing ($p = .86$), compared to Cold. Liver-derived glucose oxidation increased across time in Temperate and Cold (*Figure 4.15C*, $p = .003$) and with Clothing (*Figure 4.15D*, $p = .019$). There was no difference in the liver-derived glucose oxidation rate between Cold and Temperate ($p = .94$), or between Cold and Clothing ($p = .22$). Liver-derived glycogen oxidation was 32, 33, 35, and 32 g in Temperate, Cold (n=11), Cold (n=9), and Clothing conditions respectively for the 75 min trial.

Table 4.10. RER and rate of partitioned substrate oxidation ($\text{g}\cdot\text{min}^{-1}$; Mean \pm SD) during 75 min of $\sim 74\%$ $\dot{V}O_{2\text{ max}}$ cycling in cold without (Cold) or with thermal clothing (Clothing) and in temperate (Temperate) conditions

	Time (0-75 min)				Time (45- 75 min)			
	Temperate (n=11)	Cold (n=11)	Cold (n=9)	Clothing (n=9)	Temperate (n=11)	Cold (n=10)	Cold (n=8)	Clothing (n=9)
RER ^a	0.92 \pm 0.03	0.91 \pm 0.03	0.91 \pm 0.04	0.93 \pm 0.05	0.93 \pm 0.03	0.91 \pm 0.03	0.91 \pm 0.04	0.93 \pm 0.03
CHO oxidation	2.13 \pm 0.25	2.05 \pm 0.40	1.98 \pm 0.41	2.20 \pm 0.43	2.11 \pm 0.26	2.05 \pm 0.42	1.96 \pm 0.44	2.19 \pm 0.48
Liver-derived	0.43 \pm 0.08	0.44 \pm 0.14	0.47 \pm 0.15	0.42 \pm 0.12	0.49 \pm 0.10	0.48 \pm 0.15	0.51 \pm 0.15	0.47 \pm 0.13
Muscle	1.71 \pm 0.25	1.61 \pm 0.43	1.51 \pm 0.41	1.77 \pm 0.38	1.61 \pm 0.48	1.53 \pm 0.49	1.46 \pm 0.41	1.62 \pm 0.50‡
Fat oxidation	0.27 \pm 0.13	0.31 \pm 0.14	0.32 \pm 0.15	0.24 \pm 0.19	0.28 \pm 0.13	0.32 \pm 0.14	0.33 \pm 0.15	0.25 \pm 0.21‡
Protein ^b	0.14 \pm 0.05	0.13 \pm 0.05	0.15 \pm 0.06	0.13 \pm 0.03	0.14 \pm 0.05	0.13 \pm 0.05	0.15 \pm 0.06	0.13 \pm 0.03

All units of $\text{g}\cdot\text{min}^{-1}$ except for RER, ^a Respiratory exchange ratio corrected for protein oxidation, ^b calculated over entire trial so rate change is indistinguishable, ‡significantly different to Cold (n=9)

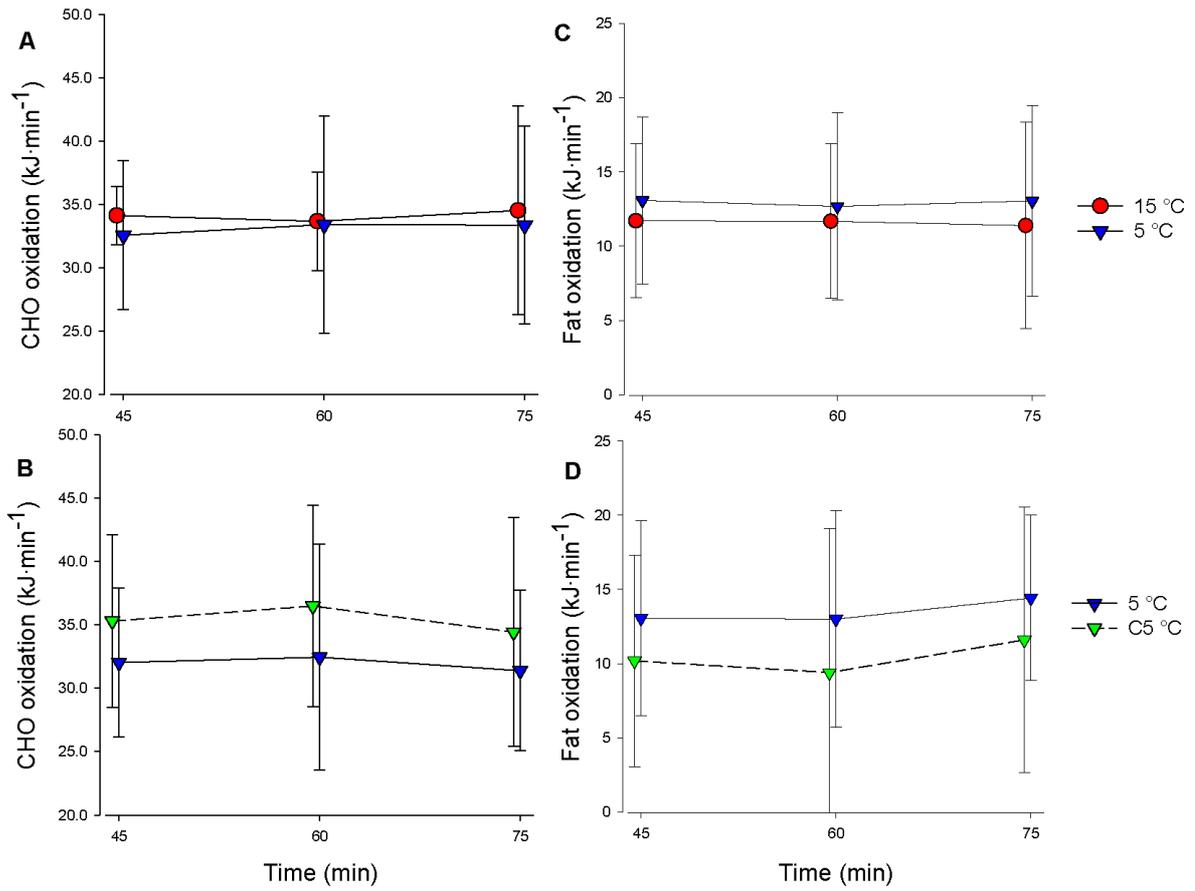


Figure 4.14. Rates of CHO (A, B) and Fat (C, D) oxidation during the last 45-min of 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A,C) 5 °C (RH 93%, Cold, n=11) and 15 °C (RH 77%, Temperate, n=11) or (B,D) 5 °C (RH 93%, Cold, n=9) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km Time trial in the same environment.

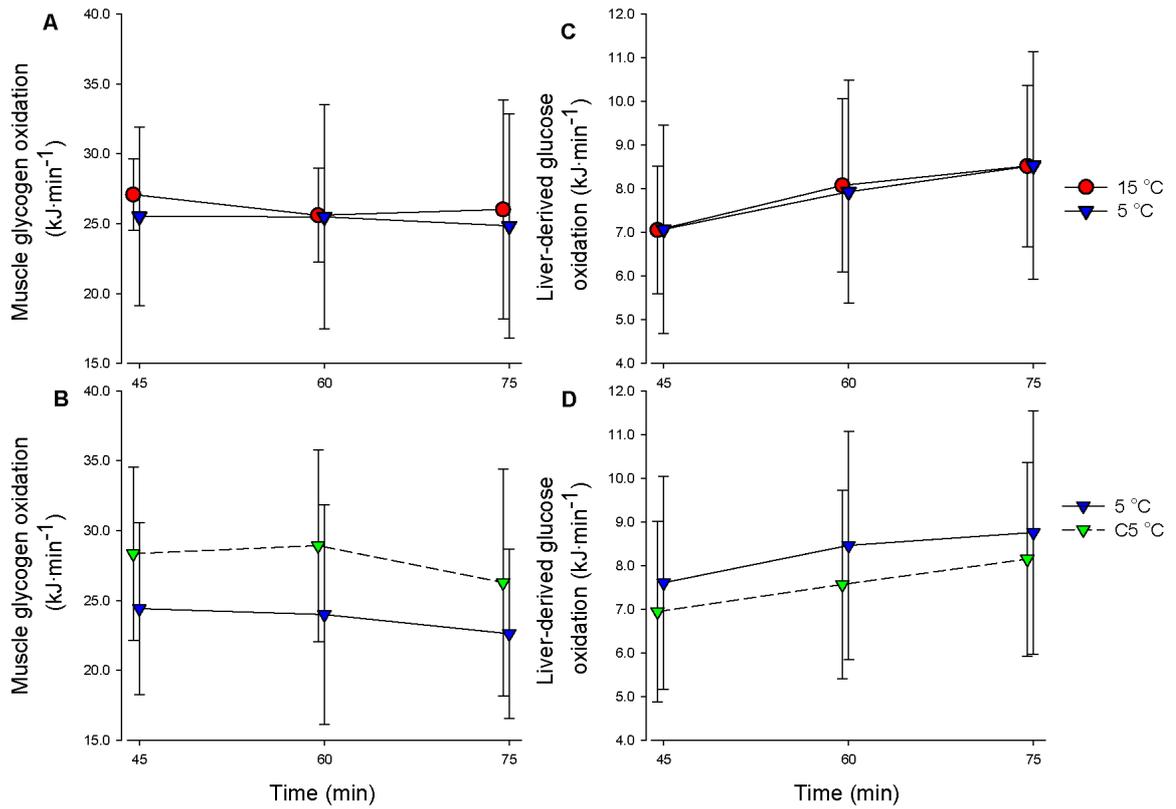


Figure 4.15. Rates of Muscle glycogen (A, B), and Liver-derived glucose (C, D) oxidation during the last 45-min of 75 min cycling at $\sim 74\% \dot{V}O_{2\max}$ in either (A,C) 5 °C (RH 93%, Cold, n=10) and 15 °C (RH 77%, Temperate, n=11) or (B,D) 5 °C (RH 93%, Cold, n=8) and C5 °C (RH 93%, Clothing in cold, n=9), immediately followed by a 4 km Time trial in the same environment.

5.0 DISCUSSION

This study is the first to examine the effects of low ambient temperature (T_a) on exercise metabolism in female athletes. The results indicate that reduced T_a (5 °C vs. 15 °C) does not affect metabolic rate or proportional substrate use when female cyclists exercise with facing air velocity (3.9 m·s⁻¹). Despite skin temperature being ~6 °C lower in the Cold condition, core temperature was similar between Cold and Temperate conditions. In both conditions, when partitioned out, carbohydrate (CHO) provided the majority of the energy (~70% total energy (TE)) for exercise, and muscle glycogen (MG) provided the majority of the CHO (~80%, or ~56% of TE). Although no studies involving female participants exercising in low T_a were available for comparison, the hypothesis – based on male data – of increased CHO oxidation in cold conditions, was not supported with this group of women. The similar core temperature between conditions may partly account for the lack of difference in metabolic responses between the Cold and Temperate conditions. Anthropometric and hormonal differences between men and women may explain why T_a may not have the same effect on exercise metabolism in both sexes. However, as discussed below, comparing between sexes was not the focus of this study, and as sex hormones were mostly controlled in this study, the female responses may be in line with previous research with male participants.

The addition of clothing is a typical behavioural response for exercising in cold conditions. Therefore, this study included an investigation on the effect of clothing in moderating core (rectal) and skin temperatures and participants' thermal perceptions. Additionally, this study examined the effect of clothing on substrate metabolism during exercise in a low T_a (5 °C). The additional clothing modified participants' perceived temperature but not their thermal comfort. There was a trend for carbohydrate metabolism, particularly muscle glycogen oxidation, to be higher with Clothing.

The non-invasive method used in this study is novel for examining metabolic responses to cold ambient conditions and has provided much more specific metabolic information regarding partitioning of CHO sources than has been published in the literature. In contrast to many lab-based studies, which are heat stressful by virtue of low or no air velocity, this study used semi-realistic air velocity ($3.9 \text{ m}\cdot\text{s}^{-1} \sim 14 \text{ km}\cdot\text{h}^{-1}$). We also required participants to wear a helmet and gloves. It might be speculated that the use of helmets in the present study may have affected thermoregulation, however, Sheffield-Moore and colleagues (1997) reported that wearing a helmet whilst exercising in the heat ($35 \text{ }^\circ\text{C}$) had no effect on thermoregulatory responses when cycling at $60\% \dot{V}\text{O}_{2 \text{ max}}$ for 90 min. Nevertheless, by having participants wear typical cycling attire, including a helmet and gloves, the results have greater ecological validity.

Given that the focus of this study was on metabolism, and that metabolism depends on thermal and cardiovascular strain, these factors will be discussed first. The effect of additional clothing will be discussed last with regard to the magnitude and nature of the thermal and cardiovascular effects, with an emphasis on the modified perceptual responses.

5.1 Effect of ambient temperature

The current literature is not in agreement concerning metabolic effects of exercise in the cold. The findings for males have shown CHO oxidation to be both increased (Febbraio et al., 1996b; Layden et al., 2002; Patterson et al., 2008) and decreased (Fink et al., 1975; Hurley & Haymes, 1982; Timmons et al., 1985; Dolny & Lemon, 1988). However, the decrease in CHO use shown by three of those four studies occurred in a wide range of T_a ranges (Fink et al., 1975; Timmons et al., 1985) and lower intensities (Hurley & Haymes, 1982; Timmons et al., 1985) than the present study, hence the most acceptable consensus may be increased CHO oxidation in cold. The present study does not support either observation for female athletes, as CHO oxidation was not different between Cold and Temperate conditions, possibly linked to the lack of change in core temperature (rectal, T_c).

Thermoregulation and cardiovascular factors

Exercise-cold stress includes scenarios such as low T_a combined with wind cause an increase in metabolic cost for thermal balance - as indexed from core and skin temperatures - to be maintained (Castellani et al., 2006). Heat balance has not been reported in this study due to the inherent errors in calculations based on estimated changes in body temperature (\bar{T}_b), as previously described (Jay, Reardon, Webb et al., 2007), and hence the following comments are based on core temperature (rectal, T_c) and mean skin temperature (\bar{T}_{sk}) observations. In the current study, T_c increased across time (*Figure 4.6*), independently of ambient conditions, indicating that exercise probably caused heat gain even in cold conditions (Castellani et al., 2006), and therefore it can be suggested that participants were not nett cold stressed. Although \bar{T}_{sk} decreased in Cold (*Figure 4.7*), the similar T_c and total energy expended in Cold compared to Temperate indicates these participants' heat generation was similar in both environments. The elevated noradrenaline concentrations observed in Cold and Temperate at 15 and 75 min of exercise compared to baseline, are related to the change in \bar{T}_{sk} as it works as a vasoconstrictor (Zouhal et al., 2008), and therefore the reduction in \bar{T}_{sk} in Cold and in Temperate (where \bar{T}_{sk} was lower than at baseline) is expected. The exacerbated reduction in \bar{T}_{sk} in the 5 °C environment was anticipated due to the extra transient heat loss to the environment and likely a reduced blood flow to the skin.

A reduction in deep body temperature has been shown to alter the metabolic response when passively immersed in cold water, due to the increase in metabolic cost of shivering (Cannon & Keatinge, 1960). However, \bar{T}_{sk} and T_c have been reported to both decrease in 3 °C compared with 20 °C (Febbraio et al., 1996b), and decrease \bar{T}_{sk} but no change in T_c in 0 and -10 °C compared to +10 and +20 °C (Layden et al., 2002). Therefore, perhaps the attenuated elevation in T_c plays the major role in altering substrate utilisation, but when T_c is unchanged and the \bar{T}_{sk} is low enough it may be that T_m is also reduced hence altering

metabolism. Skin temperature could also be somewhat a surrogate for between-condition differences in the underlying T_m , so T_m may have been slightly lower in Cold, hence using less CHO than in warmer muscle (Febbraio, 2001). Similarly, the \bar{T}_{sk} could be reflecting the change in CVS strain, and more specifically the redistribution of blood which in turn would help drive muscle and liver glycogenolysis. Also, as adrenaline concentration did not increase as much in Cold by the end of 75 min of exercise, this would indicate that CHO oxidation would be lower. Considering these observations, the participants in the current study appeared to be more heat than cold stressed, possibly due to the moderately high intensity.

Heart rate responses were not different between Cold and Temperate conditions, indicating that the cardiovascular consequence of the different temperature was minimal. The similar HR between 5 and 15 °C in this study is in contrast to Febbraio et al. (1996b), who reported a lower HR in 3 °C compared to 20 °C. Similarly, Layden et al. (2002) reported a lower HR in 0 and +10 °C compared to 20 °C, but no difference between 0 and +10 °C. Although those studies were performed at a lower relative intensity ($\sim 65\% \dot{V}O_{2\max}$) than the current study ($\sim 74\% \dot{V}O_{2\max}$), and with male participants, the results are similar when the difference in environmental temperature is taken into account. Moreover, Patterson et al. (2008) found an 8 bpm lower HR in males at the completion 60 min cycling at 70% $\dot{V}O_{2\max}$ in 0 °C compared to 20 °C with almost no forced convection ($0.36 \text{ m}\cdot\text{s}^{-1}$). In the current study, participants' HR increased similarly across the exercise period between conditions, in comparison to a higher cardiovascular drift during 45 min in 20 °C compared to 3 °C (Febbraio et al., 1996b). Therefore, the lack of heart rate average or drift effect in the current study is perhaps explained by stronger convective cooling ($3.9 \text{ m}\cdot\text{s}^{-1}$ vs. $0.36 \text{ m}\cdot\text{s}^{-1}$) along with a lower temperate-environment temperature (15 vs. 20 °C).

In this study it would be expected that muscle blood flow would be similar due to insufficient stress i.e. no differences in CVS strain or T_c . The effects of T_a on T_m diminish

as exercise intensity increases. Saltin and colleagues (1968) reported progressively less effects on the variation T_m with changes in T_a (10, 20, and 30 °C) during cycling at 25, 50 and 70% $\dot{V}O_{2\max}$ at each T_a . Saltin et al. (1968) also demonstrated that increasing intensity (from 25 to 72% $\dot{V}O_{2\max}$) increases T_m irrespective of T_a . A lack of change in T_m would indicate that blood flow was similar between conditions and therefore no difference in CHO utilisation, specifically MG oxidation, would have been expected.

Metabolism

An increased absolute $\dot{V}O_2$ and % of $\dot{V}O_{2\max}$ was observed in Cold relative to Temperate conditions, which is similar to the higher $\dot{V}O_2$ and \dot{V}_E reported during 70% $\dot{V}O_{2\max}$ cycling to exhaustion in 4 °C compared with 11 and 21 °C (Galloway & Maughan, 1997). However, there was no change in TE cost in the current study due to the calculation involved to correct the indirect calorimetry for protein oxidation.

In the current investigation, as the exercise progressed, no change in respiratory exchange ratio (RER) was observed across time (*Figure 4.10*), which is inconsistent with the typically observed reduction in RER over time as MG is used with a shift towards greater fat metabolism (Timmons et al., 1985; Galloway & Maughan, 1997; Layden et al., 2002; Roepstorff, Steffensen, Madsen et al., 2002). In previous research, in which the same exercise duration, and similar intensity (76% $\dot{V}O_{2\max}$) were used, male participants' RER decreased across time (Harvey et al., 2007). As both the duration and relative intensity are similar to Harvey et al. (2007) the greater *absolute* intensity of exercise (263 ± 50 W, Harvey et al., 2007) compared to the females in this study (158 ± 19 W) therefore a stronger force in driving CHO utilisation and depleting MG at a faster rate, may in part explain the discrepancy between results.

Muscle glycogen oxidation rates may have differed between studies also explaining the stability of RER data in the current study if MG oxidation was higher across exercise, possibly due to the differences in body composition i.e. the lower muscle mass typically

reported in females compared to males. Harvey et al. (2007) reported total MG oxidation (~125 g) which was similar to that of the current study (~113-133 g). Considering that females in this study probably had smaller muscle mass, and therefore would have less MG to use, they probably depleted at a faster rate than the males in the Harvey et al. (2007). As previous researchers have summarised (Wismann & Willoughby, 2006; Tarnopolsky, 2008), fat oxidation appears to be greater in females than males during exercise at the same relative intensity, although most studies have been at intensities below the 'crossover' point ($< 65\% \dot{V}O_{2\max}$) where it is suggested that fat oxidation should predominate regardless of sex (Brooks & Mercier, 1994). However, this crossover point is dependent on fitness and as the participants in the current study are less trained than those of Harvey et al. (2007) as measured by $\dot{V}O_{2\max}$ both absolutely and relatively then the participants in the current study would have had to have relied on CHO as they were further above the 'crossover' intensity.

Sex differences

While the intention of the study was not to compare between sexes, some comparison was inevitable due to the lack of literature pertaining to females. Toner and colleagues (1986) suggest that the insulating potential of having a larger muscle mass, i.e. in male participants, is reduced during exercise due to increased muscle perfusion. Recent research has illustrated that passive cooling in water to reduce T_c from 39.5 °C to 37.5 °C is significantly slower in male participants, possibly due to their larger muscle mass (Lemire, Gagnon, Jay, & Kenny, 2009). Hence, differences between female and male metabolic responses to exercise in the cold may be explained in part by differences in body composition, more specifically less lean body mass and greater fat mass in females versus males. In support of this notion is the fact that body composition is significantly different in the vast majority of studies where metabolic responses to exercise in males and females have been compared (see Tarnopolsky, 2008).

Specifically the difference in body composition is that females have more subcutaneous fat (Tarnopolsky et al., 1990; Horton, Pagliassotti, Hobbs, & Hill, 1998; Wallis, Dawson, Achten, Webber, & Jeukendrup, 2006). An increased thickness of this insulative layer would facilitate both lower \bar{T}_{sk} (Buskirk, Thompson, & Whedon, 1963) and warmer T_m , further minimising differences in T_m between conditions. Although it has been found that there is no direct correlation between \bar{T}_{sk} and T_m (Saltin et al., 1968) indicating that the differences in body composition affect \bar{T}_{sk} but not directly influencing T_m .

It is speculated that the female sex hormone 17 β -oestradiol is the main hormone involved in altering metabolism between the sexes, originally by altering glycogen utilisation, based on animal studies, but recently by increasing the mobilisation of fat stores and fat oxidation (Wismann & Willoughby, 2006; Tarnopolsky, 2008). This is supported by the indication that not all of the enzymes involved in regulating CHO metabolism are affected by oestrogen concentrations, and enzymes involved in fat metabolism seem to be more affected by oestrogen (see Table 2 in Tarnopolsky, 2008). The exact mechanism of the difference in metabolic response between males and females is unknown. Further research considering both body composition and sex hormones may provide key information.

Hormonal responses

Measures of T_m and catecholamines can give an indication of mechanisms involved with alterations of muscle- and liver-derived glucose oxidation with ambient temperature variation, but the relationship between such hormones is complex and may not tell the whole story. Furthermore, large variations in results from catecholamine analysis indicate that individual receptor sensitivity and responses to the hormones may have affected the observed trends within this study.

The major catecholamines, adrenaline and noradrenaline, are upregulated during exercise in combination with a reduction in insulin, which protects against low blood glucose (hypoglycemia) during exercise (Holloszy & Kohrt, 1996). Similarly, during

shivering when cold stressed, catecholamines are upregulated to stimulate various mechanisms to utilise fat and CHO stores (Stocks, Taylor, Tipton, & Greenleaf, 2004). Insulin concentrations usually fall as exercise progresses, making glucose and FFA more available to sustain the set exercise intensity (Holloszy & Kohrt, 1996). The lack of differences in insulin at T15 and T75 between conditions supports the lack of difference in metabolism between Cold and Temperate conditions. It has been found that increased circulating noradrenaline increases oxygen consumption (Hemingway & Price, 1968), however, no difference in noradrenaline concentration was observed in the current study between Cold and Temperate conditions. Therefore, although respiratory data indicate an increased $\dot{V}O_2$ consumption in Cold, due to the lack of change it is unlikely that noradrenaline influenced this observation.

The response of adrenaline to Cold and Temperate tended to differ between conditions so that there was greater concentration in Cold after 15 min, but by 75 min concentrations tended to be lower in Cold. Based on the adrenaline observation it would be assumed that liver glycogenolysis would increase in Temperate, as muscle glycogenolysis responds with increased blood lactate (Zouhal et al., 2008) which was not observed. Therefore CHO oxidation would also increase with the increase in liver glycogenolysis. However, this difference in adrenaline concentrations was obviously not large enough or was overshadowed by other regulatory mechanisms as evidenced by the lack of difference in CHO metabolism between the two conditions. These results are similar to that of Febbraio et al. (1996b) who found reduced adrenaline and noradrenaline in 3 °C compared to 20 °C. However, Febbraio et al (1996) also showed this reduced catecholamine stimulus in combination with increased CHO oxidation, which was not observed in the current study. Moreover, the responses of catecholamines in the current study are similar to Layden et al. (2002), the major difference being the larger difference in \bar{T}_{sk} and hence possibly CVS strain, via changes in blood delivery, autonomic nervous system responses altering

glycogenolysis, and underlying T_m , have some sort of joint or combination effect to change metabolism compared to that of the current study.

Methodological considerations

The indirect calorimetry used in the current study is consistent with previous research on exercise in cold environments, and the measure of muscle glycogen utilisation with the ^{13}C [glucose] tracer technique is highly correlated with that measured with biochemical analysis of muscle biopsy samples (Harvey et al., 2007). Although neither measures oxidation from one muscle and are not identical, as one represents the oxidation from one muscle whereas the other represents total body skeletal muscle oxidation, the correlation is good and so studies reporting muscle biopsy data are comparable to the method employed in the current study. The present study can be compared with studies involving female participants investigating substrate use in warmer thermal environments as discussed below, when considering dietary control and variability in the techniques used to measure metabolic responses, although the majority of studies have investigated lower intensities.

Campbell et al. (2001) studied the responses to 2 h cycling at $\sim 70\% \dot{V}O_{2\max}$ in women in the follicular phase and in the luteal phase, with and without ingesting a glucose drink (6% CHO). Although T_a was not reported, it is assumed that this exercise was performed in $\sim 20^\circ\text{C}$ laboratory conditions with relatively low airflow, and although the study did not investigate the effects of T_a , the intensity is somewhat similar to that in the current study. Campbell et al. (2001) reported total CHO oxidation of 251-256 g for the 2 h exercise study, approximately equating to 158 g of CHO for 75 min. This is very similar to the current study, albeit this comparison is very crude and does not take into consideration the expected decrease in RER and hence CHO oxidation across time. However, it is possible to conclude that the substrate oxidation measured in the current study is in line with current literature of females exercising at moderately high intensities for > 1 h duration.

The increase in CHO utilisation observed in the cold ambient conditions has only been measured via indirect calorimetry and limited use of muscle biopsies, therefore only total CHO and fat and representations of total MG utilisation are known. The present technique of measuring MG and liver-derived glucose oxidation is important in this process as muscle biopsies cannot provide alterations in oxidation rates over time. The [¹³C]glucose technique is also less invasive. In addition, many studies fail to account for protein oxidation, which requires measures of urea in the sweat and urine (Lemon & Mullin, 1980), and for fractional CHO oxidation. This lack of correction leads to less accurate calculation of fat and CHO oxidation with indirect calorimetry and thus this added source of variation may influence findings. Our study was one of the few which have corrected for protein oxidation and thereby gives a more accurate portrait of substrate metabolism in women, but may be difficult to compare with other studies in which this was not done.

The stable isotope method used in this study, gives a measurement of the CHO from the liver that is oxidised. The working muscles provide the majority of the CHO for exercise, as these stores are the largest, the subsequent glucose can only be used in the working muscle. As exercise progresses MG stores deplete and the liver must provide glucose, firstly via glycogen stores and secondly via gluconeogenesis, to sustain energy requirements. The stores of glycogen in the liver are only ~ 72 g (Trimmer, Schwarz, Casazza et al., 2002) – enough to provide sufficient CHO for ~50% of the 75 min trial – and therefore the gluconeogenesis becomes increasingly important as liver glycogen depletes, although at a slower rate of CHO provision than glycogen. Muscle glycogen is the largest volume source of endogenous CHO (Maughan et al., 1997) and was the biggest provider, in terms of rate and volume of CHO and of total energy in the current study, which is consistent with other male (van Loon, Greenhaff, Teodosiu, Saris, & Wagenmakers, 2001) and female (Romijn et al., 2000) studies. The estimated liver-derived glucose oxidation was similar in both

temperature conditions indicating that gluconeogenic pathways were not substantially affected.

In the current investigation, participants were not fasted before exercise. After resting measures were taken when fasted, each participant consumed a standardised breakfast 2 h prior to the 75 min steady state exercise. Although this methodology has been reported previously (M'Kaouar, Peronnet, Massicotte, & Lavoie, 2004), most studies of exercise metabolism that are concerned with metabolism have tested participants after fasting for a minimum of 10-12 h (e.g. Galloway & Maughan, 1997; Campbell et al., 2001; Zderic, Coggan, & Ruby, 2001; Roepstorff et al., 2002; Ruby, Coggan, & Zderic, 2002; Haman, Peronnet, Kenny et al., 2005; Devries et al., 2006). Testing participants after fasting for 10-12 h, albeit common in research, is not logical when applying results to athletic populations, as it is uncommon for athletes to train and/or especially compete without any pre-exercise nutrition. In addition, the effect of pre-exercise feeding is well documented with regard to total CHO oxidation (Coyle & Coggan, 1984; Sherman, Brodowicz, Wright et al., 1989; Chryssanthopoulos, Hennessy, & Williams, 1994; Marmy-Conus, Fabris, Proietto, & Hargreaves, 1996).

It could be argued that participants should consume some form of CHO as exercise is longer than one hour. However, athletes may purposely deplete glycogen as a strategy for the adaptation of glycogenesis or inadvertently not consume any CHO on such a ride that is of a 'short' duration for road cyclists and hence nutrition is not as important. Although 75 min of exercise at 74% $\dot{V}O_{2\max}$ would not fully deplete glycogen stores, if participants had consumed CHO overall CHO use may have been higher, including muscle glycogenolysis. However, any difference between conditions would only be caused by differences in muscle blood flow, T_m or catecholamines and as the current methods illustrate, any such effects would be minor. Therefore, the methods used in this study were intended to represent a

typical athlete's pre-exercise environment to ascertain the metabolic effects that would be more typical in an athletic population with regards to training.

As previously mentioned, the stable isotope method in the present study is slightly different to others (Campbell et al., 2001) in which isotope infusion has been used. The infusion method measures the rate of appearance (R_a) and disappearance (R_d) of labeled glucose during exercise to provide utilisation rates of fat and CHO. Specific tracers, such as [^{13}C]leucine for protein (Wagenmakers, 1999), [^{13}C]palmitate for fat, and as in the current study [^{13}C]glucose for CHO (Coggan, 1999) are used to partition substrates and hence muscle biopsies are not necessary. The stable isotope method used in the current study is reliant on indirect calorimetry, and with measures of the isotope (in this case glucose for measures of CHO) in the breath and blood, can partition out MG and liver-derived glucose oxidation. Both methods require a time period where the tracer is absorbed through the bicarbonate pool so that measurements are stable during exercise and, as calculations are heavily derived, errors can arise if the equilibrium period is not allowed for (for a more extensive review see Coggan, 1999). This requires sometimes up to 2.5 h of infusion, or in the case of this study a 60 min period from ingesting the tracer to it being stable in the breath to record accurate measurements. It is for this reason that in the current study only the last 45 min of exercise was utilised for comparing substrate utilisation between conditions. In summary, due to thermoregulatory and CVS responses to cycling at $\sim 74\% \dot{V}O_{2\text{max}}$ in Cold and Temperate conditions being similar, metabolism remained unaltered in spite of hormonal and ventilatory measures indicating that differences might exist.

Performance

A time trial was used to test participants' exercise capability after a 75-min steady state period. In theory, a decrease in CHO oxidation in the Temperate condition may have spared MG, and therefore there could have been more MG left for the time trial. This would only be a benefit if glycogen stores become limited, which in the current study appeared not

to be the case. Furthermore, as RER values exceeded 1.0 for the 4km time trial performance, the calculation of total fat and CHO, or partitioning of MG and liver-derived glycogen oxidation is not possible. As discussed below, a time to exhaustion trial may have proved more useful as participants would be less likely to have high RER values hence hindering the partitioning method but that approach would have been less ecologically valid.

In this study, time trial performance did not differ across conditions when expressed either as time to completion, or work rate in Watts, possibly due to the lack of difference in substrate metabolism during the preceding 75 min exercise at $\sim 74\% \dot{V}O_{2\max}$ and that MG stores were not depleted. There was no additional cardiac or thermal (T_c) reserve arising from the cooler condition to offset or supplement any metabolic effect in the preceding 75 min. If the Cold condition lowered T_c prior to exercise onset greater gains in T_c may have improved performance similar to the performance benefit that has been observed with pre-cooling (Lee & Haymes, 1995).

A time-to-exhaustion trial may have allowed for greater differences in performance to be measured between conditions, as participants would have been under stress for longer, but that approach was considered less valid for athletic populations. It is possible the Cold condition would prolong exercise via a greater gradient for heat loss over that of the higher temperature condition. This hypothesis is not supported by Galloway and Maughan (1997) who observed an increased time to exhaustion in 11 °C over 4 °C and 21 °C, with their measure of performance being time to exhaustion at a given set intensity (70% $\dot{V}O_{2\max}$). In contrast, results of a meta-analysis of marathon running performance indicate that 5-10 °C may be optimal (Montain, Ely, & Chevront, 2007). It may be that the intensity of the time trial in the current study, being much higher than that of the performance trial of Galloway and Maughan (1997), elicited greater heat production and therefore no difference in performance was observed between the two conditions. Furthermore, participants may experience a learning effect from a time trial test and therefore a time to exhaustion test may

be less susceptible to learning effects that may have been experienced by some participants who were unfamiliar with time trial situations.

Facing air velocity

The use of semi-realistic, forced convection is rare among research studies (Adams et al., 1992; Saunders et al., 2005). The forced convection does provide more applicable information to the athlete and sports science community, especially with regards to thermoregulatory and CVS responses (Cheuvront, Carter, Montain, Stephenson, & Sawka, 2004). Previous research has shown that facing air velocity of $3.0 \text{ m}\cdot\text{s}^{-1}$ lowered \bar{T}_{sk} and T_c (esophageal) in $24 \text{ }^\circ\text{C}$ compared to $0.3 \text{ m}\cdot\text{s}^{-1}$, without having any affect on HR (Adams et al., 1992). Saunders et al. (2005) found exacerbated T_c (esophageal), T_{re} (rectal), \bar{T}_{sk} and HR responses when wind speed was zero compared with $10 \text{ km}\cdot\text{h}^{-1}$, and was further lowered slightly when wind speed was $33 \text{ km}\cdot\text{h}^{-1}$. Hence, the effect of increasing wind speed is exponential and this study represented most of the cooling power that cyclists would get due to facing air velocity of $3.9 \text{ m}\cdot\text{s}^{-1}$ ($\sim 14 \text{ km}\cdot\text{h}^{-1}$).

Specifically, it could be that the stronger fan cooling in the present study made the Temperate environment less heat stressful and therefore the HR response was lower than that in the ‘temperate’ conditions of Febbraio et al. (1996b) and Layden et al. (2002) and, thus, less of a difference between temperatures was observed. Accordingly, the $17 \text{ }^\circ\text{C}$ (Febbraio et al., 1996b) and $20 \text{ }^\circ\text{C}$ (Layden et al., 2002) difference in environmental temperature may have had more of an effect on heart rate, and in turn metabolism during exercise than that of the $\sim 12 \text{ }^\circ\text{C}$ environmental temperature difference in the present study. Therefore it is probably a combination of the large temperature range and especially the lack of convective cooling in those studies that caused the different HR response in contrast to that observed in the current study.

In the current study, the T_a was $5 \text{ }^\circ\text{C}$, when considering the effect of the large fan 1 m directly in front of the participants at $3.9 \text{ m}\cdot\text{s}^{-1}$ ($\sim 14 \text{ km}\cdot\text{h}^{-1}$), the wind chill temperature is

closer to ~ 2 °C (Osczevski & Bluestein, 2005). Similarly 15 °C becomes closer to 14 °C when facing air velocity is considered (Osczevski & Bluestein, 2005; Castellani et al., 2006). Therefore, the temperature difference between conditions in the current study is closer to 12 °C when realistic airflow is considered, providing much more practical data.

Perceptual responses

The link between \bar{T}_{sk} and participants perception of cold is exemplified in Thompson and Hayward (1996) where more than half of the participants were ‘unable’ to continue exercising, yet participants were not hyperthermic during walking in wet and cold conditions. This illustrates that peripherally generated perception, i.e. \bar{T}_{sk} and wetness, affects exercise adherence, and perhaps also the lack of rise in T_c when it should ordinarily be higher in that circumstance. Thermal sensation – which is dominated by participants’ perceptions of their \bar{T}_{sk} (Gagge, Stolwijk, & Hardy, 1967) – was higher in the Temperate condition compared to the Cold condition in the present study. However, an increase in thermal sensation did not correspond to participants reporting feeling warmer, as participants reported feeling more “neutral” on the thermal discomfort scale in the Temperate condition. This change in perception is not directly related to a change in performance, as large decrements in performance are only seen at extremes, i.e. when thermal stress is high (Hancock, Ross, & Szalma, 2007)

As the exercise progressed in the Temperate condition, there was a greater increase in RPE than in the Cold condition. It is possible that participants perceived the exercise to be harder in the Temperate due to increased \bar{T}_{sk} . This difference in RPE between conditions was not significantly related to cardiovascular or metabolic responses. In summary, participants felt ‘warmer’, and were reportedly more comfortable with their perception of skin temperature, yet they found the exercise to be increasingly more difficult in Temperate compared to Cold.

5.2 *Effects of clothing*

As mentioned above, additional clothing in 5 °C would have similar effects to exercising in warmer temperatures, therefore the detailed physiological considerations that were discussed previously will not be repeated extensively here. Therefore, the focus in the subsequent section is on the magnitude and nature of Clothing effects, especially perceptual, and will be discussed with an integrative approach.

Thermoregulation and cardiovascular factors influencing metabolism

Cardiovascular strain, measured via HR, tended to be increased when exercising with clothing in 5 °C compared with exercise in Cold. However, this increase in CVS strain could be related to the significantly higher resting HR in the Clothing condition, accounting for the consistent higher HR observed during the 75-min exercise. The higher resting HR in Clothing compared to Cold seems to be a random effect as at that stage of measurement participants were not wearing different clothing, or in different environmental conditions. Even though the altered resting HR may explain the differences between the two conditions, it may be that the clothing layer provided a slightly higher stress due to the change in evaporative conditions and hence the effect that Clothing had on reported sensation and measured \bar{T}_{sk} .

Mean skin temperature was increased by ~1 °C when the clothing was worn and as with the ambient comparison, Σ skinfolds influenced \bar{T}_{sk} so that it was lower with larger Σ skinfolds, and therefore percentage of body fat. Interestingly, there was a trend for the rate of CHO oxidation to be higher in Clothing and the rate of fat oxidation to be lower, compared to Cold. However, total fat and CHO oxidation over the 75 min exercise was not different between conditions. This difference between Cold and Clothing could be influenced by T_m being higher in Clothing due to a change in muscle blood flow via the body having to deal with a change in evaporation.

Blood flow to the muscle is an important consideration as a hotter muscle, due to reduced muscle blood flow (Williams, Bredell, Wyndham et al., 1962), uses more CHO (Febbraio, Snow, Hargreaves et al., 1994; Bergman & Brooks, 1999). This is thought to be due to less oxygen supply to the muscle and therefore the breakdown of FFAs is reduced, as it requires a larger amount of oxygen and a higher metabolic rate due to being hotter (Q_{10} effect). However, in contradiction, the small difference in \bar{T}_{sk} (~ 1 °C) in combination with the trend towards lower adrenaline response by the end of exercise, would suggest that metabolism would be unaltered between conditions. Therefore, it is assumed that muscle blood flow was different between Cold and Clothing, although there was little difference in local vasodilatory drive, and therefore a greater Q_{10} effect at the muscular level and, thus, causing the rate of fat oxidation to be reduced.

Respiratory exchange ratio was not different between conditions but may have been skewed by extreme responses of two individuals. Participant 11 exhibited much higher RER values (> 1 SD from the mean) in the Clothing trial than any of the other participants. As this participant completed the Clothing trial as her third trial, training status over the three-month period may have resulted in these extreme, yet physiologically possible values as demonstrated by the responses of trained cyclists exercising at 75% $\dot{V}O_{2\max}$ held RER values > 1 for 45 min (Bergman & Brooks, 1999). Participant 3 exhibited much lower RER values (> 1 SD from the mean) in both the Cold and Clothing trials. However, when these participants were removed from analyses, there was no difference on the treatment effect and therefore data from all participants have been reported.

There was no difference in the response of catecholamines, adrenaline or noradrenaline to Cold and Clothing environments, in contrast to the observed trend of upregulated CHO oxidation in Clothing. This shift in metabolism away from fat and towards CHO cannot be explained by catecholamines, or insulin, since concentrations were similar in both conditions. Liver-derived glucose oxidation did not differ between conditions, however,

there was increased MG oxidation in the Clothing, suggesting that the small difference in T_c , in combination with the extra layer covering the skin, may have created a greater T_m and hence CHO oxidation was upregulated. This is supported by studies in which MG utilisation was evaluated during exercise in the heat (Fink et al., 1975; Febbraio et al., 1994), in combination with increased blood lactate levels (Williams et al., 1962). However, in the current study blood lactate concentration was similar whilst wearing Clothing compared to Cold, which does not support the increase MG oxidation in Clothing.

The addition of Clothing to the normal cycling attire in Cold, in this study's conditions with significant facing air velocity appears to have a greater effect on metabolism than when altering the T_a (Temperate vs. Cold). There was a tendency for the rate of CHO oxidation to be increased in Clothing, which does not agree with current literature if considering the Clothing condition similar to other temperate conditions. However, one must bear in mind that in these studies facing air velocity was much less than that of the current study. However the lack of difference in catecholamine response between Cold and Clothing does not support differences in sympathetic drive as a mechanism. Furthermore, insulin concentrations were also not different, and therefore it may have been that T_m was higher in Clothing that lead to an increase in temperature (due to decreased muscle blood flow) at the local muscle level to increase CHO oxidation.

Perceptual responses

When participants rated thermal discomfort, the Clothing condition did not result in a changed thermal discomfort than for the Cold condition. The lack of change in comfort could be related to the possible increased sensation of skin wetness in Clothing causing the participants to not feel any extra comfort, although they reported feeling closer to neutral in their thermal sensation responses. In a study involving a comparison between manikin thermal responses and human comfort responses to different clothing types it was found that participants were more comfortable when the clothing had a higher water vapour resistance,

and was therefore represented by sensations of permeability and skin dryness (Fan & Tsang, 2008)

In the current study, Clothing did not change how the participants rated the intensity of the exercise. It is possible that the fit of the clothing used in this investigation influenced participants' perceptions as many cyclists wear tight lycra leg and arm warmers, rather than the looser-fitting garments that were worn in this study. Therefore, they may have felt more comfortable in such an attire that is possibly made of a tighter weave than the SilkBody clothing and with the facing air facilitating convection and therefore lowering the clothing's insulative value (Castellani et al., 2006).

As previous researchers have noted (Laing, Sims, Wilson, Niven, & Cruthers, 2008) there are very few studies investigating the physical and perceptual responses to different fabric properties during sport. Their investigation of three different garments effect on participants' perception and physiological responses in cold (8 °C) and hot (32 °C) environments (Laing et al., 2008) concluded that a wool single jersey fabric put the least strain on the thermoregulatory system and was the most comfortable and would therefore be the most appropriate during adventure racing as a base layer or during short-duration walking/running. The fabric in Laing et al. (2008) was similar to that of the current study in thickness, it was lighter mass and therefore thermal resistance was less indicating less of an insulative effect in this study. However, as the focus of the study was on different garment properties, as opposed to garment vs. no garment in the current study, the participants perceptual responses to exercise in 8 °C and 32 °C were measured but not compared between conditions. Similarly, metabolism per se was not investigated and to the author's knowledge, has not been thoroughly investigated in any other studies.

5.3 Conclusions

Previous research (Galloway & Maughan, 1997) has led to the suggestion that T_a for optimal performance of exercise that is prolonged and of moderate intensity at around ~ 11 °C (Nimmo, 2004). However the lack of research in this area may have put too much influence on this one study that could be considered flawed because of lack of air velocity, which would alter heat generation, and the time to exhaustion results over representing the actual effect if taken at face value. Thus with appropriate convection cooling at similar moderate intensity the time to exhaustion results for the warmer temperatures may be closer to the performance at the ‘optimal’ 11 °C. When marathon performance in different ambient conditions was investigated, it was found that optimal performance occurred between 5-10 °C (Ely, Chevront, Roberts, & Montain, 2007). This difference in optimal T_a may be related to the higher intensity and associated greater heat production in marathon competition. In the current investigation we observed no difference in performance between 5 and 15 °C. ‘Thermoneutral’ whilst at rest has been suggested to be 27-29 °C for men without clothing (Hardy & Du Bois, 1940 cited in Cannon & Keatinge, 1960) and 23-26 °C in lightly clothed men . However, these temperatures cannot be considered ‘thermoneutral’ when exercising at intensities similar to the present study ($\sim 74\% \dot{V}O_{2\max}$). There are an overwhelming number of studies in which conditions are reported as ‘thermoneutral’ or ‘control’ exercise testing conditions with ambient temperatures above 20 °C (i.e. Buskirk et al., 1963; Jacobs et al., 1985; Graham et al., 1989; Grucza, Pekkarinen, & Hanninen, 1999; Bailey, Zacher, & Mittleman, 2000; Péronnet, Massicotte, Folch et al., 2006 as examples). These conditions are more likely to heat stress participants during moderately high exercise ($+65\% \dot{V}O_{2\max}$) than provide a ‘temperate’ condition. Therefore, in future research particular attention should be paid to the T_a of control conditions, even if it is not directly considering thermoregulation, as previous studies have illustrated that T_a can alter metabolism.

In short, the results from this study indicate that reducing the temperature by ~ 12 °C (when considering the wind chill from realistic forced convection) does not change metabolism in female cyclists. Although recent literature has indicated exercising in the cold increases CHO oxidation, the results of the present study indicate moderately intense exercise, exhibits the same metabolic stress in temperatures 2-14 °C when there is realistic airflow is incurred (i.e. cycling outdoors or with large fans) and ‘normal’ cycling attire is worn. Methodological differences between this and previous research may explain some of the discrepant findings, in which this study has used more realistic airflow and temperature that is more ‘thermoneutral’ than most studies and therefore more valid. Furthermore, as no studies involving female participants with partitioning data are available, the discrepant findings are likely to be, in part, sex-related differences.

The issue of interpreting clothing worn during metabolism studies adds another ‘layer’ surrounding the issue of exercising in cool T_a on substrate metabolism (Jett et al., 2006). There are no studies to the authors knowledge, that have investigated the effect of clothing on metabolism, and many of the studies, in which the metabolic effects of exercising in the cold have been investigated, fail to mention important specific details about the clothing in which the exercise was performed in (Jett et al., 2006). As an example, some do not refer to what participants wore at all (Febbraio et al., 1996b), even though T_a was the variable being manipulated, making comparisons with other studies difficult. Others only provide information such as ‘light’ clothing, and as Jett et al. (2006, p. 653) notes “it is difficult to interpret whether light clothing typical of summer wear, or light clothing worn in cooler climates”. The current study provides specific information regarding the baseline clothing worn, and the additional clothing, with regards to substrate metabolism during exercise in low T_a .

The information provided is highly applicable to exercise outside of the laboratory with the use of semi-realistic airflow, as well as participants wearing appropriate helmets

and short-fingered gloves in all trials. The Clothing provided a slight increase in insulation in that \bar{T}_{sk} was higher, yet T_c was similar between conditions, and as discussed in the previous section, this generally has led to little changes in T_m and therefore small CVS strain and autonomic nervous system changes may have contributed to a very small shift in metabolism.

Further research is needed to determine the effects on metabolism of exercising in cold environments to determine whether there is in fact an upregulation of CHO metabolism and if so at which temperatures, with which facing air velocities and exercise intensities. This information may prove invaluable to athletes competing in varying environments in relation to performance. In particular, metabolic responses to exercise in the cold across males and females are of interest, in order to determine if there is a difference in substrate oxidation. Further to this, differing body compositions across the sexes would be worth further examination, as this would provide information to determine if it is skinfold thickness, as a measure of body composition, that most influences substrate metabolism. The effect of training status in combination with heat acclimation on responses to exercise in the cold would also be of interest, as highly trained individuals that are used to high temperatures may have better thermoregulatory strategies that would also benefit in cooler environments and already have enhanced fat metabolism. Finally, as T_m has only been assumed in previous studies, it would be relevant to collect data involving metabolism as well as deep muscle temperature.

5.4 *Limitations*

In essence, a greater metabolic and/or performance effect may have been evident in a colder environment ($< 5\text{ }^{\circ}\text{C}$), but would have questionable practical value – i.e. cyclists tend to wear more clothing in colder environments. This study is limited in part by the number of participants; although more participants were studied than in most studies that we have compared our data with (including but not limited to Febbraio et al., 1996b; Galloway &

Maughan, 1997; Romijn et al., 2000; Mäkinen et al., 2001; Layden et al., 2002). A power analysis was conducted using variance and effect size data from a study involving male participants (see Methods p.20) however, any effect on metabolism in females that may exist may be much smaller than that of exercise intensity, duration, and participant training status. The inclusion of a direct sex comparison by having a male control group could improve the study, however, with the time restrictions the importance was put onto female responses as they are warranted in their own right, as addressed elsewhere. It is also possible that a 10 °C (to ~12 °C when accounting for wind chill) difference in T_a between conditions is not great enough to change metabolism via altering T_c and \bar{T}_{sk} . However, a 10 °C difference provides more practical information than studies with wide temperature ranges or studies that have not included a temperate environment i.e. 9 °C versus 40 °C (Fink et al., 1975).

It is acknowledged that there are limitations to every method, and although the present methodology partitions substrate use to a greater extent than most other studies, it is still based on derived metabolism measures that may have some errors associated. Previous studies have utilised muscle biopsy samples as measures of MG utilisation, which are limited by the localization of the sample, and therefore the extrapolation of results to a whole-body level. Indirect calorimetry is often biased in that protein oxidation is considered constant or negligible. Furthermore, indirect calorimetry cannot identify the source of fat or CHO utilised, hence the current method, for CHO partitioning in this instance, of stable isotope methodology allows more knowledge with regards to substrate utilisation.

The reliability of the current method was examined by Romijn, Coyle, Hibbert, and Wolfe (1992) who observed that both methods produce similar oxidation rates of CHO and fat. In their study, blood [^{13}C]glucose was not measured and hence partitioning was not reported, but it does confirm the validity of part of the method. Furthermore, the earlier studies using stable isotope methodology have acknowledged the need for an equilibrium period before partitioning can be accurately calculated due to the entrapment of $^{13}\text{CO}_2$ in the

bicarbonate pool (Coggan, 1993). Hence, in the current study comparisons between conditions for MG and liver-derived glycogen oxidation were only made with data from the last 45 min of exercise, allowing for a 60 min equilibrium period.

5.5 Implications

Firstly, the non-invasive methodology provides a much greater amount of detail to the metabolic responses, more specifically partitioning of CHO substrates, of exercise than is currently published. Furthermore, research on exercise responses in females is lacking. Specific training guidelines and recommendations for female athletes should be based on research on female participants and this study will add to the literature for female athletes. This study assists in providing more specific recommendations of CHO utilisation during moderately high training or competition sessions as ~128 g of CHO was oxidised for an hour of exercise. The majority of that was from MG, further quantifying the need for adequate MG stores prior to exercise. Also, the higher intensity used in this study is more relevant for an athletic population and as yet underrepresented in the literature. Finally, the environmental conditions used in this study are realistic training conditions in New Zealand and many other countries and therefore the information presented is relevant.

6.0 SUMMARY

In summary, there is no effect of cold (5 °C vs. 15 °C) on substrate utilisation with moderately hard ($\sim 74\% \dot{V}O_{2\text{ max}}$) cycling in women. Core temperature is unaffected despite differences in skin temperatures and perceptions of cold and discomfort at 5 °C. This lack of effect of cold on substrate metabolism is in contrast to results for males suggesting that women may be more resilient to temperature variation, possibly due to increased subcutaneous fat layer. Due to body composition differences more extreme cold temperature may be required to stress female participants.

The addition of thermal clothing increased CHO utilisation compared to normal cycling attire in 5 °C (Cold). This increase in CHO utilization during the clothing condition was due to an increased provision of energy by the MG, and consequentially less energy from liver-derived glycogen. Although the Clothing condition altered participants' perception of their body temperature whilst exercising, it failed to alleviate the discomfort from exercising in low T_a .

Future studies on exercise in cold environments should be conducted to further investigate measures of different types of exercise performance in varying cold environments, in males and females with varying body composition. Future work should specify all environmental variables including facing air velocity, clothing, humidity, and state of acclimatisation.

Although research involving female participants is becoming more prevalent, research on female metabolic responses to exercise in temperature extremes is lacking. Females and males differ in their metabolic responses to exercise, although the exact mechanism is unknown, hormones and body composition differences are probable factors and hence males and females need to be considered separately. More research involving female participants with similar training status and the role of sex hormones is needed to reduce research bias and provide accurate information for female athletes.

7.0 REFERENCES

- Adams, W. C., Mack, G. W., Langhans, G. W., & Nadel, E. R. (1992). Effects of varied air velocity on sweating and evaporative rates during exercise. *Journal of Applied Physiology*, 73(6), 2668-2674.
- Ahlborg, G., & Felig, P. (1982). Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. *Journal of Clinical Investigation*, 69(1), 45-54.
- Anderson, D. E., & Hickey, M. S. (1994). Effects of caffeine on the metabolic and catecholamine responses to exercise in 5 and 28°C. *Medicine and Science in Sports and Exercise*, 26(4), 453-458.
- Bailey, S. P., Zacher, C. M., & Mittleman, K. D. (2000). Effect of menstrual cycle phase on carbohydrate supplementation during prolonged exercise to fatigue. *Journal of Applied Physiology*, 88(2), 690-697.
- Bergman, B. C., & Brooks, G. A. (1999). Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *Journal of Applied Physiology*, 86(2), 479-487.
- Bergström, J. (1962). Muscle electrolytes in man. *Scandinavian Journal of Clinical Investigations, Suppl 68*, 1-100.
- Bergström, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and physical performance. *Acta Physiologica Scandinavica*, 71, 140-150.
- Borer, K. T. (2003). *Exercise Endocrinology*. Champaign, IL: Human Kinetics.
- Brisson, G. R., Boisvert, P., Peronnet, F., Perrault, H., Boisvert, D., & Lafond, J. S. (1991). A simple and disposable sweat collector. *European Journal of Applied Physiology*, 63, 269-272.
- Brooks, G. A. (1986). Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. *Federation Proceedings*, 45(13), 2924-2929.
- Brooks, G. A., & Mercier, J. (1994). Balance of carbohydrate and lipid utilization during exercise - the crossover concept. *Journal of Applied Physiology*, 76(6), 2253-2261.
- Buelle, Y., Peronnet, F., Charpentier, S., Lavoie, C., Hillaire-Marcel, C., & Massicotte, D. (1999). Oxidation of an oral [C-13]glucose load at rest and prolonged exercise in trained and sedentary subjects. *Journal of Applied Physiology*, 86(1), 52-60.
- Buskirk, E. R., Thompson, R. H., & Whedon, G. D. (1963). Metabolic response to cold air in men and women in relation to total body fat content. *Journal of Applied Physiology*, 18(3), 603-612.
- Campbell, S. E., Angus, D. J., & Febbraio, M. A. (2001). Glucose kinetics and exercise performance during phases of the menstrual cycle: effect of glucose ingestion. *American Journal of Physiology-Endocrinology and Metabolism*, 281(4), E817-E825.
- Cannon, P., & Keatinge, W. R. (1960). The metabolic rate and heat loss of fat and thin men in heat balance in cold and warm water. *Journal of Physiology*, 154, 329-344.
- Castellani, J. W., Young, A. J., Ducharme, M. B., Giesbrecht, G. G., Glickman, E., & Sallis, R. E. (2006). Prevention of cold injuries during exercise. *Medicine and Science in Sports and Exercise*, 38(11), 2012-2029.
- Chevront, S. N., Carter, R., Montain, S. J., Stephenson, L. A., & Sawka, M. N. (2004). Influence of hydration and airflow on thermoregulatory control in the heat. *Journal of Thermal Biology*, 29(7), 471-477.
- Chryssanthopoulos, C., Hennessy, L. C. M., & Williams, C. (1994). The influence of pre exercise glucose-ingestion on endurance running capacity. *British Journal of Sports Medicine*, 28(2), 105-109.

- Coggan, A. R. (1993). Underestimation of substrate oxidation during exercise due to failure to account for bicarbonate kinetics. *Journal of Applied Physiology*, 75(5), 2341-2342.
- Coggan, A. R. (1999). Use of stable isotopes to study carbohydrate and fat metabolism at the whole-body level. *Proceedings of the Nutrition Society*, 58(4), 953-961.
- Cotter, J. D., & Taylor, N. A. S. (2005). The distribution of cutaneous sudomotor and alliesthesial thermosensitivity in mildly heat-stressed humans: an open-loop approach. *Journal of Physiology*, 565(1), 335-345.
- Coyle, E. F., & Coggan, A. R. (1984). Effectiveness of carbohydrate feeding in delaying fatigue during prolonged exercise. *Sports Medicine*, 1(6), 446-458.
- Coyle, E. F., Coggan, A. R., Hemmert, M. K., & Ivy, J. L. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *Journal of Applied Physiology*, 61(1), 165-172.
- Derman, K. D., Hawley, J. A., Noakes, T. D., & Dennis, S. C. (1996). Fuel kinetics during intense running and cycling when fed carbohydrate. *European Journal of Applied Physiology*, 74(1), 36-43.
- Devries, M. C., Hamadeh, M. J., Phillips, S. M., & Tarnopolsky, M. A. (2006). Menstrual cycle phase and sex influence muscle glycogen utilization and glucose turnover during moderate-intensity endurance exercise. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, 291(4), R1120-R1128.
- Dolny, D. G., & Lemon, P. W. R. (1988). Effect of ambient-temperature on protein breakdown during prolonged exercise. *Journal of Applied Physiology*, 64(2), 550-555.
- Doubt, T. J. (1991). Physiology of exercise in the cold. *Sports Medicine*, 11(6), 367-381.
- Elder, P. A., Yeo, K. H. J., Lewis, J. G., & Clifford, J. K. (1987). An enzyme-linked immunosorbent assay (ELISA) for plasma progesterone: immobilised antigen approach. *Clinica Chimica Acta*, 162(2), 199-206.
- Ely, M. R., Chevront, S. N., Roberts, W. O., & Montain, S. J. (2007). Impact of weather on marathon-running performance. *Medicine and Science in Sports and Exercise*, 39(3), 487-493.
- Fan, J., & Tsang, H. W. K. (2008). Effect of clothing thermal properties on thermal comfort sensation during active sports. *Textile Research Journal*, 78(2), 111-118.
- Febbraio, M. A. (2001). Alterations in energy metabolism during exercise and heat stress. *Sports Medicine*, 31(1), 47-59.
- Febbraio, M. A., Murton, P., Selig, S. E., Clark, S. A., Lambert, D. L., Angus, D. J., et al. (1996a). Effect of CHO ingestion on exercise metabolism and performance in different ambient temperatures. *Medicine and Science in Sports and Exercise*, 28(11), 1380-1387.
- Febbraio, M. A., Snow, R. J., Hargreaves, M., Stathis, C. G., Martin, I. K., & Carey, M. F. (1994). Muscle metabolism during exercise and heat-stress in trained men - effect of acclimation. *Journal of Applied Physiology*, 76(2), 589-597.
- Febbraio, M. A., Snow, R. J., Stathis, C. G., Hargreaves, M., & Carey, M. F. (1996b). Blunting the rise in body temperature reduces muscle glycogenolysis during exercise in humans. *Experimental Physiology*, 81(4), 685-693.
- Fink, W. J., Costill, D. L., & Van Handel, P. J. (1975). Leg muscle metabolism during exercise in heat and cold. *European Journal of Applied Physiology and Occupational Physiology*, 34(3), 183-190.
- Gagge, A. P., Stolwijk, J. A. J., & Hardy, J. D. (1967). Comfort and thermal sensation and associated physiological responses at various ambient temperatures. *Environmental Research*, 1, 1-20.
- Galloway, S. D. R., & Maughan, R. J. (1997). Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Medicine and Science in Sports and Exercise*, 29(9), 1240-1249.

- Gavin, T. P. (2003). Clothing and thermoregulation during exercise. *Sports Medicine*, 33(13), 941-947.
- Graham, T. E., Sathasivam, P., & Macnaughton, K. W. (1991). Influence of cold, exercise, and caffeine on catecholamines and metabolism in men. *Journal of Applied Physiology*, 70(5), 2052-2058.
- Graham, T. E., Viswanathan, M., Van Dijk, J. P., Bonen, A., & George, J. C. (1989). Thermal and metabolic responses to cold by men and by eumenorrheic and amenorrheic women. *Journal of Applied Physiology*, 67(1), 282-290.
- Grucza, R., Pekkarinen, H., & Hanninen, O. (1999). Different thermal sensitivity to exercise and cold in men and women. *Journal of Thermal Biology*, 24, 397-401.
- Haman, F., Peronnet, F., Kenny, G. P., Massicotte, D., Lavoie, C., & Weber, J. M. (2005). Partitioning oxidative fuels during cold exposure in humans: muscle glycogen becomes dominant as shivering intensifies. *Journal of Physiology-London*, 566(1), 247-256.
- Hancock, P. A., Ross, J. M., & Szalma, J. L. (2007). A meta-analysis of performance response under thermal stressors. *Human Factors*, 49(5), 851-877.
- Hardy, J. D., & Du Bois, E. F. (1940). Differences between men and women in their response to heat and cold. *Proceedings of the National Academy of Sciences of the United States of America*, 26, 389-398.
- Harvey, C. R., Frew, R., Massicotte, D., Peronnet, F., & Rehrer, N. J. (2007). Muscle glycogen oxidation during prolonged exercise measured with oral [C-13] glucose: comparison with changes in muscle glycogen content. *Journal of Applied Physiology*, 102(5), 1773-1779.
- Hemingway, A., & Price, W. M. (1968). The autonomic nervous system and regulation of body temperature. *Anesthesiology*, 29(4), 693-701.
- Holloszy, J. O., & Kohrt, W. M. (1996). Regulation of carbohydrate and fat metabolism during and after exercise. *Annual Review of Nutrition*, 16, 121-138.
- Horton, E. S., & Terjung, R. L. (Eds.). (1988). *Exercise, Nutrition, and Energy Metabolism*. New York: Macmillan Publishing Company.
- Horton, T. J., Grunwald, G. K., Lavelly, J., & Donahoo, W. T. (2006). Glucose kinetics differ between women and men, during and after exercise. *Journal of Applied Physiology*, 100(6), 1883-1894.
- Horton, T. J., Pagliassotti, M. J., Hobbs, K., & Hill, J. O. (1998). Fuel metabolism in men and women during and after long-duration exercise. *Journal of Applied Physiology*, 85(5), 1823-1832.
- Hultman, E. (1967). Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and diet. *Scandinavian Journal of Clinical & Laboratory Investigation*, S 19, 11-&.
- Hurley, B. F., & Haymes, E. M. (1982). The effects of rest and exercise in the cold on substrate mobilization and utilization. *Aviation Space and Environmental Medicine*, 53(12), 1193-1197.
- ISO 9886. (2004). Ergonomics - Evaluation of thermal strain by physiological measurements (pp. 1-21). Geneva: ISO.
- Jacobs, I., Romet, T. T., & Kerriganbrown, D. (1985). Muscle glycogen depletion during exercise at 9-degrees-C and 21-degrees-C. *European Journal of Applied Physiology and Occupational Physiology*, 54(1), 35-39.
- Jay, O., Reardon, F. D., Webb, P., DuCharme, M. B., Ramsay, T., Nettlefold, L., et al. (2007). Estimating changes in mean body temperature for humans during exercise using core and skin temperatures is inaccurate even with a correction factor. *Journal of Applied Physiology*, 103(2), 443-451.
- Jett, D. M., Adams, K. J., & Stamford, B. A. (2006). Cold exposure and exercise metabolism. *Sports Medicine*, 36(8), 643-656.

- Jeukendrup, A. E., Raben, A., Gijzen, A., Stegen, J., Brouns, F., Saris, W. H. M., et al. (1999). Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. *Journal of Physiology*, *515*(2), 579-589.
- Kaciuba-Uscilko, H., & Grucza, R. (2001). Gender differences in thermoregulation. *Current Opinion in Clinical Nutrition*, *4*(6), 533-536.
- Keatinge, W. R. (1960). The effects of subcutaneous fat and of previous exposure to cold on the body temperature, peripheral blood flow and metabolic rate of men in cold water. *Journal of Physiology*, *153*, 166-178.
- Kuipers, H., Keizer, A., Brouns, F., & Saris, W. H. M. (1987). Carbohydrate feeding and glycogen synthesis during exercise in man. *Pflugers Archiv-European Journal of Physiology*, *410*(6), 652-656.
- Laing, R. M., Sims, S. T., Wilson, C. A., Niven, B. E., & Cruthers, N. M. (2008). Differences in wearer response to garments for outdoor activity. *Ergonomics*, *51*(4), 492-510.
- Layden, J. D., Patterson, M. J., & Nimmo, M. A. (2002). Effects of reduced ambient temperature on fat utilization during submaximal exercise. *Medicine and Science in Sports and Exercise*, *34*(5), 774-779.
- Lee, D. T., & Haymes, E. M. (1995). Exercise duration and thermoregulatory responses after whole body precooling. *Journal of Applied Physiology*, *79*(6), 1971-1976.
- Lemire, B. B., Gagnon, D., Jay, O., & Kenny, G. P. (2009). Differences between sexes in rectal cooling rates after exercise-induced hyperthermia. *Medicine and Science in Sports and Exercise*, *41*(8), 1633-1639.
- Lemon, P. W. R., & Mullin, J. P. (1980). Effect of initial muscle glycogen levels on protein catabolism during exercise. *Journal of Applied Physiology*, *48*(4), 624-629.
- Livesey, G., & Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilisation, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition*, *47*, 608-628.
- M'Kaouar, H., Peronnet, F., Massicotte, D., & Lavoie, C. (2004). Gender difference in the metabolic response to prolonged exercise with [C-13]glucose ingestion. *European Journal of Applied Physiology*, *92*(4-5), 462-469.
- Makinen, T. T., Gavhed, D., Holmer, I., & Rintamaki, H. (2001). Effects of metabolic rate on thermal responses at different air velocities in -10 degrees C. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology*, *128*(4), 759-768.
- Marfell-Jones, M., Olds, T., Stewart, A., & Carter, L. E. (2006). *International Standards of Anthropometric Assessment (ISAK)*. South Africa: International Society for the Advancement of Kinanthropometry.
- Marmy-Conus, N., Fabris, S., Proietto, J., & Hargreaves, M. (1996). Preexercise glucose ingestion and glucose kinetics during exercise. *Journal of Applied Physiology*, *81*(2), 853-857.
- Maughan, R. J., Gleeson, M., & Greenhaff, P. L. (1997). *Biochemistry of exercise and training*. New York: Oxford University Press.
- Montain, S. J., Ely, M. R., & Chevront, S. N. (2007). Marathon performance in thermally stressing conditions. *Sports Medicine*, *37*(4-5), 320-3223.
- National Institute of Water and Atmospheric Research (NIWA). (2008). Monthly climate summaries. Retrieved 4 September, 2009, from <http://www.niwa.co.nz/news-and-publications/publications/all/cs/monthly>
- Nimmo, M. A. (2004). Exercise in the cold. *Journal of Sports Sciences*, *22*(10), 898-915.
- Osczevski, R., & Bluestein, M. (2005). The new wind chill equivalent temperature chart. *Bulletin of the American Meteorological Society*, *86*(10), 1453-1458.

- Pandolf, K. B., Sawka, M. N., & Gonzalez, R. R. (Eds.). (1988). *Human Performance Physiology and Environmental Medicine at Terrestrial Extremes*. Indianapolis, IN: Brown & Benchmark.
- Patterson, S., Reid, S., Gray, S., & Nimmo, M. (2008). The response of plasma interleukin-6 and its soluble receptors to exercise in the cold in humans. *Journal of Sports Sciences, 26*(9), 927-933.
- Péronnet, F., & Massicotte, D. (1991). Table of nonprotein respiratory quotient: an update. *Canadian Journal of Sports Science, 16*(1), 23-29.
- Péronnet, F., Massicotte, D., Folch, N., Melin, B., Koulmann, N., Jimenez, C., et al. (2006). Substrate utilization during prolonged exercise with ingestion of C-13-glucose in acute hypobaric hypoxia (4,300 m). *European Journal of Applied Physiology, 97*(5), 527-534.
- Phillips, S. M., Atkinson, S. A., Tarnopolsky, M. A., & Macdougall, J. D. (1993). Gender differences in leucine kinetics and nitrogen-balance in endurance athletes. *Journal of Applied Physiology, 75*(5), 2134-2141.
- Roepstorff, C., Steffensen, C. H., Madsen, M., Stallknecht, B., Kanstrup, I. L., Richter, E. A., et al. (2002). Gender differences in substrate utilization during submaximal exercise in endurance-trained subjects. *American Journal of Physiology-Endocrinology and Metabolism, 282*(2), E435-E447.
- Romijn, J. A., Coyle, E. F., Hibbert, J., & Wolfe, R. R. (1992). Comparison of indirect calorimetry and a new breath C-13/C-12 ratio method during strenuous exercise. *American Journal of Physiology, 263*(1), E64-E71.
- Romijn, J. A., Coyle, E. F., Sidossis, L. S., Rosenblatt, J., & Wolfe, R. R. (2000). Substrate metabolism during different exercise intensities in endurance-trained women. *Journal of Applied Physiology, 88*(5), 1707-1714.
- Ruby, B. C., Coggan, A. R., & Zderic, T. W. (2002). Gender differences in glucose kinetics and substrate oxidation during exercise near the lactate threshold. *Journal of Applied Physiology, 92*(3), 1125-1132.
- Saltin, B., Gagge, A. P., & Stolwijk, J. A. (1968). Muscle temperature during submaximal exercise in man. *Journal of Applied Physiology, 25*(6), 679-688.
- Saunders, A. G., Dugas, J. P., Tucker, R., Lambert, M. I., & Noakes, T. D. (2005). The effects of different air velocities on heat storage and body temperature in humans cycling in a hot, humid environment. *Acta Physiologica Scandinavica, 183*(3), 241-255.
- Sheffield-Moore, M., Short, K. R., Kerr, C. G., Parcell, A. C., Bolster, D. R., & Costill, D. L. (1997). Thermoregulatory responses to cycling with and without a helmet. *Medicine and Science in Sports and Exercise, 29*(6), 755-761.
- Sherman, W. M., Brodowicz, G., Wright, D. A., Allen, W. K., Simonsen, J., & Dernbach, A. (1989). Effects of 4 h pre exercise carbohydrate feedings on cycling performance. *Medicine and Science in Sports and Exercise, 21*(5), 598-604.
- Sink, K. R., Thomas, T. R., Araujo, J., & Hill, S. F. (1989). Fat energy use and plasma-lipid changes associated with exercise intensity and temperature. *European Journal of Applied Physiology and Occupational Physiology, 58*(5), 508-513.
- Stevens, G. H. J., Graham, T. E., & Wilson, B. A. (1987). Gender differences in cardiovascular and metabolic responses to cold and exercise. *Canadian Journal of Physiology and Pharmacology, 65*(2), 165-171.
- Stocks, J. M., Taylor, N. A. S., Tipton, M. J., & Greenleaf, J. E. (2004). Human physiological responses to cold exposure. *Aviation Space and Environmental Medicine, 75*(5), 444-457.
- Tarnopolsky, L. J., Macdougall, J. D., Atkinson, S. A., Tarnopolsky, M. A., & Sutton, J. R. (1990). Gender differences in substrate for endurance exercise. *Journal of Applied Physiology, 68*(1), 302-308.

- Tarnopolsky, M. A. (2008). Sex differences in exercise metabolism and the role of 17-beta estradiol. *Medicine and Science in Sports and Exercise*, 40(4), 648-654.
- The Royal Women's Hospital. (2006). Prescription of oral contraceptives: women's health nurse practitioner management. Retrieved 18 February, 2009, from <http://www.thewomens.org.au/PrescriptionofOralContraceptivesWomensHealthNursePractitionerManagement#MONOPHASIC%289entries%29>
- Thompson, R. L., & Hayward, J. S. (1996). Wet-cold exposure and hypothermia: Thermal and metabolic responses to prolonged exercise in rain. *Journal of Applied Physiology*, 81(3), 1128-1137.
- Timmons, B. A., Araujo, J., & Thomas, T. R. (1985). Fat utilization enhanced by exercise in a cold environment. *Medicine and Science in Sports and Exercise*, 17(6), 673-678.
- Toner, M. M., Sawka, M. N., Foley, M. E., & Pandolf, K. B. (1986). Effects of body mass and morphology on thermal responses in water. *Journal of Applied Physiology*, 60(2), 521-525.
- Tremblay, J. H., Péronnet, F., Lavoie, C., & Massicotte, D. (2009). Fuel selection during prolonged arm and leg exercise with ¹³C-glucose ingestion. *Medicine and Science in Sports and Exercise*, 41(12), 2151-2157.
- Trimmer, J. K., Schwarz, J. M., Casazza, G. A., Horning, M. A., Rodriguez, N., & Brooks, G. A. (2002). Measurement of gluconeogenesis in exercising men by mass isotopomer distribution analysis. *Journal of Applied Physiology*, 93(1), 233-241.
- Vallerand, A. L., & Jacobs, I. (1989). Rates of energy substrates utilization during human cold-exposure. *European Journal of Applied Physiology and Occupational Physiology*, 58(8), 873-878.
- Vallerand, A. L., Jacobs, I., & Kavanagh, M. F. (1989). Mechanism of enhanced cold tolerance by an ephedrine-caffeine mixture in humans. *Journal of Applied Physiology*, 67(1), 438-444.
- van Amber, R. R. (2009). *Properties of silk and silk blend knit fabrics*. Unpublished master's thesis, University of Otago, Dunedin, New Zealand.
- van Hamont, D., Harvey, C. R., Massicotte, D., Frew, R., Peronnet, F., & Rehrer, N. J. (2005). Reduction in muscle glycogen and protein utilisation with glucose feeding during exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 15(4), 350-365.
- van Loon, L. J. C., Greenhaff, P. L., Teodosiu, D. C., Saris, W. H. M., & Wagenmakers, A. J. M. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *Journal of Physiology*, 536(1), 295-304.
- van Loon, L. J. C., Jeukendrup, A. E., Saris, W. H. M., & Wagenmakers, A. J. M. (1999). Effect of training status on fuel selection during submaximal exercise with glucose ingestion. *Journal of Applied Physiology*, 87(4), 1413-1420.
- Wagenmakers, A. J. M. (1999). Tracers to investigate protein and amino acid metabolism in human subjects. *Proceedings of the Nutrition Society*, 58(4), 987-1000.
- Wallis, G. A., Dawson, R., Achten, J., Webber, J., & Jeukendrup, A. E. (2006). Metabolic response to carbohydrate ingestion during exercise in males and females. *American Journal of Physiology-Endocrinology and Metabolism*, 290(4), E708-E715.
- Wallis, G. A., Yeo, S. E., Blannin, A. K., & Jeukendrup, A. E. (2007). Dose-response effects of ingested carbohydrate on exercise metabolism in women. *Medicine and Science in Sports and Exercise*, 39(1), 131-138.
- Wang, Y., Moss, J., & Thisted, R. (1992). Predictors of body surface area. *Journal of Clinical Anesthesia*, 4(1), 4-10.
- Williams, C. G., Bredell, G. A. G., Wyndham, C. H., Strydom, N. B., Morrison, J. F., Peter, J., et al. (1962). Circulatory and metabolic reactions to work in heat. *Journal of Applied Physiology*, 17(4), 625-638.

- Wismann, J., & Willoughby, D. (2006). Gender differences in carbohydrate metabolism and carbohydrate loading. *Journal of the International Society of Sports Nutrition*, 3(1), 28-34.
- Zderic, T. W., Coggan, A. R., & Ruby, B. C. (2001). Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. *Journal of Applied Physiology*, 90(2), 447-453.
- Zouhal, H., Jacob, C., Delamarche, P., & Gratas-Delamarche, A. (2008). Catecholamines and the effects of exercise, training and gender. *Sports Medicine*, 38(5), 401-423.

8.0 APPENDICES

Appendix A: Consent Form

Appendix B: Information sheet

Appendix C: Health screening questionnaire

Appendix D: Medical History and Training Questionnaire

Appendix E: Submaximal $\dot{V}O_{2\max}$ protocol

Appendix F: Additional Food Information Sheet

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Appendix I: Double bed ion exchange chromatography

Appendix J: Thermal discomfort scale

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Appendix L: Rate of perceived exertion scale

COLD AND CLOTHING IN THE COLD: EFFECT ON SUBSTRATE

UTILISATION IN ENDURANCE-TRAINED WOMEN

CONSENT FORM FOR PARTICIPANTS

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.

I know that:-

1. My participation in the project is entirely voluntary.
2. I am free to withdraw from the project at any time without any disadvantage.
3. The data will be destroyed at the conclusion of the project but any raw data on which the results of the project depend will be retained in secure storage for five years, after which it will be destroyed.
4. I am aware of the potential discomfort and risks.
5. I have read and signed the Health Screening Questionnaire.
6. Remuneration is not available.
7. The results of the project may be published and will be available in the library but every attempt will be made to preserve my anonymity.
8. I have had all my questions satisfactorily answered.

I agree to take part in this project.

.....
(Signature of participant)

.....
(Date)

COLD AND CLOTHING IN THE COLD: EFFECT ON SUBSTRATE UTILISATION IN ENDURANCE-TRAINED WOMEN

INFORMATION SHEET FOR PARTICIPANTS

Thank you for showing an interest in this project. Please read this information sheet carefully before deciding whether or not to participate. If you decide to participate we thank you. If you decide not to take part there will be no disadvantage to you of any kind and we thank you for considering our request.

What is the Aim of the Project?

The aim of this study is to investigate the effect that low ambient temperature will have on substrate metabolism, in particular on carbohydrate metabolism. A second purpose is to investigate the modulating effect of clothing on exercise substrate metabolism in cold temperatures (5 °C).

This research forms part of requirements for the Masters degree in Physical Education. Testing will take place at the School of Physical Education, University of Otago.

What Type of Participants are being sought?

We are looking for twelve healthy female participants in the age range of 18-39 years, and who typically train at least three to four times per week for 1.5-2 h per session. For ease of transport and communication, living in the Dunedin area is preferable. Participants will be in apparently good health, with no known injuries or illnesses that may affect safe participation. Therefore, prospective participants must complete a health-orientated questionnaire before inclusion in this study. People who meet one or more of the exclusion criteria set out in that questionnaire are requested not to participate in this project, because in the opinion of the researchers at the University of Otago Human Ethics Committee, it involves unacceptable risk to them. In particular, **you will be unable to participate if you have known injuries or illnesses involving:**

- **Cardiovascular, renal or gastrointestinal tract,**
- **Muscles, tendons, ligaments or bones, or**
- **Exercise intolerance**

What will Participants be asked to do?

Should you agree to take part in this project, you will be asked to complete a health-orientated screening questionnaire and visit the laboratory on four occasions. The purpose of the screening questionnaire is to help ensure that any risks to you associated with involvement in this study will be minimal. The first session will be to determine your maximal aerobic fitness (VO_2 max protocol), your size of thermals, and also to familiarize you with the procedure and equipment. Sessions two to four will be performed in random order, and will consist of the same exercise protocol, but either in an ambient temperature of 5 degrees with thermals or without thermals, or at 15 degrees without thermals. You will need to be in the same menstrual phase for each trial so we will ask you to record your menstrual cycle so we can attain the days available to test.

Session 1 will take ~1 hour, and involves:

- Determination of thermal size
- Cycling on a stationary bike to voluntary exhaustion (to determine maximal oxygen uptake (VO_2 max). This exercise duration will typically be less than 20 minutes.

Sessions 2-4 (test sessions) will take around ~4 hours per session (7am – 11am), with approximately one month between sessions. These sessions involve:

- Resting measurements of height, body mass, heart rate, oxygen consumption, rectal temperature, and skin temperature measurements at nine sites
- Measurements of body composition using bioimpedance
- Insertion of a cannula in your forearm for blood collection at rest and every fifteen minutes during the trial
- Exercise in normal cycling attire (helmet, glasses, gloves, shirt, shorts, socks and shoes) either wearing or not wearing thermal pants and top (provided)
 - Exercise consists of cycling on an ergometer at 70% VO_2 max for 75 minutes, then a 4km time trial;
 - Exercise will be in one of two environmental conditions;
 - Either 5 °C OR 15 °C

Standardisation of your status before and between sessions (sessions 2-4) is very important, so it is necessary that you:

- Record your dietary intake (including fluid) and exercise regime for two days prior to testing
- Replicate this for the subsequent test sessions

It will help if you can:

- Be available at the same time of day for each test **each month**;
- Abstain from alcohol and caffeine consumption for 24 hours prior to each test day
- Avoid exercise 24 hours prior to test day

If you feel unwell at any stage during the 48 hours prior to a testing session, please let us know and we will arrange with you an alternative time for that session.

Please be aware that you may decide not to take part in the project without any disadvantage to yourself of any kind.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

What Data/Information will be Collected and What Use will be made of it?

The following data will be collected from you:

Fitness Related:

- Maximal aerobic power (~aerobic fitness, or VO₂ max) based on your oxygen consumption and maximal heart rate responses to maximal workloads on a cycle ergometer. This test will be used to determine the exercise intensity for the test sessions, and for characterising participants as a group.

Physiological status (generally measured continuously):

- Core body temperature – from the rectum, using a flexible, sterile and disposable thermistor. This will be used to help monitor your safety, and to quantify thermal status and the thermal strain associated with exercise.
- Skin temperatures – from small temperature-measuring devices (thermistors) taped onto the skin at nine locations (forehead, scapula, chest, bicep, forearm, finger, thigh, calf, and foot). This will be used with the core temperatures to determine overall body temperature (and therefore heat storage) as well as any vasoconstriction (to the hands especially).

- Heart rate – from a heart rate monitor that consists of a chest strap and watch. This will also be used to help monitor your safety, and to indicate how hard your body is working in response to the exercise.
- Oxygen uptake and carbon dioxide production – non-invasive breathing through a face mask, for 3 minutes approximately every 15 minutes.
- Blood glucose content – through venous sampling. A cannula will be inserted into your forearm prior to commencement of exercise. Approximately 12ml of blood will be taken at rest, then 8mL at 15-minute intervals during the 75-minute exercise block, and a final 8ml sample after the 4km time trial. Resting measures of insulin, catecholamines and sex hormones (oestradiol and progesterone) will also be measured in the blood.
- Body composition measurements – prior to and immediately after exercise participants will be required to stand on a body composition machine (In Body) to determine muscle mass, fat mass etc
- Sweat and urine samples – a disposable sweat collector will be adhered to your lower back at the beginning of the trial. Throughout the trial small samples of sweat collected will be removed for analysis of protein metabolism. Similarly, urine will be sampled at rest and at the end of exercise also to help measure protein as well as hydration status.

Psychophysical status (measured at approximately 15-minute intervals):

- Thermal comfort (scale of 1 to 10)
- Thermal sensation (scale of 1 to 13)
- Rate of perceived exertion (scale of 6 to 20)

These data will be analysed to determine whether there is a change in substrate metabolism (in particular carbohydrate) between exercise in the cold and temperate environments, as well as whether the addition of clothing in the cold can negate the expected metabolic change.

Results of this study will be presented in a Masters Thesis, and will be available to the University of Otago Library (Dunedin, New Zealand) but anonymity will be preserved. The personal data will be accessible only by the researchers (Drs Rehrer, Cotter, Frew, and Ms Parr). Results of this project may be published and /or presented at a relevant conference but, again, any data will not be linked to any specific participant, and data will typically be for the group as a whole.

Any data collected will be securely stored in such a way that only those mentioned above will be able to gain access to it. At the end of the project, any personal information will be destroyed immediately except that, as required by the University's research policy, any raw data on which the results of the project depend will be retained in secure storage for five

years, after which it will be destroyed. Blood samples will not be used for any other purposes other than the measurements mentioned above.

You are most welcome to request a copy of the results of the project should you wish.

Reasonable precautions will be taken to protect and destroy data gathered by email. However, the security of electronically transmitted information cannot be guaranteed. Caution is advised in the electronic transmission of sensitive material.

Benefits and Risks of Participation

Benefits

Should you choose to participate, you will gain:

- Knowledge of your fitness status
- Knowledge of your body composition
- Some insight into physiological responses to exercise and cold

Risks/inconveniences

Exercise – there are risks associated with any type of exercise:

- musculo-skeletal injury (e.g. strained muscles, tendons or ligaments) is possible, but the risk is low due to the low impact nature of the exercise.
- cardiovascular injury is possible due to blockage of blood vessels in the heart (heart attack) or brain (stroke). However, the risk of a fatal episode in maximal exercise tests across all age and fitness levels is ~1 in 30,000 tests. Thus, the risk should be lower for all participants in this study because higher-risk individuals are screened out before participation.
- thermal discomfort to some degree is likely, however it will not be unlike that experienced under normal training conditions. Core temperature will be constantly monitored however it is unlikely to reach a high enough temperature to cease the exercise.

Recording equipment

- there may be some initial discomfort associated with measurement of core body temperature via the rectal thermistor. However, it should not be painful, and any initial feeling of discomfort should subside following insertion.

What if Participants have any Questions?

If you have any questions about our project, either now or in the future, please feel free to contact either:

Evelyn Parr

School of Physical Education

University Telephone No: 479 8982

Email: evelyn.parr@otago.ac.nz

Dr Nancy Rehrer

School of Physical Education

University Telephone No: 479 9128

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This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

HEALTH SCREENING QUESTIONNAIRE

Name _____

DOB: ___/___/___ Home Phone: _____ Mobile: _____

The maximal exercise test (VO_{2max} / lactate threshold) used to determine exercise intensity is strenuous in nature and can place high demands on the musculoskeletal and cardiovascular systems. Safe participation therefore requires that participants are free from observable illness or injury. This questionnaire will help determine whether there are medical reasons why you should not participate. Your responses to the following questions are therefore important for ensuring your safety. Please read each question carefully and answer every question honestly. Your confidentiality is assured.

- 1 Has a doctor ever said you have a heart condition and you should only do physical activity recommended by a doctor? Yes / No
- 2 When you do physical activity, do you feel pain in your chest or have you had *any* chest pain in the past month? Yes / No
- 3 Do you have a family history of heart disease? Yes/ No
- 4 Do you have an irregular heart beat? Yes / No
- 5 Do you have high blood pressure? Yes / No
- 6 Do you have any form of diabetes? Yes / No
- 7 Are you currently taking any medications? Yes / No

If YES; Please describe current medications:

- 8 Do you have kidney, gut or liver dysfunction? Yes / No

- 9 Do you ever lose consciousness or lose your balance because of dizziness? Yes / No
- 11 Do you smoke? Yes / No
- 12 Do you have asthma? Yes / No
- 13 Are you feeling unwell, or suffering from a viral infection or cold? Yes / No
- 14 Are you unaccustomed to strenuous exercise? Yes / No
- 15 Do you know of any other reason you should not perform a maximal exercise test? Yes / No
- 16 Did you have any difficulty in understanding any of these questions? Yes / No

I know that:-

1. My participation is entirely voluntary.
2. I am free to withdraw from testing at any time.
3. I am aware of the potential discomfort and risks.
4. I have had all my questions satisfactorily answered.

.....
(Signature of participant)

.....
(Date)

Your signature: _____ Date: __/__/__

Appendix D: Medical History and Training Questionnaire

MEDICAL HISTORY AND TRAINING QUESTIONNAIRE

Participant Code _____ Date _____

As a possible subject in this project, would you please complete the following questionnaire? Your responses to the following questions are important. Please read each question carefully and answer every question honestly. Your confidentiality is assured and your co-operation is greatly appreciated.

ANY INFORMATION CONTAINED HEREIN WILL BE TREATED AS CONFIDENTIAL.

1. How would you describe your present level of activity? (Circle one)

Sedentary Moderately active Highly active

How often do you complete vigorous activity: (Circle one)

Once a week 2/3 times a week 4 or 5 times a week 6+ times a week

2. How would you describe your present level of fitness? (Circle one)

Unfit Moderately fit Trained Highly trained

3. Are you competing/ in race season at the moment?

Yes No

If yes, how often.....

4. Do you suffer, or have you ever suffered from:

- | | | |
|------------------------------------|-----|----|
| • Heart or cardiovascular problems | Yes | No |
| • Renal diseases | Yes | No |
| • Asthma | Yes | No |
| • Diabetes | Yes | No |
| • Bronchitis | Yes | No |
| • Epilepsy | Yes | No |
| • High Blood pressure | Yes | No |

Other.....

5. Are you currently taking any form of medication other than oral contraceptive pills?

Yes No

If yes, please give brief details.....

6. Have you had any kind of illnesses, injuries or other problems in the last two weeks that may affect your ability to successfully complete the tests that have been outlined to you?

Yes No

If yes, please give brief details

7. Do you have regular menstrual cycles?

Yes No

If so, how long is your cycle (day 1 being the first day of menses): _____ days

How long does your bleeding last? (Circle one)

3-4 days 5-6 days 7+ days

8. If you are on oral contraceptive medication, what type (brand name and type of phasic) and how long have you been following an OCP regime?

<i>Pill name</i>	<i>Type</i>	<i>Months/years on OCPS</i>
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Signature of Participant..... Date.....

Signature of Witness.....

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Appendix E: Submaximal VO_{2max} Protocol

SUBMAXIMAL VO_{2max} PROTOCOL

Test date		Subject ID #	
Time		Seat Height (cm)	
Room temp/humidity		Height (m)	
Age		Weight (kg)	

Interval (min)	Power (Watts)	HR (bpm)	Cadence (RPM)	Lactate (mmol/L)	Mean VO ₂ (L/min)	Mean VCO ₂ (L/min)
Resting	-		-		-	-
5.0 min	75					
7.30min	125					
10 min	175					
11 min	200					
12 min	225					
13 min	250					
14 min	275					
15 min	300					
16 min	325					

Time at point of fatigue _____

Lactate at fatigue _____

Protocol: Two legged cycling start at 75W for five minutes, then increasing by 50W every 2 ½ minutes, when heart rate reaches 150 bpm, increase by 25W each minute until fatigue.

VO _{2max} (L/min)
VO _{2max} (mL/kg/min)
Peak power output (W)
Peak HR (bpm)

**COLD AND CLOTHING IN THE COLD: EFFECTS ON SUBSTRATE METABOLISM
DURING ENDURANCE EXERCISE IN WOMEN**

ADDITIONAL INFORMATION SHEET

Thank you for completing the first stage of this research project – the $\dot{V}O_2$ max test. The next part of the trial requires you to perform a muscle glycogen depletion training session and then avoid foods in your diet that are known to be naturally enriched with high levels of C^{13} . The exercise and dietary restrictions reduce the body's natural endogenous C^{13} levels. Using indirect calorimetry combined with the isotopic composition of breath CO_2 and blood glucose an accurate calculation for the partitioning of glucose sources is possible.

Two days before the first trial you will need to train at a reasonable intensity (~90 min) with the aim of depleting muscle glycogen stores. It is asked that you then avoid any food items containing corn (maize) and cane sugar leading up to the trial. Items such as sports drinks and sweets contain high sugar levels so you should try to minimise the use of such items. If you drink coffee or tea etc. you may use honey as a sweetener.

You are required to **fast overnight before the trial** and you should **avoid caffeine** containing drinks the morning of testing. You will also be required to accurately record your diet and exercise for a two day period prior to the first test and then follow the exercise and dietary regime as close as is possible in the two days preceding the second and third trials.

SAMPLE NUMBERING SYSTEM

Body fluids:

- Participant: 1 - 10
- Trial: A - C
 - Five degrees = A
 - Fifteen degrees = B
 - Five degrees + thermals = C
- Time: 0, 15, 30, 45, 60, 75
- Sample: 1 -4, S & U
 - Grey cap plasma for glucose: 1
 - EDTA plasma for 2-Cats: 2
 - Red cap serum for Insulin: 3
 - Red cap serum for glucose isolation: 4 &4*
 - Urine post exercise: U
 - Sweat post exercise: S
- Duplicate: *

Breath:

- Participant: 1 - 10
- Trial: A - C
- Time: 0, 15, 30, 45, 60, 75
- Duplicate: *

Further Notes:

- Where time 0 = at rest prior to warm up; and time 15 = 15 minutes into the 75 minute trial.
- Therefore the microcentrifuge flip top tubes will be labeled for example:
- 1B-30-1 for a sample from participant 1, in their 15 degree trial, at 30 minutes, where the plasma sample is for glucose.

^{13}C BREATH SAMPLING PROCEDURE

Samples of 3 ml were withdrawn from a bleeder valve of a Douglas bag. The air was mixed in the bag prior to sampling. Exetainers (containing helium) were pierced with a 22 gauge spinal tap needle. The 3 ml sample was then withdrawn through the bleeder valve into a Luerlock syringe. The valve was closed and when the syringe was removed a finger was placed quickly over the syringe end to prevent the sample escaping or air entering. A 20 gauge syringe needle is then attached to the end of the syringe. Constant pressure is applied to excrete air from the syringe down to 1 ml. Simultaneously, the needle is inserted into the top of the Exetainer when the syringe is at 1 ml. The centre of the spinal tap needle is removed to allow the helium to escape when the breath is pushed in. A finger is then placed over the needle to prevent the breath sample escaping. The syringe needle is removed, and then with a finger still over the spinal tap needle, it too is removed. This is repeated for each breath sample so duplicate samples are obtained.

DOUBLE BED ION EXCHANGE CHROMATOGRAPHY

Plasma samples are defrosted and 1 ml portions are put into test tubes. First the plasma is deprotonated with Ba(OH)₂ (1.5 ml, 0.3N) added to each sample, then vortexed. 1.5 ml of 0.3N ZnSO₄ is added to each sample, then vortexed. The contents are then centrifuged (20 min, 3000 g, 4°C) to separate the soluble phase into clean test tubes. The remaining protein precipitate is then washed with 3 ml distilled water and centrifuged again (20 min, 3000 g, 4°C). Again the soluble phase is decanted off. The glucose is then separated by double-bed ion-exchange chromatography by running the combined soluble phases (~7 ml) through superimposed columns.

The columns used for this method are 0.5 cm diameter glass pipettes. The tips of each pipette were stuffed with glass wool. The first resin (AG 50W-X8 H⁺, 200-400 mesh) is put into the column and eluted with water. The same is done with the second resin (AG 1-X8 chloride, 200-400 mesh) as with the H⁺ resin. The resins are measured to ensure 0.5 x 2 cm of each resin was in each pipette, following closely the method of Burelle *et al.* (1999). The columns are then superimposed held by clamps, with the hydrogen resin first. Fourteen samples can be run at once into glass vials of the same numbers. A small amount of distilled water (~2 ml) are used to make sure all the sample elutes. Once the samples are collected the resins and pipettes are discarded. The samples are then covered with Parafilm and frozen. The frozen samples are then freeze dried and placed into tin 500 µl tin cups. ~0.5 ml distilled water is added to resuspend the sample and then freeze dried again. The tin cups are then crushed so the sample is sealed and they can then be sent for analysis.

THERMAL DISCOMFORT SCALE

*How comfortable do you feel
with the temperature of your body?*

1.0	Comfortable
2.0	
3.0	Slightly Uncomfortable
4.0	
5.0	Uncomfortable
6.0	
7.0	Very Uncomfortable
8.0	
9.0	Extremely Uncomfortable
10.0	

Source: Adapted from Gagge, A. P., Stolwijk, J. A. J., & Hardy, J. D. (1967). Comfort and thermal sensation and associated physiological responses at various ambient temperatures, *Environmental Research*, 1, 1-20.

THERMAL SENSATION SCALE

*How does the temperature?
of your body feel?*

1.0	Unbearably Cold
2.0	Extremely Cold
3.0	Very Cold
4.0	Cold
5.0	Cool
6.0	Slightly Cool
7.0	Neutral
8.0	Slightly Warm
9.0	Warm
10.0	Hot
11.0	Very Hot
12.0	Extremely Hot
13.0	Unbearably Hot

Source: Gagge, A. P., Stolwijk, J. A. J., & Hardy, J. D. (1967). Comfort and thermal sensation and associated physiological responses at various ambient temperatures. *Environmental Research, 1*, 1-20.

RATINGS OF PERCEIVED EXERTION SCALE

*How hard do you feel you are
exercising?*

- 6
- 7 Very, very light
- 8
- 9 Very light
- 10
- 11 Fairly light
- 12
- 13 Somewhat hard
- 14
- 15 Hard
- 16
- 17 Very hard
- 18
- 19 Very, very hard
- 20

Source: Borg, G. A. V. (1970). Perceived exertion as an indicator of somatic stress.
Scandinavian Journal of Rehabilitation Medicine, 2(2), 92-98.