The Effect of Carbohydrate Ingestion Type and Timing on Substrate Utilization During Low Intensity Shivering

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SUBSTRATE UTILIZATION DURING LOW INTENSITY SHIVERING

by
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THESIS

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SUMMARY

During cold exposure, carbohydrates (CHO) play a substantial role in providing substrate for shivering muscles. To date, little is known about the effects of CHO ingestion on substrate utilization in the cold. The purpose of this study was to determine the rate of exogenous glucose oxidation ($R_{\text{Gox-exo}}$) and quantify the rate of substrate utilization during low-intensity shivering when: 1) glucose is given from the onset (G0) or after 60 min (G60) or 2) ingesting glucose alone or in equal parts with fructose (GF60). Results showed that although CHO and lipid oxidation and their relative contribution to heat production increased during cold exposure when ingesting a CHO beverage, the timing and type of CHO ingested had no effect on overall substrate utilization. However, $R_{\text{Gox-exo}}$ during cold exposure was significantly greater in the G0 and GF60 condition relative to G60 ($p<0.05$) but no significant difference was found between the G0 and GF60.
RÉSUMÉ

Les hydrates de carbone constituent un substrat énergétique important pour les muscles frissonnant. Il y a peu d'information disponible sur les effets de l'ingestion de glucides sur l'utilisation des carburants métaboliques dans le froid. L'objectif de cette étude était de déterminer le taux d'oxydation du glucose exogène et l'utilisation des substrats énergétiques au cours du frissonnement lorsque le glucose est ingéré : 1) seul, au temps 0 (G0) ou après 60 minutes (G60), 2) seul ou en quantité égale avec le fructose (GF60). Les résultats ont démontré que l'utilisation absolue et relative des glucides et lipides augmentent au cours de l'exposition au froid dans toutes les conditions. La manipulation temporelle et du type de glucides ingérés n'a pas modifié l'utilisation des carburants métaboliques. Toutefois, l'oxydation des glucides exogènes durant l'exposition était significativement plus élevée dans G0 et GF60 par rapport à G60 (p<0.05) et aucune différence significative entre G0 et GF60.
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CHAPTER I

GENERAL INTRODUCTION

Exposure to extreme environmental conditions can impose significant demands on the regulatory processes that help our bodies to maintain homeostasis. While humans are relatively well adapted to dissipate heat in hot conditions, they are less adept at conserving it when exposed to cold environments (Armstrong, 2000; Golden & Tipton, 2002). Although we can often rely on behavioural means such as finding shelter and wearing appropriate clothing to assist in the conservation of heat, these methods are not always sufficient to maintain homeostasis. When such strategies are inadequate, physiological mechanisms are activated to prevent heat loss, through the vasoconstriction of peripheral vessels, and increased heat production through involuntary muscle activity (shivering thermogenesis) to protect against hypothermia. While peripheral vasoconstriction allows humans to limit heat loss and tolerate mild cold conditions by creating a greater insulative layer (Young & Castellani, 2001), this response is not always adequate to prevent a decrease in body temperature. As a result, human survival in the cold ultimately depends upon the ability to sufficiently sustain shivering to maintain a thermal balance (Stocks et al., 2004). There is plenty of anecdotal and scientific evidence to suggest that maintaining a caloric balance is very important in sustaining shivering and to ensure survival when exposed to a cold environment (Young et al., 1998; Wittmers & Savage, 2001). This was evidenced by the death of four soldiers who were undergoing rigorous U.S. Army Ranger training, during which sleep and caloric deprivation is the norm, suffered what was considered secondary hypothermia such that the sleep deprivation combined with insufficient food intake and strenuous prolonged exercise in cold water led to their eventual hypothermia (Wittmers & Savage, 2001). This hypothesis was supported by the findings of Young et al. (1998) who
found that exertional fatigue, sleep loss and negative energy balance in United States Army Rangers reduced their metabolic heat production. The mechanism for this reduced metabolic heat production \((H)\) remained unknown; however, it is possible that the limited substrate availability may have been a contributing factor. The subsequent recommendations would suggest ingesting food could improve the response to cold exposure; however it remains unclear what the macronutrient content should be to favour survival.

Since the end of the Second World War, standardized cold survival rations used in aircraft and ship life rafts have undergone a number of important changes in total energy content and macronutrient composition. Typically provided in the form of high-energy candy, cereal bars or cookies (~400 to 1500 kcal per person per day), macronutrient composition has ranged from 100% carbohydrate (CHO) to a balanced profile [55% CHO, 40% fat and 5% protein; (Golden & Tipton, 2002)]. It is evident that further research is necessary to determine the metabolic fate of various ingested fuel sources when exposed to a cold condition. Over the last decades, most shivering studies carried out on metabolic fuel utilization have focused on CHOs since it is a limited supply of stored energy and is known to limit exercise endurance. While it is clear from previous studies that ingesting CHO will not increase overall heat production or affect core temperature (Glickman-Weiss et al., 1993; Vallerand et al., 1993; Blondin et al., 2008), the additional contribution to total heat production provided by ingesting glucose as well as its role in preserving endogenous energy stores offers clear evidence of the advantages of CHO feeding on survival in the cold (Blondin et al., 2008).

Therefore, the purpose of this thesis is to improve our understanding on the effects of CHO supplementation on the overall energy budget of non cold-acclimatized men exposed to a cold condition as well as determine the metabolic fate of the ingested CHO.
Importance of CHO availability

The energy required to maintain shivering thermogenesis is obtained primarily from the oxidation of CHO and lipids (Vallerand & Jacobs, 1989; Vallerand et al., 1995; Vallerand et al., 1999; Haman et al., 2002). Until recently, proteins have generally been thought to play a more minor role, resulting in their contribution to heat production often being unaccounted for, however more current research has shown that they may play a more substantial role than originally thought (Vallerand & Jacobs, 1989; Vallerand et al., 1995; Vallerand et al., 1999; Haman et al., 2002; Haman et al., 2004; Haman et al., 2005). The proportion in which each macronutrient supplies energy to the shivering muscles varies depending on shivering intensity, nutritional status and muscle recruitment, with CHO utilization being the most influenced by these changes, likely due to its limited reserve (represents only 1% of total energy stores) (Haman, 2006).

Carbohydrate utilization

It has been well established that the source of CHO for oxidation can be derived from two sources: 1) intravascular glucose stores from the plasma, through the release of hepatic glucose or from exogenous glucose ingestion, and 2) from intracellular stores, through the phosphorylation of muscle glycogen (Weibel et al., 1996). During low intensity shivering [<2.5 times that of the resting metabolic rate (RMR)] the greatest contribution for total CHO oxidation is derived from the intracellular/muscle glycogen stores (Haman et al., 2002; Haman et al., 2004). As Fig. 1.1 illustrates, muscle glycogen has been shown to contribute over 75% of the total CHO oxidized during shivering both at this intensity and moderate-intensity shivering (2.5-3.8 X RMR), while plasma glucose has a more limited role, only contributing up to 25% of the total CHO oxidized (Haman et al., 2002).
Further, a recent study by Haman et al. (2004) showed that by manipulating the nutritional status of an individual by modifying the CHO availability of endogenous sources, different metabolic pathways could be recruited within the same muscle fibers to maintain the same heat production during low-intensity shivering (<2.5 X RMR). When CHO-depleted and CHO-loaded participants were exposed to a cold condition inducing low-intensity shivering, there were differences in fuel utilization with the CHO-loaded group.
tending to utilize largely CHO, and the CHO-depleted group utilizing primarily lipids, combined with a higher contribution from proteins to compensate for lowered CHO reserves and rate of utilization. This compensatory shift in fuel utilization, was consistent with previous findings in studies comparing the effects of CHO availability on total heat production (Martineau & Jacobs, 1989; Young et al., 1989). While this response may simply be a reflection of substrate availability, what remained significant was that muscle glycogen remained strongly mobilized in the glycogen-depleted group (see Fig. 1.2), still contributing 75% of the total CHO oxidation, despite the reduced contribution of CHO oxidation and the concomitant increase in the contribution of lipids and proteins to total heat production, compensating for the reduced CHO oxidation. With CHOs contributing to total heat production to such an extent, and the continued mobilization of muscle glycogen in the glycogen depleted state (Haman et al., 2004), many have concluded that shivering endurance is largely dependent upon the relative use of both muscle glycogen and plasma glucose.
Figure 1.2 Effects of shivering on muscle glycogen metabolism in adult humans: (a) absolute rates of muscle glycogen oxidation in mg·kg\(^{-1}\)·h\(^{-1}\); (b) relative contribution of muscle glycogen oxidation to total heat production (%\(H\)). Adapted from Weber & Haman (2005).
While, some researchers believe that the main limiting factor for survival in the cold is the depletion of muscle glycogen, leading to a reduced cold sensitivity [lowered drive to shiver (Tikuisis et al., 2002)], no study has ever demonstrated this link between cold sensitivity and low muscle glycogen reserves. There is evidence however that clearly demonstrates that even when muscle glycogen reserves are depleted, metabolic heat production remains the same (Young et al., 1989; Martineau & Jacobs, 1991; Haman et al., 2004). Other researchers have shown that maintaining glycemia is of greater importance than the depletion of muscle glycogen stores during cold survival, as shivering thermogenesis has been reported to decrease when glycemia is artificially reduced to 2.8 mM (Haight & Keatinge, 1973; Passias et al., 1996). Gale et al. (1981) have also reported the complete inhibition of shivering, through the inhibition of cold-sensitive neurons in the hypothalamus, when glycemia is reduced to below 2.5mM. While there is no study available that has shown glycemia to be reduced to this degree through shivering alone, there is little doubt that preserving endogenous glycogen reserves is of critical importance either to maintain the shivering drive or glycemia. Although it is well documented that when muscle glycogen reserves are reduced, lipid and protein oxidation compensate to maintain a constant rate of heat production (Haman et al., 2004), it is unclear whether this mechanism is sufficient to maintain heat production for a prolonged period, particularly if muscle glycogen is still strongly mobilized. This fact, combined with the relatively limited endogenous CHO reserve, has lead some researchers to suggest that sparing CHO reserves may be an important strategy in prolonging survival time in the cold (Haman et al., 2002; Tikuisis et al., 2002).
**CHO Supplementation in the Cold**

A common practice used to preserve endogenous CHO reserves during prolonged low to moderate intensity exercise (45%-65% of VO₂ max) has been the ingestion of glucose and other CHOs prior to and throughout exercise bouts [see Jeukendrup (2004) for review]. Exogenous CHO ingestion during exercise has been shown to increase and maintain a high rate of CHO oxidation, while sparing and at times synthesizing endogenous CHO sources and maintaining glycemia (Jeukendrup, 2004). Unfortunately, most shivering studies have largely focused on fuel selection in 12h post-absorptive individuals (Vallerand & Jacobs, 1989, 1990; Vallerand et al., 1995; Haman et al., 2002; Haman et al., 2004; Haman et al., 2005), while only a few have investigated the effects of food consumption before or during cold exposure on the overall energy balance (Vallerand et al., 1988; Vallerand et al., 1992; Glickman-Weiss et al., 1993; Vallerand et al., 1993; Glickman-Weiss et al., 1994; Blondin et al., 2008). While the purpose of these respective studies varied tremendously, most centered on the link between CHO ingestion and its effect on total heat production but not on the relative contribution of the oxidation of each substrate on total heat production during shivering thermogenesis (Vallerand et al., 1992; Glickman-Weiss et al., 1993; Vallerand et al., 1993). Others were interested in identifying the link between shivering and the increased plasma glucose clearance (Vallerand et al., 1988). Of these only Vallerand, et al. (1992; 1993) and Blondin et al. (2008) reported estimates of changes in CHO, lipid and protein oxidation in men during low-intensity shivering (<2.5 X RMR) following the ingestion of CHOs.

Vallerand et al. (1993) provided the first estimates of changes in CHO, lipid and protein oxidation in men during low-intensity shivering (<2.5 X RMR) following the ingestion of a placebo (100 ml of water), a starch jelly (712 kJ or ~45g CHO) or a high-CHO
bar (712 kJ; ~30g CHO, ~4g fat and ~4 g protein) at the beginning and after 90 min in the cold. Their results indicated that CHO ingestion increased overall use of CHO and decreased that of lipids without modifying protein oxidation or thermal responses. These findings were consistent with the earlier study by Vallerand et al. (1992), that also found that when ingesting the same energy bar there was neither an effect on thermogenesis or heat loss. This earlier study also found there to be no effect on substrate utilization (no change in CHO utilization or fat oxidation).

A more recent study by Blondin et al. (2008) has provided further insight into not only the effects of ingesting glucose on fuel utilization but more importantly their relative contribution to heat production during low-intensity shivering. Their results indicated that ingesting glucose at a low (LG) and high (HG) ingestion rate (400 and 800 mg·min⁻¹ respectively) during low-intensity shivering increased the contribution of CHO oxidation to total heat production by over 10% without significantly modifying the relative contribution of lipids and proteins to total heat production. However the fuel utilization rates observed in this study further substantiate the previous results found by Vallerand et al. (1992; 1993) as the absolute rates of oxidation for CHO (546±79 for LG and 582±75 mg·min⁻¹ for HG versus 488±60 mg·min⁻¹ for C), lipid (153±27 for LG and 155±28 mg·min⁻¹ for HG versus 204±26 mg·min⁻¹ for C) and protein (70±3 for LG and 79±3 mg·min⁻¹ for HG versus 67±3 mg·min⁻¹ for C) remained the same regardless of CHO ingestion while the thermal responses also remained unaffected. Although, there was no statistical difference in the rate of CHO oxidation between experimental conditions there was a clear trend suggesting a slightly greater reliance on CHO in the glucose ingestion conditions compared to the control condition. In addition to investigating the effect of glucose ingestion on fuel utilization and their relative contribution to heat production, we will partition CHO with the purpose of
determining the metabolic fate of the ingested CHO solution and quantifying its effects on endogenous CHO reserves.

*Metabolic Fate of Ingested CHO and its Effect on CHO Reserve*

A study by Vallerand *et al.* (1988), provided the first glimpse into the possible fate of exogenous glucose when given either intravenously or orally during low-intensity shivering. Participants were given either an oral or intravenous glucose tolerance test immediately prior to cold exposure, to determine whether shivering could increase the clearance of plasma glucose. Although their major findings indicated a greater rate of appearance and clearance of plasma glucose, when exposed to a cold environment compared to thermoneutral conditions, it was not clear whether the fate of the glucose disappearance was through oxidative or nonoxidative pathways.

The more recent study by Blondin and colleagues (2008) using stable isotopes and indirect calorimetry techniques, has shed more light on determining the metabolic fate of CHOs in non-acclimatized men exposed to 4°C for 120 min using a liquid conditioned suit. In addition to determining the effects of ingesting glucose on fuel utilization and their relative contribution to heat production the purpose of this study was also to provide estimates of exogenous glucose oxidation rates during cold exposure, and to quantify the utilization rates of muscle glycogen, plasma glucose, and glucose released from the liver. Aside from increasing the contribution of CHO oxidation to total heat production without modifying overall rates of CHO and lipid oxidation relative to a control condition, ingesting glucose increased plasma oxidation by almost 40% and doubled the relative contribution of the oxidation of this CHO source to total heat production (from 15 to 28%), largely due to the 20% of total heat production provided by the oxidation of the ingested glucose. Further, the ingested glucose not only provided an additional substrate source to contribute to total
heat production but it also subsequently reduced RG_{ox-liver} and its contribution to total heat production by up to 50%, while maintaining both the oxidation rate and relative contribution of muscle glycogen to total heat production throughout the exposure period.

Until recently, no study had ever examined the metabolic fate of ingested CHO's or its effect on CHO reserve when exposed to a cold environment. Consequently, researchers relied on results from exercise studies to provide some insight on its use. Some researchers have shown that ingesting glucose and other CHO's during prolonged low to moderate intensity exercise (cycling at 50-70% VO_{2max}) can elevate and maintain a high CHO oxidation rate and glycemia, while also sparing the limited muscle and liver glycogen reserve (Coyle et al., 1983; Coyle et al., 1986). Some studies have shown that ingesting CHO can spare liver glycogen reserve to the extent of suppressing or completely inhibiting hepatic glucose output with increasing CHO ingestion (Howlett et al., 1998; Jeukendrup et al., 1999b). Whether muscle glycogen is also spared remains to be debated, with many researchers reporting no reduction in muscle glycogen breakdown (Hargreaves & Briggs, 1988; Mitchell et al., 1989; Jeukendrup et al., 1999a; Jeukendrup et al., 1999b). Others have seen a net breakdown of glycogen in type I fibres but not type II (Tsintzas et al., 1995, 1996). Further to preserving endogenous CHO sources, researchers have suggested that the CHO ingested throughout an intermittent exercise bout could be used for glycogenesis in inactive muscle fibers during low-intensity portions of intermittent exercise (Kuipers et al., 1986; Keizer et al., 1987).

While the results from Blondin et al. (2008) allowed for the utilization rates of the different CHO sources to be quantified and make inferences into the possible fate of the ingested glucose, it is unclear whether the maximal response, with regards to attaining the maximal RG_{ox-exo} and preserving endogenous CHO sources, was indeed attained. The most
significant finding from this study was that only 23 g of the total glucose load (in both conditions) was oxidized, representing ~45 and 25% of the total glucose ingested in the low glucose and high glucose conditions respectively. This finding suggests that the remaining ~30g and 80g of glucose was either not absorbed or stored as glycogen (Wagenmakers et al., 1993; Livesey et al., 1998). Despite providing further insight into the possible metabolic fate of the ingested glucose, the stability in $R_{G_{\text{ox-exo}}}$, independent of changes in the glucose feeding rate observed in this study indicates that at low shivering intensity (< 2.5 X RMR) the maximal oxidation rate of ingested glucose (~200 mg glucose·min$^{-1}$) may already be achieved at low glucose consumption rates (~400 mg·min$^{-1}$) as indicated in Fig. 1.3. This suggests that perhaps certain factors may be counteracting a further increase in $R_{G_{\text{ox-exo}}}$.

![Exogenous glucose oxidation](image)

**Figure 1.3** Exogenous glucose oxidation (mg·min$^{-1}$) during low-intensity shivering as a function of carbohydrate intake (mg·min$^{-1}$). Horizontal dotted line represents rate of CHO ingestion at which exogenous oxidation levels off. *Adapted from Blondin et al. (2008).
Limitations of Exogenous Glucose Oxidation

What remains to be determined are the possible factors that could limit the oxidation rate of ingested glucose in the cold. The available cold exposure studies currently provide limited insight to assist in answering this question, however a number of mechanisms that influence $\text{RG}_{\text{ox-exo}}$ have been identified during exercise (see Jeukendrup et al. (2004) for review). For glucose, as for a number of other types of CHOs, these limitations have been associated with factors such as 1) rate of ingestion, 2) timing of ingestion, 3) rate of gastric emptying and intestinal absorption, 4) rate of uptake by skeletal muscle and 5) exercise intensity. Similarly, it is likely that a number of these parameters are responsible for limiting $\text{RG}_{\text{ox-exo}}$ in the cold, though their relative importance in regulating maximal $\text{RG}_{\text{ox-exo}}$ remains widely unexplored. It is possible that $\text{RG}_{\text{ox-exo}}$ is limited either at the level of intestinal glucose absorption or uptake by peripheral tissues as evidenced by a study from Vallerand et al. (1988). They showed that when men were exposed to air at 10°C and given a 75 g glucose load either orally or intravenously, glucose concentration returned to normal values twice as fast when the glucose load was given intravenously (~60 min) rather than orally (~120 min). This suggests that in cold exposed humans, $\text{RG}_{\text{ox-exo}}$ was not likely to be limited by glucose uptake by the muscle or other insulin sensitive tissues but rather the rate of intestinal glucose absorption.

While the first limitation to $\text{RG}_{\text{ox-exo}}$, ingestion rate, has already been addressed in the previous study by Blondin et al. (2008), the objective of this thesis is to provide some insight into the effects of modifying both the glucose ingestion timing, and gastric emptying and intestinal absorption (by ingesting two different CHOs in combination) on $\text{RG}_{\text{ox-exo}}$. Although the timing of CHO ingestion has been shown to have little influence on $\text{RG}_{\text{ox-exo}}$ during exercise (Pallikarakis et al., 1986), it may have been a limiting factor in attaining an
elevated $RG_{ox\text{-exo}}$ in previous cold exposure studies [eg. Vallerand et al. (1992; 1993) and Glickman-Weiss et al. (1993); Blondin et al.(2008)]. In these studies, the largest glucose load was ingested at the onset of cold exposure which created an insulinemic peak within 20-30 min of exposure. However, the shivering metabolic rate may have still been increasing, as it did not appear to reach a steady state until after 60 min into cold exposure. This may have limited the potential to reach a greater $RG_{ox\text{-exo}}$, since the insulinemic response and the maximal muscle activity for this stimulus may not have reached its peak. By providing the large bolus of glucose following 1h of cold exposure, this will ensure that the insulinemic peak occurs when the muscle activity is stabilized at its peak for that particular cold stress, thus ensuring a more optimal response.

The second limiting factor, gastric emptying and intestinal absorption, will be addressed indirectly by modifying the rate of entry of CHO by ingesting glucose in combination with fructose. While the volume, osmolality and CHO content of the ingested solution have all been linked as possible limiting factors in gastric emptying (Costill & Saltin, 1974; Foster et al., 1980; Hunt et al., 1985; Mitchell et al., 1989; Noakes et al., 1991; Vist & Maughan, 1995) the maximum rate of CHO delivery to the small intestine (believed to be $\sim 5.34 \text{ kcal}\cdot\text{min}^{-1}$ or $\sim 1.3 \text{ g}\cdot\text{min}^{-1}$) during moderate intensity exercise ($\sim 70-75 \% \text{ VO2 max}$) is strongly influenced by the gastric volume, which is modulated by the volume ingested and the drinking pattern (bolus versus intermittent ingestion) (Noakes et al., 1991). Intermittently ingesting concentrated solutions throughout cold exposure ensures that gastric emptying is maintained at a rapid rate and will not be limited by the negative feedback loop inhibiting gastric emptying following the initial rapid emptying phase (Hunt et al., 1985) and will not act as a limiting factor in $RG_{ox\text{-exo}}$. 
Exercise studies have shown that the rate of exogenous glucose oxidation tends to plateau at 1.0 g·min\(^{-1}\) when ingested at a rate of 1.2 g·min\(^{-1}\) [see Jeukendrup (2004) for review]. This peak has been attributed to the progressive saturation of competing glucose molecules at the luminal membrane limiting its entry into the enterocyte (Wagenmakers et al., 1993). A number of studies have since shown that during low to moderate intensity exercise (45%-65% of VO\(_2\) max), maximal \(R_{G_{\text{ox-exo}}}\) can be increased by up to 50% when glucose or glucose polymers are given in combination with fructose or sucrose (Adopo et al., 1994; Jentjens et al., 2004; Jentjens & Jeukendrup, 2005; Burelle et al., 2006; Jeukendrup et al., 2006). The greater exogenous oxidation rate observed when ingesting fructose or sucrose in combination with glucose compared to ingesting glucose alone, has been attributed to a reduced competition for transport across the luminal membrane as both hexoses are transported independently during intestinal absorption. Although both glucose and fructose cross the basolateral membrane and enter the portal vein via GLUT-2 mediated facilitated diffusion, glucose enters the enterocyte via facilitated diffusion and active transport mediated by the sodium-glucose cotransporter (SGLUT-1) while fructose is taken up by facilitated diffusion by the fructose transporter GLUT-5 (see Frayn, (2003) for review). Therefore, ingesting glucose in combination with fructose allows for more CHO to enter the portal vein, since the saturation of the glucose transport mechanism at the luminal membrane will be offset by the continued transport of fructose. Once transported into the portal vein, part of the fructose is either already converted to lactate by the intestine (Holloway & Parsons, 1984) which will then be converted to glucose in the liver and either oxidized or stored there or utilized at the periphery. Of the remaining fructose, most will be metabolized by the liver, however since the removal of fructose from the circulation by the liver is quite rapid, it often exceeds the hepatic oxidative capacity, resulting in an accumulation of glycolytic
intermediates which is largely disposed nonoxidatively (Tappy & Jequier, 1993). This is consistent with the findings of Delarue et al. (1993) who found that over a 6h rest period about 50% of a fructose load is converted to glucose in the liver and either oxidized or stored there, or transported to the muscle either as glucose or lactate to be utilized or stored. Some have suggested that up to 20% of a fructose load is recirculated as lactate (Bjorkman & Felig, 1982) which is reconvered to glucose in the liver and stored as glycogen or taken up by the muscle to be utilized or stored. Since the ingested fructose must be converted to glucose by the liver in order to be utilized at the periphery by the muscles is likely oxidized.

Whether ingesting two different CHOs in combination will affect $\text{RG}_\text{ox-exo}$ during shivering as it does during exercise remains to be determined, but based on its metabolic fate and the significant changes observed during exercise a similar response is hypothesized.

Clearly, more research is needed to determine if higher exogenous glucose oxidation rates can be achieved during low ($< 2.5 \times \text{RMR}$) to moderate ($2.5-3.8 \times \text{RMR}$) intensity shivering, as well as to determine the effects of glucose ingestion on whole body substrate utilization. Therefore the purpose of this thesis is to improve our understanding of the effects of ingesting exogenous glucose on fuel selection during low intensity shivering. This thesis will partition the fuels being utilized when shivering at this intensity, through the use of stable isotope tracer techniques. The primary interest of this investigation is to determine whether $\text{RG}_\text{ox-exo}$ can be increased above 200 mg·min$^{-1}$ as previously reported by Blondin et al. (2008), by providing a glucose drink following the establishment of a shivering steady state and by combining two hexoses (glucose and fructose) in the same solution.
1.2 Objectives

The main objectives for the current thesis are two-fold:

1) To quantify the rate of exogenous glucose oxidation when glucose is ingested at a rate of \(~800 \text{ mg} \cdot \text{min}^{-1}\) from the onset versus after 60 minutes of low intensity shivering (<2.5 X RMR) and

2) To quantify the rate of exogenous glucose oxidation when a glucose or a combined glucose and fructose solution is ingested at a rate of \(~800 \text{ mg} \cdot \text{min}^{-1}\) following 60 minutes of low intensity shivering (<2.5 X RMR).

1.3 Hypotheses

It is hypothesized that:

1) Ingesting a glucose solution at a rate of \(800 \text{ mg} \cdot \text{min}^{-1}\) after 60 minutes of low-intensity shivering will increase the rate and amount of glucose oxidized above the 200 mg·min\(^{-1}\) and 23 g observed respectively by Blondin et al. (2008) due to a greater insulin response and muscle activity during steady state shivering compared to the ingestion from the onset of cold exposure; and

2) Ingesting a solution containing equal parts glucose and fructose at a rate of \(800 \text{ mg} \cdot \text{min}^{-1}\) after 60 minutes of low-intensity shivering will further increase the rate and amount of exogenous CHO oxidized compared to ingesting only glucose.
CHAPTER II
METHODOLOGY

Subjects

Six healthy, non-cold acclimatized men volunteered for this study approved by the Health Sciences Ethical Committee of the University of Ottawa with the written consent of all participants. Age, body mass, height and percent body fat (estimated by dual energy x-ray absorptiometry) of the subjects were 28±2 years, 81.8±4.0 kg, 180.7±2.8 cm, and 18.7±1.5 % respectively. Peak oxygen consumption, estimated by incremental treadmill exercise to volitional fatigue (Bruce Protocol), averaged 54.5 ± 3.0 ml·kg⁻¹·min⁻¹ and was measured 5-7 days before the experiments.

Experimental protocol

Each subject participated in three randomly assigned experimental trials, each separated by at least seven days. The order of the trials was randomly assigned following a cross-over design. Each trial consisted of a 120 min baseline period followed by 150 min of shivering at an intensity equivalent to 2.5-times that of the resting metabolic rate. Subjects ingested drinks containing glucose from the onset of cold exposure (G0) or 60 min into cold exposure (G60) each traced using [¹³C]-glucose, or containing equal parts glucose and fructose 60 min into cold exposure (GF60) traced using [¹³C]-glucose and [¹³C]-fructose.

Experiments were conducted between 8.00 h and 13.00 h, following 36-h without heavy physical activity. The last evening meal was standardized (3220 kJ or 770 kcal, 42% CHO, 28% fat and 30% protein) and subjects were asked to report to the laboratory at 7.30 h the next morning after a 12-14 h fast. Ingestion of carbohydrates from plants naturally rich in ¹³C (C₄ photosynthetic cycle) was avoided to maintain low ¹³C background enrichment in
plasma glucose and expired CO₂ (Peronnet et al., 1990). Upon their arrival in the laboratory, subjects wearing only shorts were instrumented with thermal probes (tympanic and skin). An indwelling catheter (22G, 25.4 mm, Medex, CA) was placed in an antecubital vein for blood sampling (left arm) once the participant was fitted with a “liquid-conditioned suit” (LCS: Three Piece, Med-Eng, Ottawa, ON). Subjects were then asked to empty their bladder (Time = 0 min) and remain seated for 120 min at ambient conditions (~22°C). Resting blood and expired gas samples were collected at the onset of the baseline period and every 15 min during the final 30 min of the baseline period, to determine the $^{13}$C/$^{12}$C ratio in plasma glucose and expired CO₂. Following this stabilization period, 4°C water began circulating through the LCS using a temperature and flow-controlled circulation bath (Endocal, NESLAB and Model 200-00, Micropump Inc., Vancouver, WA, USA). Blood and gas samples continued to be collected every 30 min throughout the cold exposure. Thermal response, metabolic rate and fuel utilization were measured at baseline prior to cooling and every 30 min during the subsequent 150 min of cold exposure.

**Thermal response**

Changes in heat production ($H$) were calculated by indirect respiratory calorimetry corrected for protein oxidation [as described in Haman et al. (2002)]. Tympanic temperature ($T_{ty}$) and mean skin temperatures ($\bar{T}_{skin}$) were monitored continuously prior to and during cold exposure using a tympanic thermocouple (Mon-a-therm Tympanic, Mallinckrodt Medical, St. Louis, MO) and skin temperature transducers respectively (area-weighted equation from 12 sites: forehead, chest, biceps, forearm, abdomen, lower and upper back, front and back calf, quadriceps, hamstrings and finger tip (Dubois & Dubois, 1916).
Metabolic rate and fuel utilization

Ventilation (\(\dot{V}_E\)), oxygen consumption (\(\dot{V}O_2\)) and carbon dioxide production (\(\dot{V}CO_2\)) were measured using a metabolic cart (MOXUS, Applied Electrochemistry Inc., Pittsburgh, PA, USA). Total protein (RP\(_{ox}\)), carbohydrate (RG\(_{ox}\)) and lipid (RF\(_{ox}\)) oxidation rates (in g·min\(^{-1}\)) were calculated as described previously (Haman et al., 2002; Haman et al., 2004):

\[
RP_{ox} (g\cdot min^{-1}) = 2.9 \times \text{UREA}_{urine} (g\cdot min^{-1})
\]

\[
RG_{ox} (g\cdot min^{-1}) = 4.59\dot{V}CO_2(l\cdot min^{-1}) - 3.23 \dot{V}O_2(l\cdot min^{-1})
\]

\[
RF_{ox} (g\cdot min^{-1}) = -1.70\dot{V}CO_2(l\cdot min^{-1}) + 1.70 \dot{V}O_2(l\cdot min^{-1})
\]

where \(\dot{V}CO_2(l\cdot min^{-1})\) and \(\dot{V}O_2(l\cdot min^{-1})\) were corrected for the volumes of O\(_2\) and CO\(_2\) corresponding to protein oxidation (1.010 and 0.843 l·g\(^{-1}\), respectively). RP\(_{ox}\) was estimated from urinary urea excretion (UREA\(_{urine}\)) in urine samples collected for 120 min during the habituation period, and 150 min in the cold. Urinary concentrations were determined using a commercial urine assay kit (BioAssay Systems, CA, USA). Energy potentials of 16.3 kJ·g\(^{-1}\) (carbohydrates), 40.8 kJ·g\(^{-1}\) (lipids), and 19.7 kJ·g\(^{-1}\) (proteins) were used to calculate the relative contributions of each fuel to total heat production (Elia, 1991; Péronnet & Massicotte, 1991).

Blood analysis

Plasma glucose concentrations were assayed using spectrophotometric analysis after conversion of glucose to glucose 6-phosphate by hexokinase. Laboratory-grade reagents
(Sigma-Aldrich Canada Ltd., Oakville, Ont; Fisher Scientific Ltd., Nepean, Ont.) were used for preparing a standard hexokinase reaction and after 30 min incubation of prepared samples at room temperature, spectrophotometric analysis of resultant NADH light absorbance was performed in duplicate using a Synergy HT Series Multi-Detection Reader (Bio-Tek Instruments Inc., Highland Park, Winoosi, Vt.), with absorbance readings of 340 nm wavelength emissions. Insulin concentration was measured by using a commercial human insulin ELISA kit (# EZHI-14K, Linco, Missouri, USA). It should be noted that all plasma samples from the same individuals were analyzed within the same kit.

**Exogenous and endogenous glucose oxidation**

During the stabilization period before cold exposure, rates of exogenous and endogenous glucose oxidation were estimated by repeated [$^{13}$C]glucose ingestion (Haman et al., 2002; Haman et al., 2004; Haman et al., 2005). This glucose solution was prepared using 5g of corn glucose [(natural enrichment of -11.03 \%o $^{13}$C/$^{12}$C Vienna Pee Dee Bilemnitella (VPDB)] further enriched with [U-$^{13}$C]glucose ($^{13}$C/C>99\%, Isotec, Miamisburg, OH, USA) to obtain a final enrichment of +200 \%o $^{13}$C/$^{12}$C VPDB. Such a high enrichment allows for the background enrichment of expired CO2, observed from the oxidation of endogenous substrates from those living on a North American diet, to be negated as the background enrichment (0.5-1.0 \%o [\delta^{13}C] PDB-1) is considered small relative to the artificially enriched beverage. Blood samples (7 ml each) and expired gas samples (10 ml) were taken just before the ingestion of each $^{13}$C-glucose solution.

During cold exposure, rates of exogenous and endogenous glucose oxidation were quantified as described by Burelle et al. (2006). A total of 120 and 80 g of corn glucose were artificially enriched with [U-$^{13}$C]-glucose to +50\%o $\delta$ $^{3}$C/$^{12}$C VPDB for G0 and G60. A total
of 40 g of glucose and fructose (80 g total) derived from corn were also artificially enriched with [U-\(^{13}\)C]-glucose and [U-\(^{13}\)C]-fructose to +50\%o \(\delta^{13}\)C/\(^{12}\)C VPDB. Isotopic solutions were then diluted in 750 ml of water in the G0 condition and 550 ml of water in the G60 and GF60 conditions. The solutions were subsequently divided into six equal doses for the G0 condition and four equal doses for the G60 and GF60 conditions and provided according to the schedule described in Fig. 2.1. Similar to the stabilization period, blood samples (7 ml each) and expired gas samples (10 ml) were taken just before the ingestion of each \(^{13}\)C-glucose dose. Upon collection, blood samples were placed on ice and spun in a refrigerated centrifuge. Plasma was separated and stored at -80\°C until analyzed.

Isotopic composition of the expired CO\(_2\) was expressed as \(\%o\) difference compared with VPDB standard (Craig, 1953):

\[
\%o\delta^{13}\text{C}\text{ VPDB} = \left[\frac{R_{\text{sp}}}{R_{\text{std}}} - 1\right] \times 1000
\]  \hspace{1cm} (4)

where \(R_{\text{sp}}\) and \(R_{\text{std}}\) are \(^{13}\)C/\(^{12}\)C in the sample and standard (1.1237\%) respectively. The isotopic compositions of expired CO\(_2\) is presented in Fig. 3.1.

The rate of exogenous glucose oxidation (\(\text{RG}_{\text{exo}}\), in mg\cdot min\(^{-1}\)) was estimated from isotopic composition of expired CO\(_2\) (\(R_{\text{exp}}\)) and the ingested exogenous glucose solution (\(R_{\text{exo}}\)) as follows:

\[
\text{RG}_{\text{ox-\text{exo}}} = \dot{V}\text{CO}_2 \left(\frac{R_{\text{exp}} - R_{\text{ref-exp}}}{R_{\text{exo}} - R_{\text{ref-exp}}}\right)(1/k1\cdot k2)
\]  \hspace{1cm} (5)

where \(\dot{V}\text{CO}_2\) is in l/min (STPD), \(R_{\text{ref-exp}}\) is the isotopic composition of expired CO\(_2\) prior to the ingestion of the first [\(^{13}\)C]glucose dose, \(k1\) (0.7426 l\cdot g\(^{-1}\)) is the volume of CO\(_2\) produced from the complete oxidation of glucose, and \(k2\) is the fractional recovery (at the mouth) of CO\(_2\) produced in tissues. Fractional \(^{13}\)CO\(_2\) recovery values (\(k2\)) of 0.8 and 1 were used.
before and during cold exposure, respectively (Coggan et al., 1993; Leese et al., 1994; Leijssen & Elia, 1996). However, because of the presence of a large bicarbonate pool in the body, $^{13}\text{C}/^{12}\text{C}$ in expired CO$_2$ only slowly equilibrates with $^{13}\text{C}/^{12}\text{C}$ in the CO$_2$ produced in the tissues (Pallikarakis et al., 1991). To take into account this delay between $^{13}\text{CO}_2$ production in the tissues and at the mouth, the above computations were only made during the last 30 min of the baseline period and last 15 min of the cold exposure period.

The rate of endogenous glucose oxidation ($\text{RG}_{\text{ox-endo}}$, in mg·min$^{-1}$) was calculated by subtracting $\text{RG}_{\text{ox-exo}}$ from $\text{RG}_{\text{ox}}$:

$$\text{RG}_{\text{ox-endo}} = \text{RG}_{\text{ox}} - \text{RG}_{\text{ox-exo}}$$  \hspace{1cm} (6)

**Statistical analyses**

Data are presented as means ± standard error (SE). Statistical significance was set at $P \leq 0.05$. The main effects of time and conditions as well as their interaction were tested by analysis of variance for repeated measures. Follow-up one-way analysis of variance for repeated measures identified the significant differences in conditions and their interaction. Differences in heat production, oxidation rates and relative contributions to total heat production of CHO ($\text{RG}_{\text{ox}}$, $\text{RG}_{\text{ox-endo}}$), lipids ($\text{RF}_{\text{ox}}$) and proteins ($\text{RP}_{\text{ox}}$), over the last 30 min of cold exposure were determined using a one-way analysis of variance to verify the main effect of temperature (Baseline vs. Cold). Follow-up paired sample t-tests determined differences in conditions.
Figure 2.1. Timeline of exogenous glucose ingestion (in grams) for glucose ingestion from onset of cold exposure (G0), after 60 min of cold exposure (G60) and a solution containing glucose and fructose in combination after 60 min of cold exposure (GF60).
Isotope solution ingestion ($^{13}$C/C enrichments of 1.33 for baseline and first 60 min of G60 and GF60 and 1.17 for G0 and after 60 min for G60 and GF60.

Figure 2.1
CHAPTER III
RESULTS

Thermal response

Changes in $H$, $T_{ty}$ and $\overline{T_{skin}}$ are presented in Fig. 3.2. Total heat production progressively increased throughout cold exposure, peaking and stabilizing at about $\sim2.5$-times that of baseline for G0 ($6.0\pm0.2$ to $15.0\pm1.2$ kJ·min$^{-1}$), G60 ($6.2\pm0.2$ to $13.6\pm0.8$ kJ·min$^{-1}$) and GF60 ($6.3\pm0.1$ to $14.7\pm1.0$ kJ·min$^{-1}$) (Fig. 3.2A). However, heat production in the G60 condition transiently increased above G0 at 75, 90 and 105 min into cold exposure, before returning to a similar rate. There was no significant change in $T_{ty}$ in any condition. $T_{skin}$ decreased by $\sim18\%$ for G0 (from $32.8\pm0.2$ to $27.0\pm0.4^\circ$C), G60 (from $32.7\pm0.3^\circ$C to $26.9\pm0.4^\circ$C) and GF60 (from $33.2\pm0.3^\circ$C to $24.8\pm0.7^\circ$C) during cold exposure (Fig. 3.2C) with no significant difference between conditions.

Metabolic response and fuel utilization

Changes in absolute rates of oxidation for CHO ($RG_{ox}$), lipids ($RF_{ox}$) and proteins ($RP_{ox}$) are shown in Fig. 3.3 and Table 3.1. In response to cold exposure $RG_{ox}$ increased significantly above baseline values in the G0 (from $78\pm23$ to $439\pm65$ mg·min$^{-1}$), G60 (from $66\pm26$ to $345\pm35$ mg·min$^{-1}$), and GF60 (from $65\pm15$ to $361\pm63$ mg·min$^{-1}$) conditions, however the $RG_{ox}$ was significantly greater in the G0 condition relative to G60 at 45 and 75 min into cold exposure (Fig. 3.3A). There was no significant difference between the G60 and GF60 condition. Similarly, $RF_{ox}$ increased significantly in response to cold exposure for all three experimental conditions peaking at $162\pm24$, $164\pm17$, and $184\pm15$ mg·min$^{-1}$ for G0, G60 and GF60 respectively; however, G60 transiently increased above G0 from 75 to 105 min.
into cold exposure before returning to levels similar to G0 by the end of the exposure period (Fig. 3.3B). Similar to RG_{ox} there was no significant difference in RF_{ox} between the G60 and GF60 condition. In contrast, RP_{ox} remained unaffected by cold exposure regardless of the exogenous glucose ingestion time or CHO ingestion type, averaging 68±10, 77±6 and 74±5 mg·min^{-1} at baseline and 63±4, 66±4 and 64±12 mg·min^{-1} during cold exposure for G0, G60, and GF60 respectively (Fig. 3.3C).

Changes in the relative contributions of CHO, lipids and proteins to total heat production are shown in Fig. 3.4 for all experimental conditions. While the percentage of heat production derived from the utilization of CHO's increased in all three conditions (Fig. 3.4A), the relative contribution of CHO's was significantly greater in the G0 condition relative G60 but only at two time points (45 and 75 min). In contrast, the relative contribution of lipids to total heat production progressively fell throughout cold exposure in all three experimental conditions (Fig 3.4B) only reaching statistical significance at the end of cold exposure. Similarly, the percentage of heat production derived from proteins decreased following cold exposure and was on average ~2.6-fold lower than during the baseline period for G0, G60 and GF60 (Fig. 3.4C).

**Plasma concentrations**

Changes in plasma concentrations of insulin, and glucose are presented in Fig. 3.5. Plasma insulin and glucose concentrations transiently increased over time from the time of ingestion for G0 (from 12.4±3.4 to a peak of 87.1±25.9 pmol·l^{-1} for insulin and from 4.4±0.2 to a peak of 5.9±0.5 mmol·l^{-1} for glucose). While the insulin concentrations remained elevated throughout the cold exposure, only remaining significantly different from G60 prior to the point of glucose ingestion for this condition, glucose concentrations in G0 returned to
baseline levels after 90 min of shivering. Although insulin and glucose concentrations both increased over time for G60 (from 12.7±1.5 to a peak of 73.2±14.8 pmol·l⁻¹ for insulin and from 4.2±0.2 to a peak of 6.6±0.5 mmol·l⁻¹ for glucose) and GF60 (from 11.4±0.9 to a peak of 57.0±14.4 pmol·l⁻¹ for insulin and from 4.3±0.1 to a peak of 6.6±0.5 mmol·l⁻¹ for glucose), glucose concentrations remained elevated longer in G60 than in GF60 (interaction).

**Exogenous and Endogenous glucose oxidation**

Table 3.1 summarizes average values for glucose oxidation rates estimated between 135 and 150 min of cold exposure compared to baseline values. Although the absolute rate of carbohydrate oxidation increased by as much as 6.3-times following glucose ingestion during cold exposure relative to the baseline period, regardless of the timing of glucose ingestion or the type of CHO ingested, the RG_{ox-exo} appeared to differ between conditions. The RG_{ox-exo} increased during cold exposure in all conditions, however when glucose was ingested from the onset of cold exposure the RG_{ox-exo} was more than 1.5-times greater than when it was ingested 60 minutes into exposure (271±20 and 175±20 mg·min⁻¹ for G0 and G60 respectively). This represented 62% of total CHO oxidation during G0 and 50% during G60. Alternatively, the RG_{ox-exo} was also significantly greater when 40 g of glucose and 40 g of fructose were ingested in combination than when the same quantity of only glucose was ingested after 60 min of low-intensity shivering. The RG_{ox-endo} also increased in all conditions during cold exposure relative to ambient conditions (main effect of time) however there was no main effect of condition as it remained between 133±52 and 171±23 mg·min⁻¹ during the last 15 min of cold exposure.

The relative contribution of RG_{ox} to total heat production increased in all of the experimental conditions following cold exposure, largely due to the increased contribution to
total heat production from the oxidation of exogenous glucose as it increased significantly from ~2% during the baseline period to up to 20-30% during cold exposure, with the greatest increase occurring in the G0 and GF60 conditions. The increase in relative contribution to total heat production from this fuel source appeared to be almost double in G0 compared to G60 and was significantly greater in GF60 relative to G60. In contrast, the relative contribution of RG_{ox-endo} to total heat production did not change from baseline to cold exposure, nor did it differ between conditions.
Table 3.1. Absolute Oxidation (mg·min⁻¹) and relative contributions (%H) of CHO (Total: \(RG_{ox}\); Exogenous glucose: \(RG_{ox\text{-exo}}\); Endogenous glucose: \(RG_{ox\text{-endo}}\)), lipid \(RF_{ox}\) and protein oxidation \(RP_{ox}\) to total heat production \(H\) in men orally ingesting glucose at the onset of cold exposure (G0) or after 60 min of cold exposure (G60) or ingesting both glucose and fructose after 60 min of cold exposure (GF60). Values are means ± SE; \(n = 6\) subjects. *Significantly different from baseline values before cold exposure, \(P < 0.05\). \(a\) Significantly different from G60, \(P < 0.05\). \(b\) Significantly different from G0, \(P < 0.05\).
Table 3.1. Absolute Oxidation (mg·min⁻¹) and relative contributions (%H) of CHO (Total: RG_{ox}; Exogenous glucose: RG_{ox-exo}; Endogenous glucose: RG_{ox-endo}), lipid (RF_{ox}) and protein oxidation (RP_{ox}) to total heat production (H) in men orally ingesting glucose at the onset of cold exposure (G0) or after 60 min of cold exposure (G60) or ingesting both glucose and fructose after 60 min of cold exposure (GF60). Values are means ± SE; n = 6 subjects. *Significantly different from baseline values before cold exposure, P < 0.05. a Significantly different from G60, P < 0.05. b Significantly different from G0, P < 0.05.

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<td>15.0 ± 1.2*^a</td>
<td>6.4 ± 0.2</td>
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<td>88 ± 13</td>
<td>439 ± 65*</td>
<td>73 ± 23</td>
<td>345 ± 35*</td>
<td>71 ± 12</td>
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<tr>
<td>% H</td>
<td>1.7 ± 0.2</td>
<td>30.1 ± 2.4*^a</td>
<td>1.6 ± 0.2</td>
<td>20.8 ± 2.0*</td>
<td>1.4 ± 0.2</td>
<td>25.3 ± 1.9*^abc</td>
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<td><strong>Endogenous (RG_{ox-endo})</strong></td>
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<tr>
<td>mg·min⁻¹</td>
<td>82 ± 13</td>
<td>168 ± 53*</td>
<td>67 ± 23</td>
<td>171 ± 23*</td>
<td>68 ± 13</td>
<td>133 ± 52*</td>
</tr>
<tr>
<td>% H</td>
<td>22.5 ± 4.2</td>
<td>17.4 ± 5.3</td>
<td>17.5 ± 5.9</td>
<td>20.7 ± 2.8</td>
<td>17.7 ± 3.0</td>
<td>14.0 ± 4.8</td>
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<td><strong>Lipids (RF_{ox})</strong></td>
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<tr>
<td>mg·min⁻¹</td>
<td>83 ± 8</td>
<td>162 ± 24*</td>
<td>89 ± 10</td>
<td>164 ± 17*</td>
<td>97 ± 5</td>
<td>184 ± 15*</td>
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<tr>
<td>mg·min⁻¹</td>
<td>68 ± 10</td>
<td>63 ± 4</td>
<td>77 ± 6</td>
<td>66 ± 4</td>
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<td>64 ± 12</td>
</tr>
<tr>
<td>% H</td>
<td>22.4 ± 3.2</td>
<td>8.4 ± 0.5*</td>
<td>24.0 ± 1.7</td>
<td>9.8 ± 0.9*</td>
<td>22.3 ± 1.3</td>
<td>9.0 ± 1.8*</td>
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Figure 3.1. Isotopic composition of expired CO$_2$ before (Baseline) and during cold exposure when given glucose solutions from onset of cold exposure (G0), after 60 min of cold exposure (G60) or when given a solution containing equal parts glucose and fructose after 60 min of cold exposure (GF60). Cold exposure started at time 120. 

$\%\delta^{13}C$ VPDB, difference in $^{13}$C Vienna Pee Dee Bilemmitella (VPDB) standard. 

Time by condition interaction, $P \leq 0.05$ for G0 vs. G60 and G60 vs. GF60. 

*Significantly different from baseline values before cold exposure, $P < 0.05$.  a 

Significantly different from G60 condition, $P < 0.05$.  a
Figure 3.1
Figure 3.2. Heat production ($H, A$) and tympanic ($B$) and mean skin ($C$) temperatures before (Baseline) and during cold exposure when given glucose solutions from onset of cold exposure (G0), after 60 min of cold exposure (G60) or when given a solution containing equal parts glucose and fructose after 60 min of cold exposure (GF60).

*Significantly different from baseline values before cold exposure, $P < 0.05$. $a$

G60 significantly different from G0 condition, $P < 0.05$. $a$
Figure 3.2
Figure 3.3. Absolute carbohydrate (A, CHO), lipid (B) and protein (C) utilization rates before (Baseline) and during cold exposure when given glucose solutions from onset of cold exposure (G0), after 60 min of cold exposure (G60) or when given a solution containing equal parts glucose and fructose after 60 min of cold exposure (GF60). *Significantly different from baseline values before cold exposure, \( P < 0.05 \).

\( a \) G60 significantly different from G0 condition, \( P < 0.05 \).
Figure 3.3
Figure 3.4. Relative contributions of carbohydrates (A), lipids (B) and proteins (C) to total heat production before (Baseline) and during cold exposure when given glucose solutions from onset of cold exposure (G0), after 60 min of cold exposure (G60) or when given a solution containing equal parts glucose and fructose after 60 min of cold exposure (GF60). *Significantly different from baseline values before cold exposure, $P < 0.05$. a G60 significantly different from G0 condition, $P < 0.05$. 
Figure 3.4
Figure 3.5. Plasma insulin (A) and glucose (B) concentrations before (Baseline) and during cold exposure when given glucose solutions from onset of cold exposure (G0), after 60 min of cold exposure (G60) or when given a solution containing equal parts glucose and fructose after 60 min of cold exposure (GF60). *Significantly different from baseline values before cold exposure, $P < 0.05$. $a$ significantly different from G60 condition, $P < 0.05$. 
Figure 3.5
CHAPTER IV
DISCUSSION

The purpose of this thesis was to determine the rate of exogenous glucose oxidation when glucose is ingested at similar rates but at different times during cold exposure and when glucose and fructose are ingested in combination after 60 min of low-intensity shivering. Although ingesting glucose at different times or in combination with fructose during cold exposure did not elicit any major changes in the absolute or relative contribution to total heat production of CHO, lipid and protein oxidation (Fig. 3.3 and 3.4; Table 3.1). The partitioning of CHO utilization was significantly modified. The rate of exogenous glucose oxidation reached a maximum of \(\sim 270 \text{ mg-min}^{-1}\) when glucose was ingested at a rate of \(800 \text{ mg-min}^{-1}\) from the onset of cold exposure, which was over 35% greater than the rate observed when glucose was ingested after 60 min of low-intensity shivering (Table 3.1). Similarly the rate of exogenous glucose oxidation was over 30% greater when glucose was ingested in combination with fructose at the same rate after 60 min of cold exposure relative to only ingesting glucose. This represented an additional 5-10% increase in the relative contribution of this fuel source to total heat production. The changes in exogenous glucose oxidation observed as a result of CHO ingestion timing did not appear to affect the rate of utilization from endogenous sources, as both G0 and G60 increased to \(\sim 170 \text{ mg-min}^{-1}\), representing up to 20% of the total heat production (Table 3.1). However, despite not reaching statistical significance, the rate of endogenous glucose oxidation was reduced by \(\sim 25\%\) in the GF60 condition while the relative contribution to total heat production fell by 6% as a result of the differences in the type of CHO ingested. Although the overall energy balance appears to be relatively similar regardless of whether glucose is ingested at different times or when given in combination with fructose there are some very important changes that
occur from ingesting CHOs during cold exposure which play a critical part in the fuel utilization patterns, relative to not ingesting any food, and the partitioning CHO sources.

**Timing of glucose ingestion during cold exposure**

During 150 min of low-intensity shivering, our results indicated that the rate of CHO oxidation and its relative contribution to total heat production was the same whether glucose was ingested 60 min into cold exposure versus from the onset of cold exposure. Similarly, lipid and protein oxidation and their relative contribution to total heat production remained constant between conditions. Despite the similarities in fuel selection and relative contribution to total heat production, there was a slight transient change in the overall thermogenic rate (Fig.3.2), differing for a period of ~30 min (from time 75-105 min). In addition, although the overall energy budget was not affected by the timing of glucose ingestion, the metabolic fate of the exogenous glucose and the contribution of this fuel source to total heat production did differ significantly such that RG_{ox-exo} and the relative contribution to heat production was significantly greater when glucose was ingested from the onset of cold exposure versus after 60 min of shivering. However, RG_{ox-endo} appeared to be significantly lower in both conditions relative to what had been previously reported by Blondin et al. (2008) (~370 mg·min⁻¹ and 35%H). This indicates that like exercise, providing glucose from the onset of cold exposure may be the most effective strategy to increase and maintain the overall CHO and exogenous glucose oxidation rate and their relative contribution to total heat production.

**Overall Energy Budget: Total CHO, lipids and proteins.** During low-intensity shivering, our results indicated that ingesting glucose at a rate of 800 mg·min⁻¹ from the onset of cold exposure did slightly, but significantly modify total heat production (15 ±1.2
KJ·min⁻¹) by the end of cold exposure compared to when glucose was ingested after 60 min (13.6±0.8 KJ·min⁻¹; Fig. 3.2; Table 3.1). However, when whole body fuel selection was estimated by indirect calorimetry, there were no differences between experimental conditions in the absolute rates of CHO (439 ±65 mg·min⁻¹ for G0 versus 345±35 mg·min⁻¹ for G60), lipid (162 ±24 mg·min⁻¹ for G0 versus 164 ±17 mg·min⁻¹ for G60) and protein oxidation (63±4 mg·min⁻¹ for G0 versus 66 ±4 mg·min⁻¹ for G60) or their relative contribution to total heat production (Fig. 3.3, Fig. 3.4 and Table 3.1). The relative contribution of both CHOs and lipids to total heat production remained relatively constant at ~45% H (47.3±5.3 and 44.3±5.2%H respectively for G0 versus 41.5±3.6 and 48.7±4.2 respectively for G60; Table 3.1). Together these results indicate that, despite ingesting glucose at different time points throughout cold exposure there are no changes in overall fuel selection. These findings are contrary to our initial hypothesis which suggested that RGox-exo and subsequently whole body fuel utilization was likely limited in previous studies [eg. Vallerand et al. (1992; 1993), Glickman-Weiss et al.(1993) and Blondin et al. (2008)] since the largest glucose load was ingested at the onset of cold exposure which created an insulineemic peak within 20-30 min of exposure prior to reaching the peak muscle activity for a particular cold stimulus. Despite the limited change in fuel utilization and relative contribution of these fuels to total heat production found in the current thesis, there was an unforeseen effect that was not apparent in previous studies such that a distinguishable change in the thermogenic rate both at the end and between times 75 and 105 min of the cold exposure period was observed.

Only four other studies have quantified the effects of CHO ingestion on modifications in whole body fuel selection during cold exposure (Vallerand et al., 1992; Glickman-Weiss et al., 1993; Vallerand et al., 1993; Blondin et al., 2008). When cold-exposed fasted subjects were fed 100% starch jellies or a high-CHO bar at the onset and 90 min into cold exposure,
Vallerand et al. (1993) showed that CHO oxidation increased while lipid oxidation decreased in both, without modifying total heat production (remained at 3.5 X RMR). These findings however were not entirely consistent with his previous findings (Vallerand et al., 1992) where the subjects ingested the same high-CHO bar but at a milder cold exposure, where they found that there was similarly no effect on heat production, but unlike the follow-up study, there was also no change in fuel utilization. It is important to note however that neither of these two studies accounted for the contribution of proteins which may represent between 10 to 20% of heat production depending on the shivering intensity and nutritional status of the cold-exposed individual (Haman et al., 2004; Haman et al., 2005).

A more recent study by Blondin et al. (2008) showed that, consistent with this study, ingesting glucose at a rate of 800 mg-min⁻¹ did not change the absolute rate of CHO or lipid oxidation compared to the control trial (Fig.4.1); however, while the contribution from lipids and proteins remained the same in both studies, the relative contribution to total heat production from the oxidation of CHO increased as a result of CHO ingestion in our earlier study (but not in the current one) without modifying the metabolic rate. The contrasts in metabolic rate brings back to light the possibility that the thermogenic effect of feeding may be sufficient to increase overall heat production during cold exposure as has originally been hypothesized by Wang et al. (1987) since the total heat production increased by up to 20% at times 75-105 min in the G60 trial relative to G0 (from 13.7±0.8 KJ-min⁻¹ in G0 to 16.4±1.2 KJ-min⁻¹ in G60) before returning to similar levels by the end of cold exposure. While it is unlikely that this difference in thermogenic rate is entirely attributable to dietary thermogenesis, researchers have shown that the thermogenesis from the ingestion of exogenous glucose represents ~8% of its energy content (Golay et al., 1982). This contribution to total heat production could be sufficient to observe a statistical difference in
thermogenesis between these conditions. The likely reason this thermic effect of food was observed in G60 and not in G0 was likely linked to the fact that a shivering steady state had been established prior to glucose ingestion in G60 and the glucose ingestion could have had an additive effect to the shivering thermogenesis; However, in G0 the thermic effect of feeding may have been masked by the concomitant rise in shivering thermogenesis. Additionally, although none of the aforementioned studies have observed a thermogenic effect from ingesting glucose, Vallerand et al. (1993) suggests that it is possible that the higher metabolic rates seen in these previous studies, relative to the present one, may have masked this effect or was negligible relative to the muscle activity. It still remains to be determined whether the thermic effect of ingesting CHOs or other macronutrients can produce sufficient heat to increase overall heat production when exposed to a cold condition.
Figure 4.1 Absolute rates of carbohydrate, endogenous glucose and exogenous glucose oxidation in mg·min^{-1} (A) and the relative contribution of carbohydrate, lipid and protein oxidation to total heat production (%H; B) during cold exposure when given glucose solutions from onset of cold exposure (G0), after 60 min of cold exposure (G60) or when given a solution containing equal parts glucose and fructose after 60 min of cold exposure (GF60) relative to a control group without glucose ingestion (C). Values are means ± SE. * Significantly different from C, P<0.05. Data adapted from Blondin et al. (2008).
Metabolic Fate of Exogenous Glucose. This study builds upon the most recent and lone previous estimates of the rates of exogenous glucose oxidation during cold exposure. The current thesis showed that in non-cold acclimatized men shivering at an intensity ranging between \(-2.0 - 2.5 \times \text{RMR}\), \(\text{RG}_{\text{ox-exo}}\) reached a maximal rate of \(-271 \text{ mg-min}^{-1}\) in GO which represents an increase of almost \(100 \text{ mg-min}^{-1}\) above the rate observed when glucose is ingested 60 min into cold exposure, following the establishment of a shivering steady state (Fig.3.2; Table 3.1). This is a 37\% increase from the previous estimates found in men exposed to a cold condition, inducing a similar shivering intensity for 120 min, ingesting glucose at the same rate [(Blondin et al., 2008); Fig. 4.2]. In that study, the maximal \(\text{RG}_{\text{ox-exo}}\) attained a plateau at \(200 \text{ mg} \cdot \text{min}^{-1}\), by the end of 120 min of cold exposure, when glucose was ingested at a low glucose consumption rate (\(400 \text{ mg} \cdot \text{min}^{-1}\); Fig. 4.2). When comparing the current results between times 90 and 120 min to those achieved during the final 30 min of the latter study, the \(\text{RG}_{\text{ox-exo}}\) appear to be the same (\(185\pm17 \text{ mg} \cdot \text{min}^{-1}\) in GO). It is unclear what the exact mechanism is involved that is increasing the \(\text{RG}_{\text{ox-exo}}\) above the previously reported values during the final 30 min of the current study. Previous limitations may be due to glucose absorption at the gut or the glucose was simply being directed towards non-oxidative disposal (Kuipers et al., 1987; Wagenmakers et al., 1993; Livesey et al., 1998). The significant increase above previous values and apparent continual rise in \(\text{RG}_{\text{ox-exo}}\) (Fig. 3.1) seen in this study suggests that perhaps the maximal rate has yet to be achieved.
Figure 4.2 Effects of quantity [Low glucose (LG) and high glucose (HG)], timing (G0 and G60) and type (G60 and GF60) of exogenous carbohydrate ingestion during low-intensity shivering: (A) absolute rates of exogenous carbohydrate oxidation in mg·min⁻¹; (B) relative contribution of exogenous carbohydrate oxidation to total heat production % $H_{prod}$. Values are means ± SE. a Significantly different from G0, $P<0.05$. b Significantly different from G60. c Significantly different from GF60, $P<0.05$. Data adapted from Blondin et al. (2008).
While this rate is still a significant increase from previous findings, a substantial amount of the ingested glucose still remains unaccounted for. Our calculations indicate that of the 80 g (G60) or 120 g (G0) of glucose ingested only 16 g and 40 g is oxidized in G60 and G0 respectively; an amount representing 20 and 33% of their respective total glucose load. The significantly greater $RG_{ox-exo}$ seen in this study relative to previous findings suggests that perhaps the additional 30 min of cold exposure in this study was sufficient to allow for a greater portion of glucose to be emptied by the gut and absorbed by the small intestines thus allowing for an additional 17 g of glucose to be oxidized. However, the fate of the unaccounted exogenous glucose still remains to be determined.

The $RG_{ox-endo}$ seen in this study combined with the findings from Blondin et al. (2008) both clearly demonstrate a decreased contribution from this fuel source relative to a control trial (Fig. 4.1). Although the current study does not demonstrate a clear difference in $RG_{ox-endo}$ as a result of glucose ingestion timing, the latter clearly shows that the rate of glucose oxidation derived from the muscle remains constant independent of changes in glucose ingestion rate. Conversely the rate of glucose oxidation derived from the liver was reduced by 35-50% compared to control values. This suggests that perhaps the fall in $RG_{ox-endo}$ found in G0 and G60 is likely a reflection of a suppression in endogenous glucose production (primarily hepatic glucose output). Although there is very limited data available to support this hypothesis in the cold, such effects of glucose ingestion on the rate of glucose oxidation from the liver and muscle are consistent with those previously observed during prolonged exercise (50% VO2 max) (Bosch et al., 1993; Jeukendrup et al., 1999b; Wallis et al., 2007). In addition to suppressing endogenous glucose production a study by Kuipers et al. (1987) has also clearly demonstrated that CHO feeding during low-intensity exercise
(40% of maximal workload) could equally provide substrate for oxidation and for glycogen synthesis in inactive muscle fibers.

While the effects of ingesting glucose from the onset of cold exposure or 60 min during cold exposure on the absolute rate of exogenous and endogenous glucose oxidation are quite substantial, their relative contribution to total heat production provides a clearer picture of their significance for cold survival. The exogenous glucose oxidation observed in both G0 and G60 provide a sizeable fraction of all the heat produced by the end of cold exposure (20-30 % H). While the relative contribution of exogenous glucose oxidation to total heat production is similar in G60 to what has previously been observed in Blondin et al. (2008) the contribution seen during G0 provides an additional 10% of total heat production compared to both G60 and the greatest contribution seen from either experimental condition in the previous study (Fig. 4.1). Conversely the relative contribution to total heat production from the utilization of endogenous glucose sources remained relatively constant at ~20% of total heat production for both G0 and G60 which is substantially lower than what has previously been observed when no glucose was ingested.

**Effect of CHO type**

This study is the first of its kind to compare the effects of ingesting glucose in combination to fructose during low-intensity shivering on fuel utilization and the metabolic fate of the ingested CHO. Similar to the effect of timing, ingesting two different types of CHO in combination after 60 min of cold exposure had no effect on overall fuel utilization relative to ingesting glucose alone. Similarly, when comparing the respective contribution from each fuel source to total heat production, it is clear that ingesting two different types of CHO in combination does not elicit significant changes relative to ingesting glucose alone.
Surprisingly however, unlike G60 the overall thermogenic rate throughout the cold exposure period was not statistically different from G0 ($P<0.1$ at times 90 and 105min) however it did follow a similar trend to G60 (Fig. 3.2). Despite fuel selection remaining the same between G60 and GF60, the rate of exogenous glucose oxidation was 30% higher in GF60 than G60, while the rate of endogenous glucose oxidation fell by 22%. Although the partitioning of CHO was slightly modified in GF60 relative to G60, the tendencies of ingesting glucose in combination with fructose appeared to follow a similar trend to G0. Such similarities can have substantial implications for cold survival and can shed some light into the advantages of ingesting hexoses in combination.

**Overall Energy Balance: Total CHO, lipids and proteins.** The current results and those previously reported in Blondin et al. (2008) would strongly suggest that ingesting glucose during low-intensity shivering does not invoke the same effects on whole body fuel utilization as what is seen during exercise potentially due to the increased competition for absorption. This led us to believe that perhaps ingesting glucose in combination with fructose could be the necessary impetus to stimulate these modifications, since exercise studies have shown that during 120-150 min of exercise ingesting a CHO beverage containing glucose and fructose can increase the $\text{RG}_{\text{ex}}$ by up to 55% compared to the ingestion of a glucose solution (Adopo et al., 1994; Jentjens et al., 2006) and subsequently increase $\text{RG}_{\text{ex}}$ and reduce $\text{RF}_{\text{ox}}$ (Jentjens & Jeukendrup, 2005). During 150 min of low-intensity shivering, our results indicated that ingesting glucose in combination with fructose at a rate of 800 mg·min$^{-1}$ did not modify the absolute rate of oxidation as the oxidation of CHO (345±35 mg·min$^{-1}$ for G60 versus 361±63 mg·min$^{-1}$ for GF60), lipids (164±17 mg·min$^{-1}$ for G60 versus 184±15 mg·min$^{-1}$ for GF60) and proteins (66±4 mg·min$^{-1}$ for G60 versus 64±12 mg·min$^{-1}$ for GF60) remained the same in both conditions (Table 3.1). Even when the relative contribution of
each fuel to total heat production was calculated, there were no significant differences observed between the two conditions as the relative contribution of $RG_{ox}$ increased 22%, while $RP_{ox}$ decreased 14% and $RF_{ox}$ remained the same from the baseline period to cold exposure for both conditions. These absolute rates and relative contribution to heat production from the oxidation of these fuels is similar to what has previously been observed when only a placebo was ingested in the study by Blondin et al. (2008) (Fig. 4.1). We have previously suggested that perhaps the changes in absolute rates and relative contribution to heat production that are typically observed during exercise, do not occur during low-intensity shivering as a result of the significantly lower $RG_{ox-exo}$ seen during low-intensity shivering, which is possibly a product of a reduction in gastric emptying and intestinal absorption, rate of uptake by skeletal muscle and/or shivering intensity. However, by lessening the competition for absorption by ingesting glucose in combination with fructose, we expected to see more significant changes to both the utilization rate and the relative contribution to heat production from CHO oxidation, particularly since the rate of exogenous glucose oxidation was significantly greater in GF60 than G60 and did approach the values observed in G0. Further, ingesting fructose has been shown to have an effect on overall substrate utilization relative to ingesting glucose alone such that the rise in insulinemia observed when ingesting glucose alone is significantly lower when ingesting fructose which could offset the inhibitory response seen from glucose ingestion thus favouring lipid utilization relative to ingesting glucose (Massicotte et al., 1986). While the insulminemic response was transiently lower in GF60 relative to G60 this difference failed to reach statistical significance. Similarly, the absolute rate of fat utilization was slightly higher in GF60 relative to G60, but this too failed to reach statistical significance. Perhaps by ingesting a solution containing either only fructose or a larger proportion of fructose to glucose, this response could be amplified.
Of even greater significance was that despite obtaining similar absolute rates of oxidation and relative contribution to total heat production from the oxidation of CHO, lipids and proteins as what was observed in G60, the rate of heat production was not significantly altered as result of CHO ingestion and unlike G60 did not appear to be statistically different from G0, despite following a similar trend. This is surprising since previous research has shown an increase in energy expenditure following the ingestion of fructose (Schwarz et al., 1992; Blaak & Saris, 1996), representing an increase in energy expenditure that is up to 62% greater than an equivalent dose of glucose (Tappy et al., 1986). This was largely attributed to a higher cost of glycogenesis and a stimulation of gluconeogenesis from fructose ingestion (Tappy & Jequier, 1993). While it appears as though ingesting fructose could provide a thermogenic effect, the quantities ingested in this study may not be substantial enough to elicit this effect.

*Metabolic Fate of Exogenous Glucose.* As previously indicated, exercise studies have shown that during 120-150 min of exercise ingesting a CHO beverage containing glucose and fructose can increase the $\text{RG}_{\text{ox-exo}}$ by up to 55% compared to the ingestion of a glucose solution (Adopo et al., 1994; Jentjens et al., 2006). Despite the similarities in fuel selection between G60 and GF60, the $\text{RG}_{\text{ox-exo}}$ was 30% greater in GF60 ($228\pm23 \text{ mg-min}^{-1}$) than when only glucose was ingested ($175\pm20 \text{ mg-min}^{-1}$) which provided an increase in the relative contribution of this fuel source to total heat production of 5% ($25.3\pm1.9\%H$; Table 3.1). Further, the maximal rate of exogenous glucose oxidation approached the rate as that seen when glucose was given from the onset of cold exposure and also exceeded the previous maximal rates reported by Blondin et al. (2008) by 17% ($195\pm15 \text{ mg-min}^{-1}$), despite having ingested less CHO (same rate of ingestion).
Subsequent to the increase in RG_{ox-exo} seen in GF60 relative to G60, there was also a marked reduction in RG_{ox-endo} and its relative contribution to heat production (from 171±23 mg·min^{-1} and 20.7%H for G60 to 133±52 mg·min^{-1} and 14%H for GF60; Table 3.1). Although it did not reach statistical significance, this apparent sparing effect could preserve over 3 g of glucose over a 90 min period which is sufficient to provide an additional 25 min of shivering, which may have significant implications for survival in a cold condition. Based on previous findings (Blondin et al., 2008) it is unlikely that muscle glycogen utilization would have changed as a result of CHO ingestion, however it is very possible that the oxidation rate of glucose from the liver may have partially been reduced as a result of the glucose ingestion in this combined beverage. Unfortunately, there is insufficient data in the current study to determine the effects of ingesting fructose in combination with glucose on the relative contribution of each endogenous fuel source to total RG_{ox}, since the use of indirect calorimetry combined with stable isotope methodologies will not allow for the calculation of liver-derived glucose and muscle glycogen oxidation (Jentjens et al., 2006). These findings combined with the results from our previous study suggest that roughly 55-80% of a CHO load is not oxidized during 120-150 min low-intensity shivering which further suggests that the remaining glucose must be either not entirely absorbed or stored as glycogen (Keizer et al., 1987; Kuipers et al., 1987; Wagenmakers et al., 1993; Livesey et al., 1998). While it is likely that a large proportion of the ingested glucose in G60 may not be absorbed, there is a high probability that a large proportion of the CHO ingested in GF60 is being directed towards nonoxidative disposal in the liver and muscle (Nilsson & Hultman, 1974). This has been attributed to the fact that fructose is rapidly metabolized in the liver, due to the high activity of fructokinase and due to it bypassing the first rate-limiting step of glycolysis thus often exceeding the oxidative capacity in the liver. Consequently this often
results in the accumulation of the three-carbon substrates (from the cleaving of fructose 1-phosphate) which stimulates a direct formation of glycogen in the liver or gluconeogenesis, with some estimating that up to 20% of ingested fructose is recirculated in the systemic circulation as lactate while 54% appears as glucose synthesized from fructose, which can then be subsequently oxidized at the muscle or reconstituted to glycogen in either the muscle or liver (Delarue et al., 1993; Tappy & Jequier, 1993; Blaak & Saris, 1996). Clearly more research is required to determine the metabolic fate of ingested fructose when given in combination with glucose as well as their effect on the partitioning of CHO reserves during low-intensity shivering.
CHAPTER V

GENERAL CONCLUSION

The primary interest of this thesis was to determine whether $\text{RG}_{\text{ox-exo}}$ could be increased above 200 mg·min$^{-1}$ as previously reported by Blondin et al. (2008), by providing a glucose drink following the establishment of a shivering steady state and by combining two hexoses in the same solution. Of secondary interest was determining the effects of ingesting exogenous CHO on fuel utilization as well as determining its metabolic fate. The following conclusions can be drawn from the results of this thesis.

**Timing of glucose ingestion during cold exposure**

*Overall Energy Budget: Total CHO, lipids and proteins.*

1. Although the absolute rates of $\text{RG}_{\text{ox}}$ and $\text{RF}_{\text{ox}}$ increase 5-fold from baseline during cold exposure in both conditions, ingesting glucose from the onset versus 60 min into cold exposure does not increase these rates any further.

2. The relative contribution of each substrate to total heat production is also not affected by the time in which a glucose solution is ingested. Total heat production is however, as it increases transiently following glucose ingestion 60 min into cold exposure.

*Metabolic Fate of Exogenous Glucose.*

1. The $\text{RG}_{\text{ox-exo}}$ exceeded the values previously reported by Blondin et al. (Blondin et al., 2008), peaking at 271 mg·min$^{-1}$ when glucose was ingested from the beginning of cold exposure, which also exceeded the rate observed when glucose was ingested after 60 min of cold exposure by 35% (Fig. 5.1A; Fig. 5.2A).
2. The difference in \( \text{RG}_{\text{ox-exo}} \) observed from ingesting glucose from the beginning of cold exposure also provided an additional 10% to the contribution of this fuel source to total heat production, which helped increase the contribution of this CHO source for thermogenesis (30% \( H \); Fig. 5.2B).

3. Despite significant differences in \( \text{RG}_{\text{ox-exo}} \) and the relative contribution to heat production, \( \text{RG}_{\text{ox-endo}} \) and its contribution to total heat production was not affected by the timing of glucose ingestion.

**Effect of CHO type**

*Overall Energy Budget: Total CHO, lipids and proteins.*

1. Ingesting fructose in combination with glucose did not affect the absolute rates of CHO, lipid or protein oxidation relative to ingesting only glucose.

2. The relative contribution of each substrate to total heat production was also not affected by the type of CHO ingested however, unlike G60 the total heat production did not increase following CHO ingestion relative to when glucose was ingested from the onset of cold exposure.

*Metabolic Fate of Exogenous Glucose.*

1. The \( \text{RG}_{\text{ox-exo}} \) from ingesting glucose in combination with glucose also exceeded values previously reported by Blondin et al. (2008), peaking at 228 mg·min\(^{-1}\), which also exceeded the rate observed when glucose was ingested after 60 min of cold exposure by 30% (Fig. 5.1A; Fig. 5.3A).

2. The difference in \( \text{RG}_{\text{ox-exo}} \) observed from ingesting fructose with glucose, provided an additional 5% to the contribution of this fuel source to total heat production (Fig. 5.3B).
3. Although $R_{G_{\text{ox-exo}}}$ did not differ between conditions, it is unclear whether the ingested CHO is being partitioned differently when glucose is ingested alone or in combination with fructose.

Although it is apparent that a large proportion of the ingested CHO appears to be destined to non-oxidative disposal or simply not absorbed, there is a clear sparing effect that occurs just from simply ingesting CHO during cold exposure regardless of the quantity, timing of ingestion or type of CHO ingested (see figures 5.1 to 5.3). This fact combined with the possible thermogenic effect of ingesting CHO during a mild cold exposure in addition to the supplemental contribution to total heat production from its oxidation may provide the necessary support to suggest this macronutrient as the critical fuel source for survival in a cold condition. Determining the limitations to the unaccounted CHO could also provide some insight into improving the contribution of exogenous CHO on both sparing endogenous reserves as well as contributing to total heat production.
Figure 5.1 Effect of glucose ingestion quantity on absolute rates of CHO oxidation, mg·min\(^{-1}\) (A) and relative contribution to total heat production (%H; B) during 120 min of low-intensity shivering when given glucose solutions at a rate of 400 mg·min\(^{-1}\) (LG) or 800 mg·min\(^{-1}\) (HG) relative to a control group without glucose ingestion (C). Values are means ± SE * Significantly different from C, \(P<0.05\). Adapted from Blondin et al. (2008).
Figure 5.1

A

CHO oxidation rate (mg min⁻¹)

Exogenous

Endogenous

Total CHO

B

% Heat Production

Exogenous

Endogenous

Total CHO

C

LG

HG

Quantity

35%

34%

36%
**Figure 5.2** Effect of glucose ingestion timing on absolute rates of CHO oxidation, mg·min$^{-1}$ (A) and relative contribution to total heat production (%H; B) during 150 min of low-intensity shivering when given glucose solutions from onset of cold exposure (GO) or after 60 min of cold exposure (G60) relative to a control group without glucose ingestion (C). Values are means ± SE * Significantly different from C, $P<0.05$. ** Significantly different from G60, $P<0.05$. Adapted from Blondin et al. (2008).
Figure 5.2
Figure 5.3 Effect of CHO ingestion type on absolute rates of CHO oxidation, mg·min⁻¹ (A) and relative contribution to total heat production (%H; B) during 150 min of low-intensity shivering when given glucose solutions after 60 min of cold exposure (G60) or when given a solution containing equal parts glucose and fructose after 60 min of cold exposure (GF60) relative to a control group without glucose ingestion (C). Values are means ± SE * Significantly different from C, P<0.05. ** Significantly different from G60, P<0.05. Adapted from Blondin et al. (2008).
Figure 5.3
REFERENCES


Dubois D & Dubois EF. (1916). A formula to estimate the approximate surface area if height and weight be known. *Arch Inter Med* 17, 863-871.


