

IMPACT OF POSTEXERCISE HYPEREMIA ON
GLUCOSE REGULATION IN HUMANS

by

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Title: IMPACT OF POSTEXERCISE HYPEREMIA ON GLUCOSE REGULATION
IN HUMANS

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An acute bout of moderate-intensity dynamic exercise results in a sustained rise in skeletal muscle blood flow from that of pre-exercise levels. This postexercise skeletal muscle hyperemia is mediated by two histamine receptors (subtypes, H₁ and H₂). Skeletal muscle glucose uptake is also enhanced, in an insulin-independent manner, following moderate-intensity dynamic exercise. The impact of skeletal muscle hyperemia on glucose regulation following exercise has yet to be examined. Therefore, the purpose of this dissertation was to determine if postexercise skeletal muscle hyperemia plays a substantial role in glucose regulation in humans. In Chapter III I tested my ability to block local H₁- and H₂-receptors located in the vastus lateralis muscle in humans. The results demonstrate that I was able to successfully block the increase in local blood flow evoked by compound 48-80 with the combination of the H₁-receptor antagonist pyrilamine and the H₂-receptor antagonist cimetidine, administered via skeletal muscle microdialysis. In Chapter IV I sought to determine the effect of local

combined H₁- and H₂-receptor blockade, administered via skeletal muscle microdialysis, on postexercise interstitial glucose concentrations. My findings indicate postexercise delivery of glucose to the interstitial space of the previously active skeletal muscle is mediated, in part, by local H₁- and H₂-receptors. In Chapter V I examined the effect of oral administration of H₁- and H₂-receptor antagonists on glucose regulation following a postexercise oral glucose load. The results showed that the glycemic and insulin responses to postexercise oral glucose load were more sustained with H₁- and H₂-receptor blockade versus control, suggesting a histaminergic effect on postexercise glucose regulation.

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CHAPTER I

INTRODUCTION

Statement of the Problem

An acute bout of moderate-intensity dynamic exercise results in a sustained rise in skeletal muscle blood flow from that of pre-exercise levels (Halliwill, 2001; Pricher *et al.*, 2004). This skeletal muscle hyperemia is found in both men and women (Senitko *et al.*, 2002) and in both sedentary and endurance exercise trained individuals (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). Recent evidence suggests this postexercise skeletal muscle hyperemia is mediated by two (H₁ and H₂) histamine receptor subtypes (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). The function, however, of this histamine-receptor-mediated hyperemia found after an acute bout of dynamic exercise has yet to be elucidated.

It is well established that skeletal muscle glucose uptake is enhanced, in an insulin-independent manner, during the first 90 minutes postexercise (Wasserman & Halseth, 1998; Richter *et al.*, 2001a; Henriksen, 2002). Much research has focused on the role of glucose transporters, primarily the GLUT4 isoform found in skeletal muscle, on glucose uptake during and immediately following exercise (Kennedy *et al.*, 1999; Thorell *et al.*, 1999). However, while the mechanisms surrounding both postexercise skeletal muscle hyperemia and postexercise glucose regulation have been explored, the

relationship between these factors, as well as the implications of this relationship, remains poorly understood.

The purpose of the work described in this dissertation was to determine if postexercise skeletal muscle hyperemia plays a substantial role in glucose regulation in humans. More specifically, these studies were designed to determine the effect of combined local and oral H₁- and H₂-receptor blockade on interstitial and blood glucose concentrations following a bout of dynamic exercise. The studies described in Chapter III were conducted to test our ability to both measure and block increases in regional blood flow by local H₁- and H₂-receptor blockade administered via skeletal muscle microdialysis. The study described in Chapter IV was designed to determine the effect of blocking both local H₁- and H₂ receptors in the skeletal muscle on postexercise interstitial glucose concentrations. Finally, in conducting the experiment described in Chapter V, we sought to determine the effect of blunting postexercise hyperemia by comparing blood glucose and plasma insulin concentrations in response to postexercise oral glucose tolerance tests (OGTT) in combined oral H₁ and H₂-receptor blockade versus control conditions.

Significance

The findings from the work described in this dissertation, via their mechanistic and clinical relevance, have the potential to make an impact on a variety of populations, ranging from the apparently healthy to those suffering from cardiovascular and metabolic disease.

First of all, this research expands our understanding of the relationship between cardiovascular and metabolic regulation. It provides evidence that postexercise hyperemia plays a significant role in skeletal muscle glucose delivery and therefore uncovers a potential mechanism of both glucose uptake and maintenance of systemic glucose levels following exertion.

Furthermore, the apparent effects of postexercise hyperemia on glucose delivery may have implications on our understanding of recovery following dynamic exercise. Elevated blood flow to the previously active muscles may be imperative for timely postexercise glycogen repletion, as well as for optimal performance during subsequent bouts of exercise.

Perhaps most importantly, these findings have revealed potential clinical implications of common medications for those who depend on exercise to regulate their blood glucose levels. This may be particularly relevant to populations which have reduced skeletal muscle perfusion, such as the aged, obese individuals and those suffering from diabetes. For example, it is possible that by increasing glucose delivery, postexercise hyperemia may be an important mechanism by which to circumvent insulin resistance in diabetes. Furthermore, these findings have highlighted some potentially problematic effects on blood glucose regulation of commonly used medications, such as those that alleviate allergy symptoms via blockade of histamine-₁ receptors. These effects may confound exercise as a “treatment” for both the healthy population and those suffering from various diseases. This research is important, therefore, not only to

enhance our understanding of glucose regulation, but also because of its potential wide-ranging clinical implications.

Review of the Literature

Regional changes in vascular conductance following exercise

Following a bout of dynamic exercise, humans experience a sustained drop in systemic vascular resistance (Kenney & Seals, 1993; Halliwill, 2001; MacDonald, 2002). Postexercise reductions have been found in calf vascular resistance (Hara & Floras, 1992; Halliwill *et al.*, 1996a; Halliwill *et al.*, 2000) and forearm vascular resistance in both healthy individuals (Coats *et al.*, 1989) and those with mild to moderate hypertension (Cleroux *et al.*, 1992). These findings indicate vasodilation is found in both previously active and inactive regions following dynamic exercise.

Pricher *et al.* found that both postexercise splanchnic and renal vascular conductance was not different from preexercise levels, indicating that these vascular beds do not contribute significantly to elevated systemic vascular conductance following exercise (Pricher *et al.*, 2004). Interestingly, in the same investigation they found an increase in leg vascular conductance from preexercise levels that lasted 100 minutes and was virtually parallel to the increase in systemic vascular conductance. A companion study examined the role of cutaneous vascular conductance in changes in systemic vascular conductance and mean arterial blood pressure following 60 minutes of moderate exercise (60% of $\text{VO}_{2\text{peak}}$) on a cycle ergometer. Despite an elevation in core body temperature that persisted for 90 minutes postexercise, cutaneous vascular conductance, as measured in four sites, quickly returned to the level prior to the exercise bout (Wilkins

et al., 2004b). A recent investigation examining the relationship between regional cerebral blood flow and mean arterial blood pressure following exercise found *reduced* blood flow to some regions of the cerebral cortex (e.g., insular cortex) that could play a role in postexercise hypotension (Williamson *et al.*, 2004). Thus, it does not appear that vasodilation of the cerebral, renal, splanchnic, or cutaneous vasculature contributes to the rise in systemic vascular conductance following dynamic exercise. Rather, the primary contributors to increases in postexercise systemic vascular conductance seem to be elevations in blood flow to previously exercised and non-exercised skeletal muscles (skeletal muscle hyperemia).

Mechanisms of increased systemic vascular conductance postexercise

Previous research indicates that both neural and vascular factors mediate sustained vasodilation in humans following dynamic exercise (Halliwill *et al.*, 1996a). Neural factors refer to reduced sympathetic nerve activity to skeletal muscle vascular beds for given diastolic pressures, whereas the vascular factors refer to reduced vasoconstrictor responses to given levels of sympathetic outflow and/or the effects of local vasodilators on the vasculature following exercise (Hara & Floras, 1992; Halliwill *et al.*, 1996a).

Role of the arterial baroreflex

The arterial baroreflex maintains tight regulation of arterial blood pressure under resting conditions. Transient reductions in arterial pressure diminish the transmural pressure exerted on carotid and aortic baroreceptors which results in decreased afferent activity to the cardiovascular control center, thereby triggering increases in sympathetic

outflow which act to raise arterial pressure. At the onset of exercise, central command mediates an upward resetting of the baroreflex which results in an increase in mean arterial pressure and redistribution of blood flow via cardiac parasympathetic withdrawal and augmented sympathetic outflow.

To assess baroreflex control of sympathetic nervous outflow following exercise, Halliwill and coworkers infused vasoactive drugs (nitroprusside and phenylephrine) while measuring muscle sympathetic nervous activity (microneurography) after 60 minutes of dynamic exercise and after 60 minutes of seated rest (sham). They discovered that across the entire range of diastolic pressures, muscle sympathetic nervous activity was blunted in the postexercise condition compared to the sham condition, indicating a downward resetting of the baroreflex following exercise (Halliwill *et al.*, 1996a). These findings confirmed the presence of altered baroreflex control of sympathetic outflow following exercise, as suspected by previous investigators who found reductions in muscle sympathetic nervous activity in borderline hypertensive men following exercise, accompanied by postexercise hypotension (Floras *et al.*, 1989).

To evaluate postexercise baroreflex control of heart rate, Halliwill and colleagues employed sequential neck pressure and neck suction before and after 60 minutes of moderate intensity aerobic exercise. They found that baroreflex heart rate gain was augmented postexercise, indicating that baroreflex control of heart rate opposes rather than contributes to reductions in blood pressure following exercise (Halliwill *et al.*, 1996b). Furthermore, these results suggest postexercise baroreflex resetting probably occurs at the level of the central nervous system, as opposed to the level of the

baroreceptors themselves. Combined with the results from the aforementioned investigations (Floras *et al.*, 1989; Hara & Floras, 1992; Halliwill *et al.*, 1996a), these findings suggest that changes in the arterial baroreflex, via reductions in systemic sympathetic nervous activity, play a role in elevations in systemic vascular conductance following exercise.

Blunted transduction of sympathetic nervous activity

As previously noted, in addition to a downward resetting of the baroreflex following exercise, local vascular factors have also been cited as potential contributors to elevated postexercise systemic vascular conductance. Halliwill *et al.* examined the relationship between muscle sympathetic nerve activity and calf vascular resistance in response to sympatho-excitation induced by isometric handgrip exercise (held to fatigue) after 60 minutes of dynamic exercise and non-exercise sham (Halliwill *et al.*, 1996a). They found for a given level of muscle sympathetic nerve activity, calf vascular resistance was attenuated postexercise compared to post-sham, demonstrating a blunted transduction of sympathetic nerve activity to vasoconstriction following exercise (Halliwill *et al.*, 1996a). This blunted transduction of sympathetic outflow into vasoconstriction may be the result of an inhibition of pre-synaptic neurotransmitter release, hypo-responsive post-synaptic alpha receptors, or active vasodilation via circulating or local factors (Kenney & Seals, 1993; Halliwill, 2001; MacDonald, 2002).

Pre-synaptic inhibition

Sympathetic neurons release norepinephrine which, when bound to α -adrenergic receptors on vascular smooth muscle of arterioles, leads to vasoconstriction. It is

possible that the release of norepinephrine from these pre-synaptic neurons may be blunted following exercise. If so, less vasoconstriction would occur for a given level of sympathetic outflow, contributing to elevated postexercise vascular conductance.

Previous research indicates that plasma β -endorphin levels are elevated in humans following dynamic exercise (Farrell *et al.*, 1982; Goldfarb *et al.*, 1987). Animal research has shown that opioids may inhibit sympathetic transmission (Konishi *et al.*, 1979) by acting on the central nervous system (Thoren *et al.*, 1990), leading to the hypothesis that opioids play a role in altered postexercise hemodynamics. Hara and Floras administered the opioid receptor antagonist naloxone or vehicle to normotensives (Hara & Floras, 1992, 1995) and young hypertensives (Hara & Floras, 1995) prior to moderate intensity treadmill exercise. Interestingly, although naloxone had no effect on muscle sympathetic nerve activity and postexercise hypotension, it did attenuate the reduction in calf and total peripheral resistance in normotensives but not in hypertensives (Hara & Floras, 1992, 1995). Thus it seems that although opioid receptor activation is not crucial to reductions in blood pressure following exercise, it may mediate postexercise vasodilation in healthy adults via peripheral, as opposed to central mechanisms.

In addition to opioids, there are other substances or receptors that may mediate pre-synaptic inhibition. It is well established that adenosine can produce vasodilation in a variety of vascular beds (Nowak *et al.*, 1987; Edlund *et al.*, 1990). Furthermore, it appears adenosine 5'-triphosphate (ATP) and norepinephrine are co-released from sympathetic nerves (Burnstock & Sneddon, 1985), prior to the prompt conversion of ATP to adenosine within the synaptic cleft. Research by Rongen and colleagues indicates

adenosine inhibits the release of norepinephrine from sympathetic nerves during sympatho-excitation (Rongen *et al.*, 1996). Moreover, recent animal research suggests this norepinephrine inhibition is mediated by adenosine-2 and adenosine-3 receptors located on pre-synaptic nerve terminals (Donoso *et al.*, 2006). Although these studies present compelling evidence that adenosine is involved in inhibition of norepinephrine release, it has yet to be determined if this process mediates increased vascular conductance after exercise.

Since first being discovered in the rat cerebral cortex (Arrang *et al.*, 1983), histamine-3 receptors have been found on autonomic nerve terminals in guinea pigs (Ishikawa & Sperelakis, 1987) and sympathetic nerve terminals in the human heart (Imamura *et al.*, 1995). Moreover, in vitro investigations have shown that histamine-3 receptor activation attenuates norepinephrine exocytosis (Molderings *et al.*, 1992; Silver *et al.*, 2002; Seyedi *et al.*, 2005), so it is feasible that histamine-3 receptors contribute to postexercise hyperemia via pre-synaptic inhibition of norepinephrine release. However, with no histamine-3 receptor antagonist approved for use in humans, it is not currently possible to test this theory in humans.

Hypo-responsive post-synaptic alpha receptors

Transduction of sympathetic outflow into vasoconstriction could be blunted if post-synaptic alpha-adrenergic receptors are hypo-responsive to sympathetic stimulation, so a given degree of norepinephrine binding would result in less vasoconstriction following exercise. When the α_1 -adrenergic receptor agonist phenylephrine was infused locally in rabbits (Howard & DiCarlo, 1992) and rats (Patil *et al.*, 1993; Rao *et al.*, 2002),

it produced substantially less vasoconstriction in the postexercise versus sham condition. Thus in the animal model it appears that α_1 -adrenergic receptors are hypo-responsive following exercise (Howard & DiCarlo, 1992; Patil *et al.*, 1993; Rao *et al.*, 2002) and nitric oxide appears to mediate part of this postexercise attenuated α_1 -adrenergic receptor response (Patil *et al.*, 1993; Rao *et al.*, 2002).

In a study performed on dogs, Buckwalter and colleagues discovered that even during mild exercise α_2 -adrenergic receptor responsiveness was reduced, whereas heavy exercise was required to reduce the responsiveness of α_1 -adrenergic receptors (Buckwalter *et al.*, 2001). Furthermore, Dinunno *et al.* concluded that α_2 -adrenergic receptors contribute more than α_1 -adrenergic receptors to resting vascular tone in humans, suggesting differential responsiveness of the two α -receptor sub-types (Dinunno *et al.*, 2002). The question remained, however, if hypo-responsiveness of either α_1 - or α_2 -adrenergic receptors contributed to the postexercise hyperemia in humans. To address this question, Halliwill *et al.* used intraarterial infusions of α_1 -agonist phenylephrine and α_2 -agonist clonidine before and 30 minutes after moderate cycling exercise. They found that vasoconstrictor responses following exercise were intact in both the forearm and the leg, demonstrating that blunted alpha-adrenergic receptor responsiveness does not contribute to enhanced postexercise vascular conductance (Halliwill *et al.*, 2003).

Possible vasodilators

It is feasible that one or more vasodilator substances - released either systemically or locally - may contribute to postexercise hyperemia. Several substances have been suspected to contribute to enhanced vascular conductance following exercise, most

notably nitric oxide, epinephrine, adenosine, atrial natriuretic peptide, and prostaglandins (Kenney & Seals, 1993; Halliwill, 2001; MacDonald, 2002). The vasodilator substance could be a circulating hormone or locally released substance (Kenney & Seals, 1993) capable of competing with vasoconstrictor substances at the arteriole smooth muscle (Halliwill, 2001).

Dynamic exercise results in increased blood flow and vascular shear stress, which has been shown to facilitate nitric oxide formation from vascular endothelial cells (Cooke *et al.*, 1991; Miller & Burnett, 1992). Along these lines, Jungersten *et al.* measured elevated plasma nitrate, an index of overall formation of nitric oxide, in healthy humans following dynamic exercise (Jungersten *et al.*, 1997). As previously noted, nitric oxide has also been implicated as a mediator of reduced vascular responsiveness to adrenergic receptor stimulation in animal models (Patil *et al.*, 1993; Rao *et al.*, 2002). It seems reasonable, therefore, to predict nitric oxide plays a role in postexercise hyperemia in humans. However, systemic nitric oxide synthase inhibition failed to abolish postexercise peripheral vasodilation in healthy humans, which suggests that postexercise hyperemia is not dependent upon enhanced nitric oxide production (Halliwill *et al.*, 2000).

Epinephrine released during exercise binds to β_2 -adrenergic receptors on arterioles, resulting in moderate vasodilation. In a study conducted on men with primary hypertension, Wilcox and colleagues found postexercise hypotension after β -adrenergic receptor antagonism with epanolol and atenolol (Wilcox *et al.*, 1987). Although postexercise forearm blood flow was only reported during distal body sub-atmospheric

pressure, this postexercise hypotension, combined with the fact that plasma epinephrine quickly returns to pre-exercise levels in hypertensives (Wilcox *et al.*, 1987; MacDonald *et al.*, 2002) and normotensive men and women (Perrault *et al.*, 1989; Horton *et al.*, 2006), argues against epinephrine and/or β_2 -adrenergic receptor-mediated vasodilation following exercise.

Evidence suggests adenosine inhibits pre-synaptic release of norepinephrine (see *Pre-synaptic inhibition*, above), but it is possible it also contributes to postexercise hyperemia via postsynaptic inhibition of α -adrenergic mediated vasoconstriction. When compared with equipotent doses of the vasodilator sodium nitroprusside, brachial arterial infusions of adenosine attenuated the increase in forearm vascular resistance induced by local infusions of both norepinephrine and tyramine, which stimulates endogenous release of norepinephrine (Smits *et al.*, 1991b). Similar attenuation of vasoconstrictor responsiveness to lower body negative pressure (Smits *et al.*, 1991b) and cold pressor test (Smits *et al.*, 1991a) was found with adenosine versus sodium nitroprusside infusions, despite similar levels of forearm production of norepinephrine (Smits *et al.*, 1991a; Smits *et al.*, 1991b). These results suggest a post-synaptic inhibition of α -adrenergic vasoconstriction, although whether this is a factor in postexercise hyperemia remains unknown.

During dynamic exercise, enhanced venous return leads to increased atrial filling. The resulting distention of the atria stimulates the secretion of atrial natriuretic peptide (Ledsome *et al.*, 1985; Perrault *et al.*, 1989), which is known to cause profound vasodilation (Cody *et al.*, 1986). It appears that atrial natriuretic peptide is not

accountable for postexercise hyperemia, however, as reductions in calf vascular resistance actually correspond with reductions in atrial natriuretic peptide concentrations following exercise in humans (Hara & Floras, 1992, 1995).

The arteriolar sheer stress that accompanies dynamic exercise elicits the release of prostaglandins, leading to vasodilation (Koller & Kaley, 1990). Cowley and colleagues measured calf and forearm blood flow before and immediately after treadmill exercise on control and cyclooxygenase inhibition (aspirin) days. They found aspirin, which inhibits prostaglandin synthesis, attenuated the increase in forearm and calf blood flow immediately following exercise (Cowley *et al.*, 1984; Cowley *et al.*, 1985). To determine if prostaglandin-dependent vasodilation is responsible for the long lasting reductions in systemic vascular conductance underlying postexercise hypotension, Lockwood *et al.* measured changes in vascular conductance before and through 90 minutes following an hour of moderate intensity cycling after cyclooxygenase inhibition via ibuprofen. They found cyclooxygenase inhibition had no effect on either femoral or systemic vascular conductance following exercise, which suggests prostaglandin-mediated vasodilation is not responsible for the extended hyperemia following exercise (Lockwood *et al.*, 2005a).

Our understanding of the mechanisms underlying postexercise hyperemia was recently advanced by the discovery that ingestion of the H₁-receptor antagonist fexofenadine hydrochloride attenuates the elevated femoral vascular conductance following exercise (Lockwood *et al.*, 2005b). The effects of H₁-receptor blockade on hemodynamics were most pronounced during the first 30 minutes following exercise. This observation, combined with the previous findings indicating similar vasodilatory but

different time-course effects of H₁- and H₂-receptors (Black *et al.*, 1975; Chipman & Glover, 1976), led to an investigation into the role of H₂-receptors in postexercise vasodilation (McCord *et al.*, 2006). H₂-receptor antagonist ranitidine blunted the increases in femoral and brachial vascular conductance following exercise, with the effect most pronounced from 60 -90 minutes postexercise (McCord *et al.*, 2006). Subsequently, when combined H₁- and H₂-receptor antagonists were ingested by both sedentary and endurance trained individuals, the hyperemia seen through 90 minutes postexercise in the control condition was reduced by 80% (McCord & Halliwill, 2006). Thus it has become clear H₁- and H₂-receptors play a role in mediating postexercise hyperemia in both trained and untrained men and women.

The question remains, however, whether histamine is the active vasodilator which contributes to postexercise hyperemia. Elevated histamine concentrations have been found in whole blood of humans both during (Campos *et al.*, 1999) and after (Harries *et al.*, 1979) dynamic exercise. It has been suggested that stimuli such as vibration and heat elicit histamine release from mast cells (Atkinson TP, 1992), which implies that exercise could be a stimulus for elevations in blood histamine concentrations. Furthermore it appears that sympathetic withdrawal can evoke histamine release (Powell & Brody, 1976) and, as discussed previously, sympathetic withdrawal plays a role in postexercise hyperemia (Floras *et al.*, 1989; Halliwill *et al.*, 1996a).

Thus, it seems likely that H₁- and H₂-receptors are bound by histamine to contribute to postexercise hyperemia. However, recent findings from our lab indicate interstitial histamine concentrations in the biceps brachii, as determined via muscle

microdialysis, were unaffected by 60 minutes of moderate intensity cycling (unpublished observations). It is plausible that histamine release sufficient to cause measurable increases in the interstitium, may be confined to previously active skeletal muscles. Another feasible explanation is exercise could increase the sensitivity of the H₁- and H₂-receptors, so a given level of histamine would induce greater vasodilation following exercise than produced prior to exercise. Along these lines, reductions in pH, which can accompany moderate to high intensity exercise, have been found to increase the sensitivity of H₁-receptors to histamine (Gannellin & Parsons, 1982). Although the specific effect of histamine has yet to be elucidated, it is evident that both H₁- and H₂-receptors, along with the aforementioned neural and vascular factors, mediate postexercise hyperemia in humans.

Function of postexercise hyperemia

While strides have been made in understanding the mediators of postexercise hyperemia, it is unclear what purpose this increased blood flow might serve. Recently, Williams *et al.* investigated the relationship between leg blood flow and oxygen uptake following 60 minutes of moderate-intensity exercise. They found the time course of leg blood flow recovery did not match that of oxygen uptake, suggesting the skeletal muscle hyperemia does not subservise oxygen delivery to the previously exercised muscle (Williams *et al.*, 2005).

Previous research indicates that, following exercise, elevations in glucose uptake and glycogen repletion follow a similar time-course as that of skeletal muscle blood flow. It is plausible postexercise hyperemia functions to enhance glucose delivery and

subsequent glycogen repletion to previously exercised muscle. The remainder of this dissertation will address the relationship between elevated blood flow and glucose regulation following dynamic exercise.

Glucose regulation following exercise

It is well known that during dynamic exercise there is an increase in glucose uptake from the circulation to the working muscles. While glucose oxidation predominates during exercise, the glucose taken up following exercise contributes to glycogen repletion in healthy individuals (Richter *et al.*, 1989; Casey *et al.*, 2000) and diabetics (Maehlum *et al.*, 1977, 1978).

Glucose uptake

Glucose uptake by skeletal muscle is a complex process, with three general rate-limiting steps: glucose supply to skeletal muscle cells, glucose transport into skeletal muscle cells, and subsequent intracellular metabolic flux (Rose & Richter, 2005). Glucose delivery from the blood to the interstitium is determined by a combination of skeletal muscle blood flow, blood glucose concentration, and endothelial permeability to glucose. Transport of glucose into the cells is determined by the presence of a glucose gradient, as well as by the number and activity of glucose transporters in the sarcolemma. Finally, intracellular metabolism is determined by substrate flux and concentration of hexokinase in the skeletal muscle (Wasserman & Halseth, 1998; Rose & Richter, 2005; Wasserman & Ayala, 2005). Once glucose enters the cell, it is quickly converted by hexokinase to glucose-6-phosphate, which insures low intracellular free glucose stores, thereby maintaining a concentration gradient that favors transport of glucose into the cell

(Ivy, 1991). Subsequently, glucose-6-phosphate is a substrate for subsequent metabolic reactions (O'Doherty *et al.*, 1994; Wasserman & Ayala, 2005).

The rate-limiting step in skeletal muscle glucose uptake can vary depending on the specific conditions, such as subject activity (rest, exercise, postexercise) and nutritional (fasted vs fed) status. For instance, by studying transgenic mice with overexpression of either GLUT4 transporters or hexokinase II, investigators have concluded that glucose transport is the principal barrier to muscle glucose uptake under basal conditions but phosphorylation of glucose is more of a rate-limiting factor during physiological hyperinsulemia (Fueger *et al.*, 2005; Wasserman & Ayala, 2005). Moreover, this model has been used to pinpoint the sites where interventions such as high fat diet and exercise training alter glucose uptake and metabolism (Kim *et al.*, 2000). An over-arching theme generated by this line of research is that resistance to muscle glucose uptake is generally distributed among multiple sites and that this distribution changes in response to different physiological conditions.

Following exercise, there is a well-documented increase of insulin action on skeletal muscle glucose uptake (Mikines *et al.*, 1988; Richter *et al.*, 1989). This enhanced insulin sensitivity has been found to persist up to 48 hours following 60 minutes of moderate-intensity exercise (Mikines *et al.*, 1988). Increased insulin sensitivity and resulting skeletal muscle glucose uptake following exercise is known to be mediated, in part, by translocation of GLUT4 glucose transporters to the plasma membrane (Hansen *et al.*, 1998), although GLUT4 activity is increased during and directly after exercise, even in the absence of insulin. Studies utilizing muscle biopsies of

the vastus lateralis have shown increases in skeletal muscle plasma membrane GLUT4 protein following moderate intensity cycling exercise in healthy humans and non-insulin dependent diabetics (Kennedy *et al.*, 1999; Thorell *et al.*, 1999). In fact, exercise induces GLUT4 translocation equivalent to that evoked by euglycemic-hyperinsulinemic clamp (Thorell *et al.*, 1999). Moreover, the increases in plasma membrane GLUT4 glucose transporter after exercise are comparable between diabetics and non-diabetics (Kennedy *et al.*, 1999), indicating a mechanism of GLUT4 translocation capable of circumventing insulin resistance.

Glycogen synthesis

Once glucose is transported into skeletal muscle cells after exercise, glycogenesis, mediated by the enzyme glycogen synthase, insures repletion of glycogen stores. Glycogen synthase activity is known to be increased following dynamic exercise (Garetto *et al.*, 1984; Mikines *et al.*, 1988), an effect which can be amplified when glycogen stores are low (Maehlum *et al.*, 1977; Maehlum & Hermansen, 1978; Wojtaszewski *et al.*, 2001). Thus the rate of glycogen synthesis is typically highest the first 30 minutes following exercise (Price *et al.*, 1994) and, given adequate glucose availability, restoration of glycogen stores is usually complete within 24 hours (Bergstrom & Hultman, 1966; Ivy, 1991; Casey *et al.*, 1995). As illustrated by studies involving electrical stimulation of the rat hindlimb (Richter *et al.*, 1984) and single-legged exercise in humans (Bergstrom & Hultman, 1966; Richter *et al.*, 1989), glucose uptake and glycogen repletion occurs preferentially in the previously exercised skeletal muscle. Moreover, postexercise glycogen synthesis is augmented by glycogen depletion,

especially when accompanied by carbohydrate and/or protein ingestion (Fell *et al.*, 1982; Carrithers *et al.*, 2000; Wojtaszewski *et al.*, 2001).

Maehlum found that insulin-dependent diabetics, despite being deprived of their insulin, had similar glycogen synthesis rates in the first hour following exercise as their non-diabetic counterparts (Maehlum, 1978). When postexercise glycogen recovery time was extended, investigators found glycogen repletion in response to postexercise feeding to be similar in diabetics with and without insulin during the first four hours after exercise. However, only in the diabetics receiving insulin did muscle glycogen concentration continue to rise over the next eight hours, reaching 95% of pre-exercise values by 12 hours postexercise (Maehlum *et al.*, 1978). These results suggested diabetics experienced biphasic glycogen recovery following exercise, with the first phase being insulin-independent and the latter phase requiring insulin. Researchers confirmed an early insulin-independent phase of glycogen synthesis in an animal model, finding enhanced glycogen repletion in rats following hind-limb electrical stimulation (Richter *et al.*, 1984) and treadmill running (Garetto *et al.*, 1984) despite the absence of insulin.

Price and colleagues used an elegant approach to ascertain if the biphasic postexercise glycogen resynthesis found in rats and insulin-dependent diabetics existed in healthy humans. They infused somatostatin intravenously to inhibit endogenous insulin secretion following different degrees of gastrocnemius glycogen depletion induced by toe raises (Price *et al.*, 1994). Using natural abundance ^{13}C -nuclear magnetic resonance spectroscopy, they discovered somatostatin infusion had no effect on early intramuscular glycogen repletion in response to glucose infusion. In contrast, there was a plateau in

intramuscular glycogen concentration approximately 90 minutes postexercise with somatostatin infusion, while glycogen levels continued to rise in the control condition (Price *et al.*, 1994). These findings confirmed an early insulin-independent phase of rapid glycogen resynthesis in healthy humans after glycogen-depleting exercise, which is followed by a prolonged period of glycogen synthesis characterized by increased insulin sensitivity.

Effect of enhanced blood flow on glucose uptake

Impact of exogenous vasodilators

Given the insulin-independent nature of early postexercise glucose uptake and glycogen resynthesis, as well as their similar time course with postexercise skeletal muscle hyperemia, it is intuitive to suspect this hyperemia plays a role in enhanced glucose uptake following exercise. The plausibility of this idea has been bolstered by numerous studies which have suggested blood flow as a rate-limiting factor in glucose regulation. For example, early animal research found glucose uptake was enhanced with increases in isolated rat hindlimb perfusion, even when blood glucose and insulin concentrations were maintained (Grubb, 1977; Schultz, 1977).

Turning to the human model, Baron *et al.* employed hyperinsulinemic euglycemic clamp and leg glucose balance techniques to examine the effect of enhanced leg perfusion, evoked by intrafemoral arterial infusions of the endothelium-dependent vasodilator methacholine hydrochloride, on leg glucose uptake. They discovered both leg blood flow and leg glucose uptake were substantially greater in the combined insulin and methacholine trials versus the insulin only trials, indicating skeletal muscle perfusion

can independently impact insulin-mediated glucose uptake in humans (Baron *et al.*, 1994). Subsequently, Durham and coworkers infused the nitric oxide donor sodium nitroprusside into the femoral arteries of young, healthy subjects while measuring arteriovenous glucose differences and leg blood flow. They measured profound increases in leg glucose uptake in response to exogenous nitric oxide-mediated increases in leg blood flow (Durham *et al.*, 2003). It is worth noting that this enhanced glucose uptake was found in the absence of insulin stimulation, indicating either the increased blood flow itself or some other effect of nitric oxide mediated the increased glucose uptake.

Examinations of interstitial glucose

Investigations utilizing skeletal muscle microdialysis have enabled researchers to examine glucose regulation from a unique perspective, as the glucose in the interstitium is at the crossroads of glucose delivery from the blood and glucose transport to the muscle cells (Wasserman & Ayala, 2005). As such, interstitial glucose concentrations give a good indication of nutritive blood flow – that is flow through blood vessels supplying muscle cells so that nutrient exchange may occur (Newman *et al.*, 2002). Healthy, young males had muscle microdialysis probes inserted into their vastus lateralis muscles prior to performing progressive single leg knee extensions. Interstitial glucose concentrations rose almost 30% upon the initiation of exercise in these subjects, suggesting a flow-limitation for glucose uptake at rest (MacLean *et al.*, 1999). In response to heating and perfusion of a local vasodilator Hickner and coworkers saw a rise in dialysate glucose levels that was negatively correlated with the ethanol outflow-inflow ratio, which suggests the increase in local blood flow mediated glucose delivery to the

interstitial space (Hickner *et al.*, 1992). Henriksson and Knol inserted muscle microdialysis probes in each leg of healthy subjects who had just completed two hours of one-legged cycling (Henriksson & Knol, 2005). They tracked interstitial glucose concentrations and local leg blood flow in each leg over the next eight hours and observed interstitial glucose levels in the previously exercised leg to be appreciably reduced compared to the control leg through five and a half hours postexercise. In addition, local blood flow, as estimated by ethanol outflow-to-inflow ratio, had stabilized by this time. Taken together, these results imply that the notable reduction in interstitial glucose concentrations after prolonged exercise may limit skeletal muscle glucose uptake when blood flow is no longer elevated.

Role of insulin delivery

In addition to its well established effects on skeletal muscle glucose uptake, insulin is known to induce significant effects on the vasculature (Laakso *et al.*, 1990; Baron, 1994). Along these lines, there is considerable evidence physiological concentrations of insulin evoke increased limb blood flow in both rats (Rattigan *et al.*, 1997) and humans (Laakso *et al.*, 1990). Work by Steinberg and colleagues suggests insulin-mediated vasodilation is dependent upon nitric oxide (Steinberg *et al.*, 1994). Moreover, they found inhibition of nitric oxide synthase blunted skeletal muscle insulin-mediated glucose uptake by approximately 25% (Steinberg *et al.*, 1994).

Investigations using both the rat (Vincent *et al.*, 2004) and human model (Coggins *et al.*, 2001) have shown insulin increases capillary recruitment. It appears the mechanism behind insulin-mediated capillary recruitment involves insulin signaling to

endothelial cells which, in turn, produce nitric oxide to induce vasodilation (Rattigan *et al.*, 2005). Insulin binds primarily to insulin-like growth factor-I receptors and insulin receptors in the vascular endothelium to initiate this process (Wang *et al.*, 2006).

Vincent *et al.* measured microvascular recruitment in the rat hindlimb using both metabolism of exogenously infused 1-methylxanthine and contrast-enhanced ultrasound. They found nitric oxide synthase inhibition attenuated microvascular recruitment and limb glucose uptake, as measured by arterial-venous concentration differences, in response to insulin infusion (Vincent *et al.*, 2003). It makes sense that capillary recruitment would enhance muscle glucose uptake, as it functions to both increase the surface area and decrease the distance for diffusion to the skeletal muscle cells. Thus, as previously suggested, it seems insulin is not only a mediator of its own delivery (Vincent *et al.*, 2005), but it appears both insulin-mediated vasodilation and capillary recruitment are key regulators of muscle glucose uptake. Moreover, as indicated by research conducted on lean versus obese Zucker rats, attenuations in insulin-mediated limb blood flow and capillary recruitment are components of insulin resistance (Wallis *et al.*, 2002), but may be circumvented via skeletal muscle contractions (Wheatley *et al.*, 2004).

In order for insulin to aid in glucose delivery, it must move from the central circulation to the skeletal muscle microvasculature and subsequently to the interstitium; therefore anything that can facilitate insulin transport to the interstitium is likely to expedite muscle glucose uptake. Insulin concentrations within the skeletal muscle interstitial space have typically been found to be approximately 50% of that in the plasma, so there is a large gradient for diffusion into the interstitium (Vincent *et al.*,

2005). Plasma insulin concentrations can change quickly, whereas interstitial insulin concentrations change rather slowly (Miles *et al.*, 1995). Moreover, skeletal muscle glucose uptake tracks the timeframe of interstitial insulin levels better than plasma insulin levels, which suggests insulin delivery to the interstitium is crucial in timely muscle glucose uptake (Miles *et al.*, 1995; Vincent *et al.*, 2005). This notion is supported by a recent study by Chiu and coworkers who found intramuscular injection of insulin resulted in an immediate rise in hindlimb glucose uptake in dogs (Chiu *et al.*, 2008)

Evidence suggests insulin-mediated capillary recruitment occurs quickly, while insulin-mediated increases in limb blood flow are slower, and delayed relative to muscle glucose uptake (Vincent *et al.*, 2004). Along these lines, research on dogs indicates approximately 30% of capillaries are perfused at rest (Honig *et al.*, 1982), which implies a flow limitation to skeletal muscle insulin and/or glucose delivery under resting conditions (Vincent *et al.*, 2005), as opposed to conditions in which capillary recruitment is increased, such as exercise. It has been suggested, in fact, in situations where skeletal muscle glucose uptake is elevated, increases in limb blood flow will increase insulin delivery to facilitate further glucose delivery, especially when capillary recruitment is augmented (Clark *et al.*, 2003; Clerk *et al.*, 2004). In other words, since insulin mediates glucose uptake via metabolic and vasculature actions, postexercise skeletal muscle hyperemia likely “mediates the mediator” by enhancing delivery of insulin to the microvasculature when the stimulus for glucose uptake is high.

Impact on glucose effectiveness

Glucose effectiveness refers to the ability of glucose to mediate glucose disposal independent of the influence of circulating insulin (Bergman *et al.*, 1979). The effect of acute exercise on glucose effectiveness and insulin sensitivity was examined by Brun *et al.* by administering an intravenous glucose tolerance test 25 minutes after a 15 minute bout of cycling exercise. They found both glucose effectiveness and insulin sensitivity were greater in the postexercise versus non-exercise control condition (Brun *et al.*, 1995). Subsequently, Sakamoto and colleagues conducted a similar investigation, but used 60 minutes of cycling at lactate threshold intensity as their exercise stimulus and measured leg blood flow via venous occlusion plethysmography. They also measured a substantial increase in glucose effectiveness following exercise that corresponded to elevated leg blood flow, implying postexercise hyperemia promotes glucose effectiveness (Sakamoto *et al.*, 1999).

Role of vascular and metabolic training adaptations

Postexercise glycogen resynthesis and glycogen repletion rates are greater in trained versus untrained individuals (Hickner *et al.*, 1997). Furthermore, Greiwe and colleagues found both postexercise muscle glycogen content and glycogen accumulation rates to be markedly increased after 10 weeks of endurance training (Greiwe *et al.*, 1999), which indicates faster glycogen repletion is a result of training per se, as opposed to being a purely genetic phenomenon. Furthermore, an increase in GLUT4 glucose transporter protein and glycogen synthase activity appear to be adaptations to endurance training that

facilitates this augmented glycogen repletion (Hickner *et al.*, 1997; Greiwe *et al.*, 1999; Richter *et al.*, 2001b).

It is worth mentioning that increased skeletal muscle capillary density is also a well-documented adaptation to endurance training (Andersen & Henriksson, 1977). In context of the aforementioned importance of insulin delivery to the skeletal muscle microvasculature and interstitial space, it is reasonable to suggest greater training-induced capillary density also functions to improve skeletal muscle glucose uptake. This notion falls in line with idea of postexercise skeletal muscle hyperemia sub-serving glucose uptake, as increased perfusion, combined with an increased capacity of the microvasculature, would likely result in enhanced skeletal muscle glucose uptake and subsequent glycogen repletion following dynamic exercise.

Specific Aims

The studies discussed in Chapters III through V were designed to address the following specific aims:

1. The study entitled “Local H₁- and H₂-receptor blockade, administered via muscle microdialysis, abolishes the increase in local blood flow evoked by compound 48-80” (Chapter III) was designed to test our ability to block local H₁- and H₂-receptors located in the vastus lateralis muscle in humans. Specifically, we sought to determine the dosages of the H₁-receptor antagonist pyrilamine and the H₂-receptor antagonist cimetidine required to block the increase in local blood flow evoked by compound 48-80.

2. The study entitled “Local H₁- and H₂-receptor blockade blunts postexercise interstitial glucose concentrations in the vastus lateralis” (Chapter IV) was designed to determine the effect of local H₁- and H₂-receptor blockade, administered via skeletal muscle microdialysis, on postexercise interstitial glucose concentrations.
3. The study entitled “H₁- and H₂-histamine receptor blockade augments the glycemic response to postexercise oral glucose load” (Chapter V) was designed to determine if postexercise skeletal muscle hyperemia influences glucose regulation via enhanced glucose delivery following a bout of exercise.

Hypotheses

The studies discussed in this dissertation tested the following hypotheses:

1. In Chapter III, we tested the hypothesis that local H₁- and H₂-receptor blockade, administered via muscle microdialysis, would abolish the increase in local blood flow evoked by compound 48-80.
2. In Chapter IV, we tested the hypothesis that postexercise interstitial glucose concentrations, as determined by muscle microdialysis, would be higher in the control versus the combined H₁ and H₂ histamine receptor antagonist sites.
3. In Chapter V, we tested the hypothesis that the glycemic response to an oral glucose load (OGTT) following exercise would be more sustained with H₁- and H₂-receptor blockade versus control.

CHAPTER II

OVERVIEW OF THE METHODOLOGIES

Subject characterization

All study protocols described in this dissertation were approved by the Institutional Review Board of the University of Oregon (Protocols #C2-116-08F, #C2-294-08F-3, #A548-07F) and were conducted in accordance with the guidelines set forth by the Office for Protection of Human Subjects of the University of Oregon. Each subject gave his or her written consent prior to participation in the study.

Data from 44 subjects (29 men: 15 women, age 20-35 years) who volunteered to participate in the three protocols described are included in this dissertation. Subjects were healthy, normotensive, non-smokers, ranging in fitness from sedentary to moderately aerobically trained, and taking no medications with the exception of oral contraceptives. For all visits, subjects reported to the laboratory at least three hours post-prandial, having refrained from alcohol consumption and exercise for 24 hours and consumption of caffeine for 12 hours. Female subjects had a negative pregnancy test on the screening visit. In addition, female subjects were studied during the early follicular phase of their menstrual cycle or during the placebo phase of the oral contraceptive cycle to minimize the potential effects of reproductive hormones on cardiovascular and/or metabolic regulation.

Peak aerobic power test

Subjects participating in exercise protocols initially visited the laboratory to perform a peak aerobic power test on a cycle ergometer, in addition to self-reporting activity levels on two questionnaires. Subjects performed an incremental cycle exercise test to exhaustion to determine peak oxygen uptake ($\text{VO}_{2 \text{ peak}}$), measured via a mixing chamber integrated with a mass spectrometry system. A similar computerized system has been validated versus traditional Douglas bag method, at rest and during graded exercise testing (Bassett *et al.*, 2001). Peak aerobic power was determined to aid in quantifying the proper intensity of exercise for the subsequent exercise days, as well as to use for subject characterization.

Moderate intensity cycling exercise

After a brief rest (~ 5 min), subjects returned to the cycle ergometer to determine the workload corresponding to a steady-state oxygen consumption of 60% of $\text{VO}_{2 \text{ peak}}$. This workload was used on the exercise study day(s) for the 60-minute exercise bout. Exercise of this intensity and duration has proven to evoke sustained (~100 minutes) postexercise skeletal muscle hyperemia (Pricher *et al.*, 2004), which was crucial in the undertaking of the studies discussed in Chapters IV and V of this dissertation.

Heart rate

Heart rate was monitored throughout all protocols via 5-lead electrocardiogram (Quinton Instruments, Bothell, WA), as a safety measure, as well as to assess hemodynamic changes throughout the protocols.

Arterial pressure

Arterial pressure was measured with an automated oscillometric device (Dinamap Pro100 vital signs monitor, Critikon Inc, Tampa, FL) during resting conditions. Arterial pressure readings from a similar unit are highly correlated with intra-arterial measurements, which are established as the “gold standard” in arterial pressure evaluation (Hossack *et al.*, 1982). Arterial pressure during exercise was determined via manual auscultometry. Mean arterial pressure was calculated as:

$$\text{MAP} = \text{diastolic BP} + [(\text{systolic BP} - \text{diastolic BP})/3]$$

Limb and skeletal muscle blood flow

There are numerous methods to measure both limb and skeletal muscle blood flow in humans. The proper technique to use depends on a number of factors, including technical requirements, cost, level of invasiveness, and validity of the technique. The most commonly used methods appropriate for use in research on humans are reviewed below, with special emphasis placed on the methods employed in the protocols discussed in this dissertation.

Limb blood flow

For the protocol described in Chapter V of this dissertation, Doppler ultrasound was used to measure leg blood flow, as calculated using mean blood velocities and diameters of subjects' femoral arteries. Doppler ultrasound measurements are derived from changes in frequency (Doppler shift) produced via the scattering of transmitted sound waves by red blood cells (Gill, 1985). The direction and velocity of red blood cell

movement is determined by this frequency change, as detected by the ultrasound transducer (Burns & Jaffe, 1985).

In order to obtain an accurate full parabolic blood velocity profile, it is crucial the Doppler sampling volume spans the entire vessel lumen (Burns & Jaffe, 1985; Gill, 1985; Radegran, 1999). Moreover, imaging with a consistent angle of insonation, preferably $\leq 60^\circ$, insures flow estimate errors are minimized (Gill, 1985; Hoskins, 1990). Finally, since flow rate is the product of mean blood velocity and lumen cross-sectional area, accurate measurement of vessel lumen diameter is essential for valid determination of blood flow (Gill, 1985; Hoskins, 1990).

There are a number of advantages to using Doppler ultrasound for measurement of limb blood flow. It is a non-invasive method to obtain continuous blood flow measurements under a variety of conditions (Radegran, 1999). As demonstrated by Radegran (1997), Doppler ultrasound blood flow measurements were strongly correlated with those simultaneously obtained by thermodilution. This held true during resting conditions, as well as during intense dynamic knee extension exercise, during which time Doppler ultrasound detected blood flow changes with a high temporal resolution (Radegran, 1997). Furthermore, although the equipment is expensive and the method is technically advanced, once trained, researchers can acquire reproducible mean blood velocity and vessel diameter measurements under both resting and exercising conditions (Shoemaker *et al.*, 1996).

Several indicator methods have been used to measure blood flow in humans, thermodilution and indo-cyanine green dye being two of the most common techniques.

The thermodilution technique is used to determine blood flow by measuring temperature changes within a vein during infusion of saline cooled to a known temperature (Ganz *et al.*, 1964; Andersen & Saltin, 1985). A thermistor inserted into the vein allows temperature measurement of the blood both before and after being mixed with the saline. The temperature change resulting from the saline infusion, along with the known saline and blood temperatures and rate of saline infusion, allow for calculation of blood flow (Ganz *et al.*, 1964; Radegran, 1999). Ideally used in steady state conditions, *in vitro* experiments have indicated the technique, when carefully controlled, can accurately measure blood flow from resting conditions and during dynamic exercise (Ganz *et al.*, 1964; Andersen & Saltin, 1985), although frequency and volume of saline infusion must be maintained such that tissue cooling does not occur (Radegran, 1999). Although relatively inexpensive to implement and conduct studies with thermodilution, it is an invasive procedure.

The indo-cyanine green dye technique entails the infusion of a known concentration of dye into a blood vessel and subsequent sampling of blood downstream from the infusion rate, which enables investigators to measure the concentration of the dye once it has thoroughly mixed with the blood (Wahren & Jorfeldt, 1973). Under steady state conditions, if the infusion rate and sample concentrations are known, blood flow may be calculated (Wahren & Jorfeldt, 1973; Radegran, 1999; Pricher *et al.*, 2004). Dye infusion techniques allow investigators to obtain reliable blood flow measurements at rest and during steady state exercise (Wahren & Jorfeldt, 1973), but the method is invasive and conducting experiments can be expensive (Radegran, 1999).

Venous occlusion plethysmography involves the use of a strain-gauge to detect changes in limb girth when arterial inflow is allowed but venous outflow is prevented (Conrad & Green, 1961; Bygdeman *et al.*, 1971). Thus while the venous outflow is arrested, the rate of limb swelling is used to assess arterial inflow rate. In order to obtain accurate results, venous occlusion must be complete and arterial inflow must be maintained (Conrad & Green, 1961). Advantages of venous occlusion plethysmography include its low cost, relative ease of use and non-invasiveness (Radegran, 1999), but due to problems with motion artifacts (Benjamin *et al.*, 1995), it is only suitable for resting measurements.

For the protocol described in Chapter V, mean blood velocities and diameters of the common femoral artery were measured using a linear ultrasound probe (10MHz linear-array vascular probe, GE Vingmed System 5, Horton, Norway) placed distal to the inguinal ligament, approximately 2–3 cm proximal to the bifurcation. The entire width of the artery was insonated with an angle of 60 degrees and velocity measurements were taken immediately before diameter measurements. Leg blood flow was calculated as artery cross-sectional area multiplied by femoral mean blood velocity, doubled to represent both legs, and reported as $\text{ml} \cdot \text{min}^{-1}$. Leg vascular conductance was calculated as flow for both legs/mean arterial pressure and expressed as $\text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$.

Muscle blood flow

For the protocols described in Chapter III and IV of this dissertation, microdialysis ethanol outflow-inflow ratio was used to measure local blood flow in the vastus lateralis. This technique requires the insertion of a microdialysis probe into

skeletal muscle. A portion of the microdialysis probe is composed of a semi-permeable membrane, which is positioned in the muscle belly. The microdialysis probe is then perfused, at a steady rate, with a physiological perfusion medium (perfusate) containing a known concentration of ethanol. Ethanol readily diffuses through the semi-permeable membrane and is not metabolized by the muscle tissue (Lieber, 1977). Therefore as local capillary blood flow increases, more ethanol is removed from the interstitial space, the ethanol concentration gradient in the vicinity of the probe increases, and less ethanol is collected as out-flowing dialysate (Hickner *et al.*, 1991, 1992). Thus the ethanol outflow-inflow ratio is regarded as an inverse surrogate of local blood flow.

Efforts to quantify local blood flow using the ethanol removal technique have been made, based on studies conducted with cats (Hickner *et al.*, 1995; Wallgren *et al.*, 1995) and humans (Hickner *et al.*, 1994). Hickner and colleagues have found the ethanol clearance technique to be strongly correlated with Xenon-133 (^{133}Xe) clearance (Hickner *et al.*, 1994), although there has been concern that ^{133}Xe clearance is non-linear and underestimates blood flow (Cerretelli *et al.*, 1984; Radegran, 1999). Subsequently, on a study conducted in cats, they found when blood flow was held constant at various flow rates, ethanol outflow-inflow ratios and flow rates were negatively and linearly correlated in a flow range of 4 - ~45 ml · 100 g⁻¹ · min⁻¹, although the ethanol removal technique was less sensitive at higher flow rates (Hickner *et al.*, 1995). In a companion study, a mathematical model for measuring local blood flow was derived using the aforementioned ethanol outflow-inflow ratios (Wallgren *et al.*, 1995). Although the model-based blood flow predictions tracked relatively well with the known blood flows,

these results were applicable only when specific probes were used (Wallgren *et al.*, 1995). An additional concern exists regarding the effect of muscle contractions, per se, on ethanol outflow-inflow ratio measures, independent of changes in blood flow (Radegran *et al.*, 1998). Nevertheless, in the absence of skeletal muscle contractions, when local blood flow rates are low to moderate, the ethanol removal technique can supply investigators with valuable information regarding the relative effect of various interventions on local blood flow.

Another traditional method of estimating local blood flow is the isotope clearance technique, which is based on the assumption that clearance of an inert isotope is dependent upon local blood flow (Kety, 1951; Lassen *et al.*, 1964). The isotope, usually ^{133}Xe , is injected directly into the muscle and its disappearance rate is tracked externally (Lassen *et al.*, 1964; Cerretelli *et al.*, 1984). The slope of the resulting wash-out curve is used to calculate flow, as the steeper the slope, the greater the local blood flow (Lassen *et al.*, 1964). Although it may be used to measure local blood flow during exercise, when compared against direct venous outflow and microsphere trapping flow, the isotope clearance technique was found to drastically underestimate local blood flow in canine muscles (Cerretelli *et al.*, 1984). Combined with the requisite radioactive exposure, as well as the expense associated with the technique, isotope clearance is a less than ideal method of measuring local blood flow in humans.

Positron emission tomography (PET), combined with the intravenous infusion of a tracer substance labeled with a positron-emitting isotope, can be also used to measure muscle blood flow (Raitakari *et al.*, 1996). In order to obtain accurate blood flow results,

it is important that the tracer (e.g., ^{15}O -labelled water) used is inert and able to diffuse from the blood and surrounding tissues (Raitakari *et al.*, 1996). High resolution PET scanners are able to track the distribution of the tracer, as well as quantify its distribution temporally, which is an advantage of using positron emission tomography (Radegran, 1999). However, this method necessitates that positron-emitting isotopes be injected into the subject. Moreover, the equipment required and expense involved in this procedure, in addition to the logistical barriers for use during exercise, make positron emission tomography an impractical method of muscle blood flow measurement for most investigators (Radegran, 1999).

Near-infrared Spectroscopy (NIRS) is a relatively new tool that can be used to measure tissue oxygenation, muscle metabolism and blood flow in the microcirculation (Boushel *et al.*, 1998; Kalliokoski *et al.*, 2006). It is a noninvasive method based on the absorption of light at wavelengths in the near-infrared range, which enables determination of oxygenated and deoxygenated hemoglobin. An advantage of NIRS is that it can be used during exercise. NIRS can be used in conjunction with a light-absorbing tracer to quantify blood flow in discrete areas of the microcirculation, however, it has a limited capacity for discriminating between numerous, closely arranged microvessels (Kalliokoski *et al.*, 2006).

Muscle microdialysis

In order to determine glucose concentrations in the interstitial space, as well as measure local blood flow (see previous section) and deliver drugs of interest to the interstitium, I employed muscle microdialysis. First developed to study the animal brain

(Delgado *et al.*, 1972; Ungerstedt & Pycock, 1974), microdialysis is a unique technique in that it permits scientists to monitor dynamic changes in concentrations of various substances within the interstitium (Hickner, 2000). Whereas the classic muscle biopsy technique (Bergstroem *et al.*, 1965; Hultman, 1967) allows investigators to evaluate muscle composition (e.g., glycogen) under various conditions, its invasive nature limits this method to a few samples per study, thus disallowing continuous monitoring of the muscle tissue.

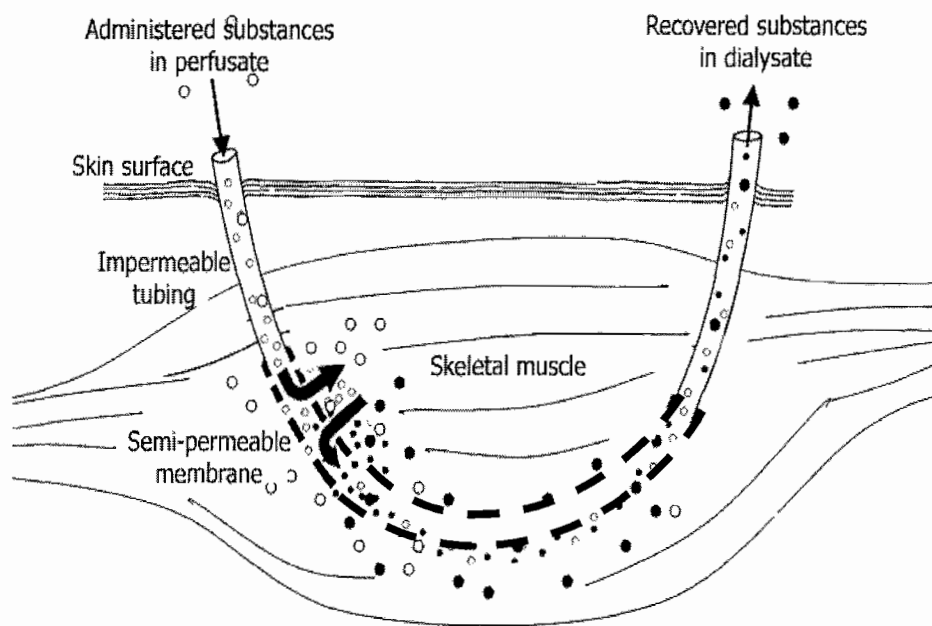


Illustration 1. Skeletal muscle microdialysis schematic. The open circles represent perfusate being delivered through the probe. The filled circles represent the dialysate collected from the probe. The dashed line represents the semi-permeable membrane that allows the concentration-dependent exchange of molecules between the probe and the interstitium.

Microdialysis, on the other hand, entails the continuous delivery of perfusion fluid through a probe at a constant rate, as well as continuous collection and subsequent analysis of the outflowing dialysate (Plock & Kloft, 2005). The composition of the dialysate is dictated by concentration gradients of various substances around the microdialysis probe's semi-permeable membrane. In other words, depending on the size of the pores in the semi-permeable membrane, substances with a small enough molecular weight will either diffuse out of or into the perfusion fluid based on the relative concentrations of those substances within both the probe and the interstitial space (Hickner, 2000; Plock & Kloft, 2005)

Muscle microdialysis is an invasive technique, so great care must be taken to minimize tissue trauma and risk of infection (Plock & Kloft, 2005). In addition, microdialysis probes are quite fragile, so using this technique during exercise increases the risk of probe kinking or breaking, although researchers have addressed this by constructing reinforced probes (MacLean *et al.*, 2000). Although commercially available microdialysis probes can be rather expensive (~ \$150.00 per probe) (Hickner, 2000), researchers can circumvent this problem by assembling and sterilizing their own probes.

For the protocols described in Chapter III and IV of this dissertation, microdialysis probes were constructed in our laboratory. The membranes used in the microdialysis probes were obtained from artificial dialysis kidney (GFS 18) with a molecular mass cutoff of 3 KD and an inner diameter of 0.20 mm. Each end of the membranes were advanced approximately 1 cm into hollow polyimide tubing with an

inner diameter of 0.36 mm and glued in place. The length of exposed membrane in each fiber used was precisely 4 cm. The proximal end of the polyimide tubing was then advanced approximately 2 cm into polyethylene tubing and glued in place. Subsequently a luer tip adapter stub was attached to the proximal end of the polyethylene tubing in order to accommodate a perfusate-filled syringe during the microdialysis procedure. Each probe was tested by attaching an empty syringe to the luer tip adapter stub with the distal tip of the microdialysis probe submerged in a beaker of dionized water. If gentle advancement of the syringe plunger did not produce a steady stream of bubbles, the probe was discarded. Microdialysis probes that were deemed operational were then gas sterilized (Anprolene AN74i) for 12 hours prior to use.

Four microdialysis probes were inserted approximately 2-3 cm apart in the vastus lateralis muscle of each subject's right leg. Prior to insertion, a sterile field was established and the entire lateral thigh region was cleansed with an alcohol-based antiseptic (Chloraprep). The skin and subcutaneous tissue at both the probe insertion and exit sites were then anesthetized via local administration of approximately 1 ml 2% lidocaine (20 mg/ml) plus epinephrine 1:100,000 (10 µg/ml) and sodium bicarbonate per site. The epinephrine produced a local cutaneous vasoconstriction to minimize bleeding and the sodium bicarbonate stabilized the pH to minimize subject discomfort during lidocaine administration. Care was taken to not to introduce the lidocaine solution into the muscle itself, thereby avoiding any alterations in the intramuscular environment. The microdialysis probes were inserted in the vastus lateralis, in a direction parallel to muscle fiber orientation, via a 20-gauge cannula. The probe insertion and exit sites were

approximately 9 cm apart. Immediately after needle insertion, a sterilized microdialysis probe was threaded through the needle lumen. The needle was then removed, leaving only the microdialysis probe in place.

Following placement, each microdialysis probe was attached to a perfusion pump (CMA 102 Microdialysis pump, CMA, North Chelmsford, MA) and perfused at a rate of 5 μ L/min with a Ringer's solution containing 3.0 mM glucose, 0.5 mM lactate and 5.0 mM ethanol. Due to its similar composition of interstitial fluid, this perfusate served as the control solution in each experiment, thus minimizing the potential draining of the interstitial space (Lonnroth *et al.*, 1987). For the protocol described in Chapter IV of this dissertation, [6-³H] glucose (2000 dpm/10 μ l) was included in the perfusate to serve as an internal reference marker for recovery of glucose (Scheller & Kolb, 1991; MacLean *et al.*, 2001) (see *Assessment of probe recovery* below). In addition, for protocols described in both Chapters III and IV, additional drugs were included to the perfusate to either evoke or antagonize increases in local blood flow (see *Drugs administered via muscle microdialysis* below). Throughout the protocols described in Chapters III and IV of this dissertation, the distal tips of the microdialysis probes were placed in microcentrifuge tubes for dialysate collection. Non-porous tape was placed over the top of the microcentrifuge tubes to prevent evaporation of ethanol. The tubes were weighed before and after dialysate collection to verify fluid balance across the microdialysis probe membrane. Microcentrifuge tubes were sealed, placed in a refrigerator, and dialysate was analyzed within 48 hours of collection.

Heated hand vein

In order to obtain “arterialized” blood samples, in lieu of placing more invasive arterial cannulas, a heated hand vein method was used (Morris *et al.*, 1997). An intravenous catheter was inserted retrogradely in the dorsal vein of the left hand, which was then placed in a custom-made heating chamber (“hot-box”), which was flushed with air at 55° C. Actual skin temperature was raised to approximately 42° C, which is below the temperature that evokes sensations of pain. Heating the hand in this way induces the opening of arterial-venous anastomoses in the hand circulation, thereby allowing investigators to sample “arterialized” venous blood with higher and less variable oxygen saturations and higher glucose concentrations compared to non-arterialized blood samples (Morris *et al.*, 1997).

Arterialized venous blood samples

Arterialization of venous blood samples was confirmed by immediate measurement of oxygen saturation in duplicate by a co-oximeter (Radiometer Copenhagen). Glucose concentrations of arterialized venous blood were measured in duplicate with a clinical glucose analyzer (YSI 2300 Stat Plus Glucose and Lactate Analyzer, YSI Life Sciences). Blood samples were promptly placed on ice, centrifuged at 4° C, separated, and stored at -80° C until analyzed.

Insulin sensitivity and glucose tolerance tests

Researchers and clinicians have used numerous approaches to quantify insulin sensitivity and glucose uptake in humans (Monzillo & Hamdy, 2003; Cobelli *et al.*, 2007). The approach taken is usually dictated by the specific objectives of the study, as

well as by the resources available to those conducting the investigation. What follows is a brief review of the most common methods used to assess insulin sensitivity and glucose uptake, with special emphasis placed on the oral glucose tolerance test (OGTT), which was employed in the protocols discussed in Chapters IV and V of this dissertation. Elaboration regarding models and formulas used in conjunction with the OGTT is included below in the section entitled “Estimation of glucose uptake and insulin sensitivity”.

Developed by DeFronzo and colleagues, the hyperinsulinemic euglycemic glucose clamp technique is considered the “gold standard” in the assessment of tissue insulin sensitivity (DeFronzo *et al.*, 1979). During this procedure insulin is intravenously infused at a constant rate in order to maintain a steady state insulin level greater than that of fasting baseline. This hyperinsulinemic state evokes a marked increase in skeletal muscle glucose uptake, while presumably suppressing hepatic glucose production. Intravenously administered dextrose ensures maintenance of normal (euglycemic) blood glucose concentration. Over the course of hours of insulin infusion, steady state blood glucose, plasma insulin, and glucose infusion rate can be obtained. Thus, assuming complete suppression of endogenous glucose production, glucose infusion rate and rate of glucose uptake under these conditions must be equal (DeFronzo *et al.*, 1979; Muniyappa *et al.*, 2008). This information can then be used to compute insulin sensitivity in a precise and highly reproducible manner (DeFronzo *et al.*, 1979; Muniyappa *et al.*, 2008). However, the glucose clamp technique is costly, time consuming, and requires constant careful subject monitoring, which deems it impractical for use in many settings.

The frequently sampled intravenous glucose tolerance test (FSIVGT), in conjunction with Bergman's minimal model (Bergman *et al.*, 1979), is another method that has been utilized to assess insulin sensitivity and glucose effectiveness in humans. This procedure consists of an intravenous bolus of glucose of 0.3 g/kg body weight and measurement of plasma insulin and glucose at 31 time points through three hours post-infusion. The glucose and insulin data are then analyzed via the minimal model program to produce an insulin sensitivity index, which is defined as the glucose clearance rate per change in plasma insulin concentration (Bergman *et al.*, 1979; Monzillo & Hamdy, 2003; Muniyappa *et al.*, 2008). Some investigators have used a modified version of the FSIVGT, adding either insulin (Finegood *et al.*, 1990) or tolbutamide infusion (Beard *et al.*, 1986) 20 minutes after the glucose infusion, as this method has produced better correlation of insulin sensitivity index with the hyperinsulinemic euglycemic glucose clamp technique. A reduced version of the FSIVGT has also been used, which has shown strong correlation with, but slightly weaker reproducibility than the standard version, while requiring less than half the number of blood draws (Duysinx *et al.*, 1994; Steil *et al.*, 1994).

Although the FSIVGT has been a valuable tool in determining insulin sensitivity and glucose effectiveness, there are a few drawbacks to this method. In comparison to oral administration of glucose, the FSIVGT is a less physiological stimulus. Moreover, the FSIVGT has been found to consistently underestimate insulin sensitivity and overestimate glucose effectiveness (Quon *et al.*, 1994; Cobelli *et al.*, 1998), which has been attributed to the relatively short exposure of peripheral tissues to hyperinsulinemia,

as well as the oversimplification of the physiology by the minimal model. The concern over the oversimplification of the model is based on the model utilizing one compartment to assess glucose dynamics, as well as its combination of the effects of insulin to elevate glucose uptake and quell hepatic glucose production (Muniyappa *et al.*, 2008). Finally, the FSIVGT is both invasive and labor intensive, due its duration and high number of blood draws.

The oral glucose tolerance test (OGTT) has been widely used to assess glucose tolerance, defined as the ability of the body to dispose of glucose following a glucose load, in a variety of populations (Cobelli *et al.*, 2007). When combined with various indices of insulin sensitivity and/or modeling techniques, the OGTT can be used to determine β -cell function and insulin sensitivity in humans. The method entails the administration of an oral glucose tolerance beverage (usually containing 75 g glucose), followed by blood draws for subsequent determination of glucose, insulin, and sometimes c-peptide concentrations. The number and distribution of blood draws has varied across investigations, depending on the primary objective of the investigation, as well as the particular formula or model employed. The OGTT glucose and C-peptide minimal models have been validated against tracer protocols (Dalla Man *et al.*, 2004) and the euglycemic hyperinsulinemic clamp technique (Dalla Man *et al.*, 2005b). Recently, Dalla Man and colleagues discovered a two-hour seven sample oral glucose tolerance test protocol resulted in comparable minimal model assessment of β -cell responsiveness and insulin sensitivity to that of longer versions requiring more blood samples (Dalla Man *et al.*, 2005a).

Several factors associated with the OGTT have the potential to affect its reproducibility. Chief among these are variations in glucose absorption, neuro-hormonal interactions, splanchnic glucose uptake, and possible effects on incretin hormones, which are known to stimulate insulin secretion in response to glucose ingestion (Muniyappa *et al.*, 2008). However, significant advantages of OGTT include its more physiological delivery of glucose, relative ease of use, and reduced invasiveness, relative to the FSIVGT and euglycemic hyperinsulinemic clamp.

In the protocol described in Chapter V, each subject consumed 0.1 oz per kg body weight (up to 10 oz maximum) Oral Glucose Tolerance Beverage (Trutol 100, NERL Diagnostics, East Providence, RI), which contained 10 g of glucose per oz. Therefore, subjects received an oral glucose dose of 1 g per kg body weight up to 100 g maximum.

Drug administration

Drugs were administered orally in the protocol described in Chapter V of this dissertation. In the protocols described in Chapters III and IV, several drugs were administered via muscle microdialysis.

Orally administered drugs

H₁-receptor antagonism was produced at the onset of randomized study days via oral administration of 540 mg fexofenadine hydrochloride (Allegra; Aventis Pharmaceuticals Inc, Kansas City, MO). This oral dose of fexofenadine adequately and selectively blocks H₁-receptors, with a half-life of approximately 12 hours and a time to peak concentration between 0.83 – 1.33 hours (Russell *et al.*, 1998). Fexofenadine is non-sedating, does not appear to cross the blood-brain barrier or have anti-muscarinic

effects (Hardman JG, 2001). Oral H₁-receptor antagonism via 540 mg fexofenadine has been shown to attenuate elevations in postexercise femoral vascular conductance in humans (Lockwood *et al.*, 2005b).

H₂-receptor antagonism was produced at the onset of randomized study days via oral administration of 300 mg ranitidine hydrochloride (Zantac; Pfizer Consumer Healthcare, Morris Plains, NJ). This oral dose of ranitidine adequately and selectively blocks H₂-receptors, with a half-life of 2.6 hours and a time to peak concentration of 2.2 hours (Garg *et al.*, 1985). Ranitidine is non-sedating, does not appear to cross the blood-brain barrier or have any cardiovascular effects (Hardman JG, 2001). Oral H₂-receptor antagonism via 300 mg ranitidine has been shown to attenuate elevations in postexercise femoral vascular conductance in humans (McCord *et al.*, 2006).

Drugs administered via muscle microdialysis

In the protocol described in Chapter III of this dissertation 10mM compound 48-80 (Sigma-Aldrich, St. Louis, MO) was administered, via skeletal muscle microdialysis, to induce local histamine-mediated vasodilation. Compound 48-80 degranulates mast cells, resulting in their release of histamine (Diamant & Dahlquist, 1970). This compound 48-80-induced vasodilation was used to verify blockade of local H₁- and H₂-receptors.

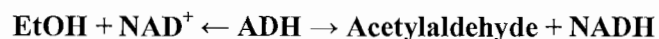
In the protocols described in Chapters III and IV of this dissertation, local H₁-receptor antagonism was achieved with 1 mM pyrilamine maleate and local H₂ – receptor antagonism was achieved with 3 mM cimetidine, both administered via muscle

microdialysis. These doses were titrated from those used previously to achieve H₁- and H₂ –receptor blockade in the skin (Wilkins *et al.*, 2004a; Wong *et al.*, 2004).

Dialysate analysis

Ethanol assay

Ethanol analysis was performed in duplicate using a modified version of the enzymatic analysis described by Hickner *et al.* (Hickner *et al.*, 1992). The assay uses alcohol dehydrogenase to break down ethanol (EtOH) per the following reaction:



Briefly, 1 ml Glycine-Hydrazine Buffer with NAD, 50 µl Alcohol Dehydrogenase Solution, and 5 µl dialysate were combined in test tubes, covered, and incubated in a 25°C bath for 75 minutes. Immediately following incubation, the contents of each test tube was transferred to a cuvette and read by a digital filter fluorometer (Barnstead-Turner Quantech) against a standard curve. Ethanol concentrations in the dialysate and perfusate were used to determine the ethanol outflow-inflow ratio, which is an inverse-surrogate to local blood flow, as discussed in the above section entitled *Muscle blood flow*.

Glucose analysis of dialysate

Dialysate glucose concentrations were determined, in duplicate, using a clinical glucose analyzer (YSI 2300 Stat Plus Glucose & Lactate Analyzer, YSI Life Sciences). Subsequently, dialysate glucose concentrations, coupled with probe recovery values (see

Assessment of probe recovery below) were used to determine interstitial glucose concentrations.

Assessment of probe recovery

[6-³H] glucose was included in the microdialysis perfusate to serve as an internal reference for probe glucose recovery (Scheller & Kolb, 1991; MacLean *et al.*, 2001). 10 µl samples of perfusate or dialysate were pipetted into a 5 ml scintillation vial, then combined with 3 ml of scintillation fluid (Ultima Gold, Shelton, CT). Scintillation vials were then vortexed and read by a scintillation counter (Beckman LS 6800). Probe recovery, per the internal reference method, was calculated as:

$$\text{Recovery (\%)} = (\text{Perfusate activity (dpm)} - \text{Dialysate activity (dpm)}) / \text{Perfusate activity (dpm)}$$

Assessment of interstitial glucose concentrations

Interstitial glucose concentrations were determined using the glucose concentrations from the perfusate and dialysate, as well as the probe recovery, using the internal reference method. The internal reference method has been validated against the no-net flux method (MacLean *et al.*, 1999) and uses the following calculation to derive interstitial glucose concentrations:

$$[\text{Glucose}]_{\text{interstitial}} = (([\text{glucose}]_{\text{dialysate}} - [\text{glucose}]_{\text{perfusate}}) / \text{recovery} + [\text{glucose}]_{\text{perfusate}})$$

Blood hormone analysis

Analysis of insulin, glucagon and C-peptide was handled by the core lab at the Oregon Clinical and Translational Research Institute (OCTRI) by standard methods.

Estimation of glucose uptake and insulin sensitivity

Several indices to estimate insulin sensitivity and/or glucose uptake have been developed for use with the oral glucose tolerance test (OGTT). This is a key development, so the OGTT, as both a research and clinical tool, can be utilized to its full capacity. Therefore, the most commonly used and well-validated indices are reviewed below, with special emphasis on the MCR est and ISI est indices, developed by Stumvoll and colleagues (2000).

The Matsuda insulin sensitivity index uses OGTT data to produce a composite estimate of muscle and hepatic insulin sensitivity. The two components of this index consider basal insulin sensitivity and insulin sensitivity following the glucose load. This index is relatively easy to use and correlates well with the euglycemic hyperinsulinemic clamp (Matsuda & DeFronzo, 1999). The formula used is:

$$ISI_{(Matsuda)} = 10,000/\sqrt{[(G_{fasting} \times I_{fasting}) \times (G_{OGTT\ mean} \times I_{OGTT\ mean})]}$$

The Gutt index ($ISI_{0,120}$) is a relatively simple model that uses basal plasma glucose and insulin levels, as well as those at 120 minutes following OGTT to estimate glucose uptake rate and insulin sensitivity. It correlates well with the euglycemic hyperinsulinemic clamp (Gutt *et al.*, 2000) and, when compared to other common indices, was the best at predicting onset of type 2 diabetes (Hanley *et al.*, 2003). The $ISI(0,120)$ index is defined as:

$$ISI_{0,120} = MCR/\log MSI = m/MPG/\log MSI$$

where m (mg/min) is the glucose uptake rate in the peripheral tissues, based on the formula:

$$m = [75,000 \text{ mg} + (\text{Glucose } 0 - \text{Glucose } 120) \times 0.19 \times \text{BW}] / 120 \text{ min}$$

Mean plasma glucose (MPG) is the mean of the 0 and 120 min glucose values and mean serum insulin (MSI) is the mean of the 0 and 120 min insulin values. Finally, the metabolic clearance rate (**MCR**) = m/MPG , as this corrects for the potential influence of different blood glucose levels on the rate of glucose uptake.

The MCR est and ISI est indices, developed by Stumvoll and coworkers use results from the OGTT to estimate metabolic clearance rate of glucose and insulin sensitivity. Using subject BMI, plasma glucose, and plasma insulin data, it has the highest correlation ($r = 0.80$) with the euglycemic hyperinsulinemic clamp (Stumvoll *et al.*, 2000) of all the established indices. It uses the following equations:

$$\text{MCR est (OGTT)} = 18.8 - 0.271 \text{ BMI} - 0.0052 \times \text{I } 120 - 0.27 \times \text{G } 90$$

$$\text{ISI est (OGTT)} = 0.226 - 0.0032 \times \text{BMI} - 0.0000645 \times \text{I } 120 - 0.0037 \times \text{G } 90$$

where BMI = body mass index, I 120 = plasma insulin at 120 min OGTT, and G 90 = plasma glucose at 90 min OGTT.

CHAPTER III

LOCAL H₁- AND H₂-RECEPTOR BLOCKADE, ADMINISTERED VIA MUSCLE MICRODIALYSIS, ABOLISHES THE INCREASE IN LOCAL BLOOD FLOW EVOKED BY COMPOUND 48-80

Introduction

An acute bout of moderate-intensity dynamic exercise results in a sustained rise in skeletal muscle blood flow from that of pre-exercise levels (Halliwill, 2001; Pricher *et al.*, 2004). Recent evidence suggests this postexercise skeletal muscle hyperemia is mediated by two (H₁ and H₂) histamine receptor subtypes (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). To date, this has only been investigated using oral administration of H₁- and H₂-receptor antagonists, which may result in systemic effects. Circumventing this problem necessitates the isolated blockade of local H₁ and H₂-receptors in the skeletal muscle.

Along these lines, skeletal muscle microdialysis is a unique tool that permits researchers to deliver drugs of interest to the interstitial space, while simultaneously monitoring concentrations of various substances within the interstitium (Hickner, 2000). In order to determine if postexercise skeletal muscle hyperemia is mediated by local H₁ and H₂-receptors, it is first necessary to confirm adequate receptor blockade. Thus, the current investigation was designed to determine the dosages of the H₁-receptor antagonist

pyrilamine and the H₂-receptor antagonist cimetidine required to block local H₁- and H₂-receptors located in the vastus lateralis muscle in humans. We tested the hypothesis that local H₁- and H₂-receptor blockade, administered via muscle microdialysis, would abolish the increase in local blood flow evoked by compound 48-80.

Methods

This study was approved by the Institutional Review Board of the University of Oregon and was conducted in accordance with the guidelines set forth by the Office for Protection of Human Subjects of the University of Oregon. Each subject gave his or her written consent prior to participation in the study.

Subjects

10 healthy, non-smoking, normotensive subjects (8 men; 2 women), between the ages of 20 and 26, participated in this study. Subjects reported to the laboratory at least three hours post-prandial, having refrained from alcohol consumption and exercise for 24 hours and consumption of caffeine for 12 hours. Subjects were taking no medications with the exception of oral contraceptives. In addition, female subjects were studied during the early follicular phase of their menstrual cycle or during the placebo phase of the oral contraceptive cycle to minimize the potential effects of reproductive hormones on cardiovascular regulation.

Microdialysis probes

All microdialysis probes used in this study were constructed in our laboratory. The membranes used in the probes were obtained from artificial dialysis kidney (GFS 18) with a molecular mass cutoff of 3 KD and an inner diameter of 0.20 mm. Each end of the

membranes was advanced approximately 1 cm into hollow polyimide tubing with an inner diameter of 0.36 mm and glued in place. The length of exposed membrane in each fiber used was precisely 4 cm. The proximal end of the polyimide tubing was then advanced approximately 2 cm into polyethylene tubing and glued in place. Subsequently a luer tip adapter stub was attached to the proximal end of the polyethylene tubing in order to accommodate a perfusate-filled syringe during the microdialysis procedure. Microdialysis probes were then gas sterilized (Anprolene AN74i) for 12 hours prior to use.

Microdialysis probe insertion

Four microdialysis probes were inserted approximately 2-3 cm apart in the vastus lateralis muscle of each subject's right leg. Prior to insertion, a sterile field was established and the entire lateral thigh region was cleansed with an alcohol-based antiseptic (ChloroPrep). The skin and subcutaneous tissue at both the probe insertion and exit sites were then anesthetized via local administration of approximately 1 ml 2% lidocaine (20 mg/ml) plus epinephrine 1:100,000 (10 µg/ml) and sodium bicarbonate per site. The epinephrine produced a local cutaneous vasoconstriction to minimize bleeding and the sodium bicarbonate stabilized the pH to minimize subject discomfort during lidocaine administration. Care was taken not to introduce the lidocaine solution into the muscle itself, thereby avoiding any alterations in the intramuscular environment. A flexible 20-gauge needle was inserted in the vastus lateralis, in a direction parallel to muscle fiber orientation. The insertion and exit sites were approximately 9 cm apart. Immediately after needle insertion, a sterilized microdialysis probe was threaded through

the needle lumen. The needle was then removed, leaving only the microdialysis probe in place. Placement of all four probes was accomplished within 25 minutes of beginning the procedure.

Experimental protocol

Following placement, each microdialysis probe was attached to a perfusion pump (CMA 102 Microdialysis pump, CMA, North Chelmsford, MA) and perfused at a rate of 5 μ L/min with a modified Ringer's solution containing 3.0 mM glucose and 0.5 mM lactate. Concentrations of glucose and lactate in the perfusion solution were verified using a clinical glucose analyzer (YSI 2300 Stat Plus Glucose & Lactate Analyzer, YSI Life Sciences). By design, the perfusion solution had a composition similar to that of the interstitial fluid, thereby minimizing osmotic differences across the probe membrane so as to avoid net fluid movement between the interstitial space and the perfusion solution within the probe (Lonnroth *et al.*, 1987). In addition, all perfusate included 5 mM ethanol. Ethanol readily diffuses through the semi-permeable membrane and is not metabolized by the muscle tissue (Lieber, 1977). Therefore as local capillary blood flow increases, more ethanol is removed from the interstitial space, the ethanol concentration gradient in the vicinity of the probe increases, and less ethanol is collected as outflowing dialysate (Hickner *et al.*, 1991, 1992). Thus the ethanol outflow-inflow ratio was used as an inverse surrogate to determine relative local blood flow.

In order to verify the stability of the ethanol outflow-inflow ratios over time, we collected dialysate from one control probe through 120 minutes after probe insertion in five subjects. For the remaining probes and subjects who participated in this protocol,

probes were randomly assigned to be perfused with the control solution (no drug), solution plus 1 mM H₁-receptor antagonist pyrilamine maleate, solution plus 3 mM H₂-receptor antagonist cimetidine, or solution plus pyrilamine and cimetidine combined. These doses were titrated from those used previously to achieve H₁- and H₂ -receptor blockade in the skin (Wilkins *et al.*, 2004a; Wong *et al.*, 2004). The distal tips of the microdialysis probes were placed in microcentrifuge tubes for dialysate collection. Non-porous tape was placed over the top of the microcentrifuge tubes to prevent evaporation of ethanol.

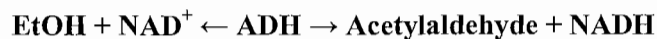
For all subjects, the first collection of dialysate began 10 minutes following the completion of insertion of all four probes, allowing time to verify probe function, set up collection of dialysate, and flush out probe dead space. The microcentrifuge tubes were replaced every 20 minutes, through 70 minutes following probe insertion, with the 70 minute collection serving as baseline for each probe. This equilibration period allowed recovery from any cellular trauma induced by probe insertion and allowed for equilibration of interstitial and dialysate fluxes and concentrations. Thus, baseline collection periods were 10-30, 30-50, and 50-70 minutes following completion of probe insertion.

As noted previously, we collected dialysate from one control probe through 120 minutes post probe insertion in five subjects. For the remaining subjects and probe sites, following the baseline collection, the perfusion solution for each probe was changed 70 minutes after completion of probe insertion. The new perfusion solution was identical to that used during the equilibration period, except for the addition of 10mM compound 48-

80 (Sigma-Aldrich, St. Louis, MO). Compound 48-80 degranulates mast cells, resulting in their release of histamine (Diamant & Dahlquist, 1970), thereby evoking H₁ and H₂ – receptor mediated vasodilation. The change in perfusion solution was followed by three more 15 minute collection periods (75-90, 90-105, and 105-120 min after completion of probe insertion).

Analysis

All microcentrifuge tubes were weighed before and after dialysate collection to verify fluid balance across the microdialysis probe membrane. Microcentrifuge tubes were sealed, placed in a refrigerator, and dialysate was analyzed within 48 hours of collection. Ethanol analysis was performed in duplicate using a modified version of the enzymatic analysis described by Hickner *et al.* (Hickner *et al.*, 1992). The assay uses alcohol dehydrogenase (ADH) to break down ethanol (EtOH) per the following reaction:



Briefly, 1 ml Glycine-Hydrazine Buffer with NAD, 50 µl Alcohol Dehydrogenase Solution, and 5 µl dialysate were combined in test tubes, covered, and incubated in a 25 °C bath for 75 minutes. Immediately following incubation, the contents of each test tube were transferred to a cuvette and read by a digital filter fluorometer (Barnstead-Turner Quantech) against a standard curve. Ethanol concentrations in the dialysate and perfusate were then used to determine the ethanol outflow-inflow ratio, thereby enabling qualification of local blood flow.

Statistics

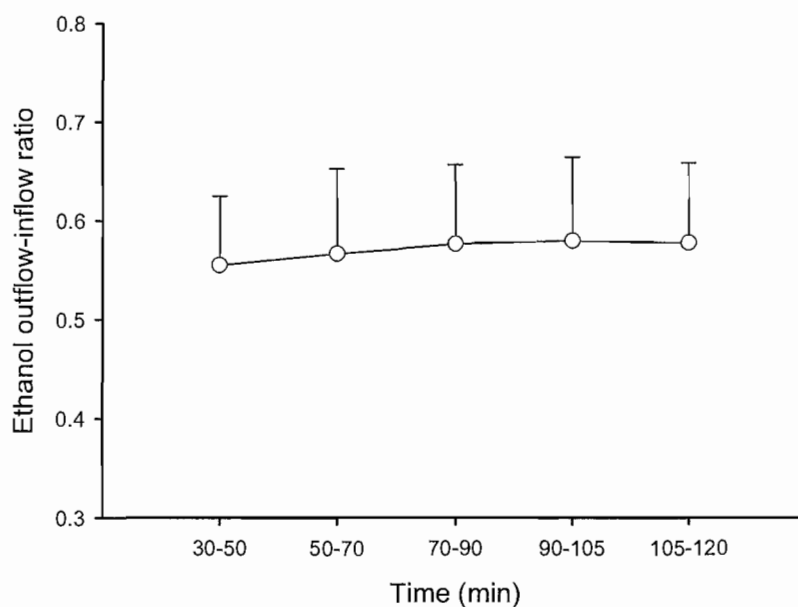
The results were analyzed with a Two-Way Repeated Measures ANOVA (effect of drug and time) with SigmaStat Version 2.0 (SPSS Inc.). Differences were considered significant when $P < 0.05$. All values are reported as means \pm SE unless otherwise noted.

Results

Muscle microdialysis control sites

Figure 1 illustrates the ethanol outflow-inflow ratios in the muscle microdialysis control sites starting from 30 minutes through 120 minutes after completion of probe insertion. There were no differences between the ethanol outflow-inflow ratio during the 30-50 minute collection period (0.555 ± 0.070) and ethanol outflow-inflow ratios at subsequent dialysate collection periods (all $P \geq 0.111$).

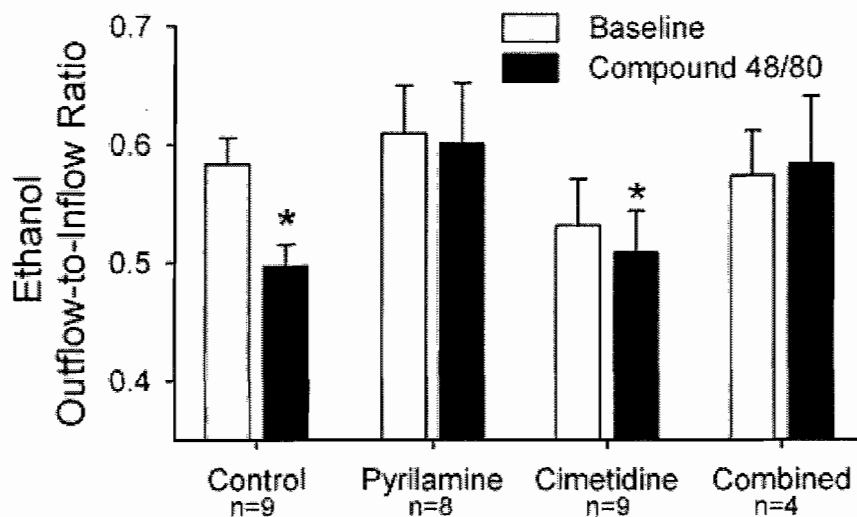
Figure 1. Ethanol outflow-inflow ratios over time from muscle microdialysis control probes inserted in the vastus lateralis. $n = 5$ subjects



Verification of local H₁- and H₂-receptor blockade

Figure 2 illustrates the effect of 10 mM Compound 48-80, delivered via skeletal muscle microdialysis, on ethanol outflow-inflow ratio in control sites, as well as sites with 1 mM pyrilamine, 3 mM cimetidine, or pyrilamine and cimetidine combined. There were no statistically significant differences between the 70 minute baseline ethanol outflow-inflow ratios at any of the sites (all $P \geq 0.051$). In the control site, ethanol outflow-inflow ratio fell from 0.583 ± 0.022 at baseline to 0.497 ± 0.018 after the addition of Compound 48-80 to the perfusate ($P < 0.01$). In the pyrilamine sites, ethanol outflow-inflow ratio went from 0.609 ± 0.040 at baseline to 0.601 ± 0.051 with the addition of Compound 48-80 ($P = 0.636$). In the cimetidine sites, ethanol outflow-inflow ratio went from 0.531 ± 0.040 at baseline to 0.508 ± 0.036 with the addition of Compound 48-80 ($P = 0.049$). In the combined H₁- and H₂-receptor antagonist sites, ethanol outflow-inflow ratio went from 0.574 ± 0.038 at baseline to 0.584 ± 0.057 with the addition of Compound 48-80 ($P = 0.552$).

Figure 2. Ethanol outflow-inflow ratios in response to Compound 48-80 in control and local histamine receptor blockade sites. $n = 4-9$ subjects, as indicated; $* P < 0.05$ vs. baseline.



Discussion

The goal of this study was to test our ability to block local H₁- and H₂-receptors, located in the vastus lateralis muscle in humans, via muscle microdialysis administration of the H₁-receptor antagonist pyrilamine and the H₂-receptor antagonist cimetidine. Furthermore, we wanted to explore the stability of the ethanol outflow-inflow ratios over time following muscle microdialysis, in the absence of drugs. Our findings suggest a combination of local H₁- and H₂-receptor blockade, administered via muscle microdialysis, blocked the increase in local blood flow evoked by compound 48-80. In addition, we found that under resting conditions, the ethanol outflow-inflow ratio remained relatively stable from 30 to 120 minutes following insertion of muscle microdialysis probes.

This is the first investigation to demonstrate local blockade of H₁- and H₂-receptors in human skeletal muscle. As such, this finding presents a new approach in the investigation of potential H₁- and H₂-receptor-mediated blood flow and metabolic regulation, as previous investigations on this front have utilized oral administration of H₁- and H₂-receptor antagonists (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006) which result in systemic blockade of these receptors. The ability to selectively block H₁- and H₂-receptors located in specific regions enables researchers to better control for possible confounds associated with orally administered H₁- and H₂-receptor antagonists. Moreover, it permits more integrative experimentation, as the interstitial space is at the junction between cardiovascular and metabolic regulation in humans (Hickner *et al.*, 1991; Hickner *et al.*, 1997; MacLean *et al.*, 1999; Newman *et al.*, 2002).

It is worth noting that the microdialysis ethanol outflow-inflow ratio is not considered a quantitative measure, per se, of local blood flow (Radegran, 1999). Factors independent of blood flow, such as capillary permeability and lymph flow, may affect clearance of ethanol to some degree (Hickner *et al.*, 1995). In addition, initial trauma caused by probe insertion may affect ethanol clearance. However, ethanol outflow-inflow ratios have been shown to be sensitive to perturbations designed to increase and decrease local blood flow (Hickner *et al.*, 1991, 1992; Hickner *et al.*, 1995). Moreover, ethanol outflow-inflow ratios have been shown to be stable within 20 minutes of probe insertion (Hickner *et al.*, 1994), which is consistent with our findings of stable ethanol outflow-inflow ratios in the control sites, starting with the collection period from 30-50

minutes after completion of probe insertion. Furthermore, this time frame is well before the start of baseline dialysate collection in the compound 48-80 portion of the current study. Thus, the ethanol outflow-inflow ratio appears to be a sound indicator of relative changes in local blood flow, and has been found to be especially sensitive in the absence of skeletal muscle contraction and during lower blood flow conditions (Hickner *et al.*, 1995).

In conclusion, our results demonstrate that we were able to block the increase in local blood flow evoked by compound 48-80 with the combination of the H₁-receptor antagonist pyrilamine and the H₂-receptor antagonist cimetidine, administered via skeletal muscle microdialysis. Furthermore, we verified stability of the ethanol outflow-inflow ratio under resting conditions, starting at the 30 minutes following the completion of muscle microdialysis probe insertion. These findings set the stage for researchers to explore how local H₁- and H₂-receptors impact the intramuscular environment under a variety of conditions.

In the study discussed in Chapter III, we verified our ability to block local H₁- and H₂-receptors in the vastus lateralis, using a combination of the H₁-receptor antagonist pyrilamine and the H₂-receptor antagonist cimetidine, administered via skeletal muscle microdialysis. In addition, we confirmed the stability of the ethanol outflow-inflow ratio under resting conditions within 30 minutes following the completion of muscle microdialysis probe insertion. These findings allowed us to proceed to the investigation discussed in Chapter IV, during which we employed skeletal muscle microdialysis to

determine the effect of local H₁- and H₂-receptor blockade on postexercise interstitial glucose concentrations.

CHAPTER IV

LOCAL H₁- AND H₂-RECEPTOR BLOCKADE BLUNTS POSTEXERCISE INTERSTITIAL GLUCOSE CONCENTRATIONS IN THE VASTUS LATERALIS

Introduction

Following 60 minutes of moderate intensity dynamic exercise, blood flow to previously active skeletal muscle remains elevated above pre-exercise levels for approximately 100 minutes (Halliwill, 2001; Pricher *et al.*, 2004). This postexercise skeletal muscle hyperemia is found in both men and women (Senitko *et al.*, 2002) and in both sedentary and endurance exercise trained individuals (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). Recent studies employing oral administration of H₁- and/or H₂-receptor antagonists suggest this hyperemia is histaminergic in nature (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). While strides have been made in understanding the mediators of postexercise hyperemia, it is unclear what purpose this increased blood flow might serve. Along these lines, Williams *et al.* investigated the relationship between leg blood flow and oxygen uptake following 60 minutes of moderate-intensity exercise. They found the time course of leg blood flow recovery did not match that of oxygen uptake, suggesting skeletal muscle hyperemia does not subservise oxygen delivery to the previously exercised

muscle (Williams *et al.*, 2005). The function, therefore, of this histamine-receptor-mediated hyperemia found after an acute bout of dynamic exercise has yet to be elucidated.

It is well established that skeletal muscle glucose uptake is enhanced, in an insulin-independent manner, during the first 90 minutes postexercise (Wasserman & Halseth, 1998; Richter *et al.*, 2001a; Henriksen, 2002). The glucose taken up following exercise contributes to glycogen repletion in humans (Richter *et al.*, 1989; Casey *et al.*, 2000) and the rate of glycogen synthesis is typically highest the first 30 minutes following exercise (Price *et al.*, 1994). Furthermore, as illustrated by studies involving electrical stimulation of the rat hindlimb (Richter *et al.*, 1984) and single-legged exercise in humans (Bergstrom & Hultman, 1966; Richter *et al.*, 1989), glucose uptake and glycogen repletion occurs preferentially in the previously exercised skeletal muscle.

This begs the question: Does postexercise skeletal muscle hyperemia serve to aid glucose delivery to previously active skeletal muscles in humans? Numerous studies have suggested blood flow as a rate-limiting factor in glucose regulation. Early animal research found glucose uptake was enhanced with increases in isolated rat hindlimb perfusion, even when blood glucose and insulin concentrations were maintained (Grubb, 1977; Schultz, 1977). Baron *et al.* discovered both leg blood flow and leg glucose uptake were substantially greater in combined insulin and methacholine hydrochloride trials versus the insulin only trials, indicating skeletal muscle perfusion can independently impact insulin-mediated glucose uptake in humans (Baron *et al.*, 1994). Subsequently, Durham and coworkers measured substantial increases in leg glucose uptake in response

to exogenous nitric oxide-mediated increases in leg blood flow (Durham *et al.*, 2003). It is worth noting that this enhanced glucose uptake was found in the absence of insulin stimulation, indicating either the increased blood flow itself or some other effect of nitric oxide mediated the increased glucose uptake.

Thus, it appears that elevations in blood flow can contribute to increased skeletal muscle glucose uptake, in both animals and humans. Whether this relationship holds true in the postexercise condition is not known. Moreover, while previous investigations have employed orally administered H₁- and H₂-receptor blockade, the effect of blockade at the level of the skeletal muscle has not been examined. Thus, while the mechanisms surrounding both postexercise skeletal muscle hyperemia and postexercise glucose regulation have been explored, the relationship between these factors remains poorly understood. Therefore, the current investigation was designed to determine the effect of local H₁- and H₂-receptor blockade, administered via skeletal muscle microdialysis, on postexercise interstitial glucose concentrations. Skeletal muscle microdialysis is uniquely suited to probe this relationship, as it allows delivery of drugs to the interstitial space, and simultaneously monitors concentrations of various substances within the interstitium (Hickner, 2000). We hypothesized that postexercise interstitial glucose concentrations, as determined by muscle microdialysis, would be higher in the control versus the combined H₁- and H₂-receptor antagonist sites.

Methods

This study was approved by the Institutional Review Board of the University of Oregon and was conducted in accordance with the guidelines set forth by the Office for

Protection of Human Subjects of the University of Oregon. Each subject gave his or her written consent prior to participation in the study.

Subjects

14 healthy, non-smoking, normotensive subjects (8 men; 6 women), between the ages of 20 and 27, participated in this study. Seven subjects (4 men; 3 women) participated in an exercise protocol and seven subjects (4 men; 3 women) participated in a sham (no exercise) protocol. For all study visits, subjects reported to the laboratory at least three hours post-prandial, having refrained from alcohol consumption and exercise for 24 hours and consumption of caffeine for 12 hours. Subjects were taking no medications with the exception of oral contraceptives. In addition, female subjects were studied during the early follicular phase of their menstrual cycle or during the placebo phase of the oral contraceptive cycle, to minimize the potential effects of reproductive hormones on cardiovascular and metabolic regulation.

Screening visit

Subjects participating in the exercise protocol initially visited the laboratory to perform a peak aerobic power test on a cycle ergometer, in addition to self-reporting activity levels on two questionnaires. Subjects performed an incremental cycle exercise test (Lode Excaliber, Groningen, The Netherlands) comprised of 1-minute workload increments to determine peak oxygen uptake ($\text{VO}_{2 \text{ peak}}$). Specifically, after a 2-minute warm-up period of easy cycling (20–30 watts), workload was increased by 20, 25, or 30 watts every minute. Selection of the workload increment was based on self-reported subject activity levels, with the goal of producing exhaustion within 8–12 minutes.

Subjects were instructed to maintain 60-80 revolutions per minute to maximize muscular efficiency. Whole body oxygen uptake was measured via a mixing chamber (Parvomedics, Sandy, UT) integrated with a mass spectrometry system (Marquette MGA 1100, MA Tech Services, St. Louis, MO). Peak aerobic power was determined as either: 1) when subjects were unable to maintain 60 revolutions per minute, 2) had obtained a respiratory exchange ratio of greater than 1.15, or 3) had reached subjective exhaustion [rating of perceived exertion on the Borg (Borg, 1970) scale of 19–20] within the 8- to 12-minute period.

After resting for approximately 10 minutes, subjects returned to the cycle ergometer to determine the workload corresponding to a steady-state oxygen consumption of 60% of $\text{VO}_{2\text{ peak}}$. The workload was titrated, as needed, to reach a steady state oxygen consumption of 60% of $\text{VO}_{2\text{ peak}}$, which was usually attained within five minutes. This workload was used on the exercise study day for the 60-minute exercise bout, as this intensity and duration of exercise has been shown to evoke sustained (~100 minutes) postexercise skeletal muscle hyperemia (Pricher *et al.*, 2004).

Microdialysis probes

All microdialysis probes used in this study were constructed in our laboratory. The membranes used in the probes were obtained from artificial dialysis kidney (GFS 18) with a molecular mass cutoff of 3 KD and an inner diameter of 0.20 mm. Each end of the membranes was advanced approximately 1 cm into hollow polyimide tubing with an inner diameter of 0.36 mm and glued in place. The length of exposed membrane in each fiber used was precisely 4 cm. The proximal end of the polyimide tubing was then

advanced approximately 2 cm into polyethylene tubing and glued in place. Subsequently a luer tip adapter stub was attached to the proximal end of the polyethylene tubing in order to accommodate a perfusate-filled syringe during the microdialysis procedure. Microdialysis probes were then gas sterilized (Anprolene AN74i) for 12 hours prior to use.

Experimental protocol

On the study day, subjects were laid in the supine position for instrumentation. Heart rate was monitored throughout the protocol via 5-lead electrocardiogram (Quinton Instruments, Bothell, WA). Arterial pressure was measured with an automated oscillometric device (Dinamap Pro100 vital signs monitor, Critikon Inc, Tampa, FL) during pre-exercise resting conditions and 55, 75, 95, 115, and 135 minutes postexercise.

Heated hand vein

In order to obtain “arterialized” blood samples, a heated hand vein method was used (Morris *et al.*, 1997). An intravenous catheter was inserted retrogradely in the dorsal vein of the left hand, which was then placed in a custom-made heating chamber (“hot-box”), which was flushed with air at 55° C. Actual skin temperature was raised to approximately 42° C, which is below the temperature that evokes sensations of pain. Heating the hand in this way induces the opening of arterial-venous anastomoses in the hand circulation, thereby allowing investigators to sample “arterialized” venous blood with higher and less variable oxygen saturations and higher glucose concentrations compared to non-arterialized blood samples (Morris *et al.*, 1997). Arterialization of venous blood samples was confirmed by immediate measurement of oxygen saturation in

duplicate by a co-oximeter (Radiometer Copenhagen). Glucose concentrations of arterialized venous blood were measured in duplicate with a clinical glucose analyzer (YSI 2300 Stat Plus Glucose and Lactate Analyzer, YSI Life Sciences) pre-exercise, 30 minutes into the exercise bout, and 55, 75, 95, 115, and 135 minutes postexercise.

Preparation for microdialysis

In preparation for the postexercise insertion of muscle microdialysis fibers, a sterile field was established and the entire lateral thigh region was cleansed with an alcohol-based antiseptic (ChloroPrep). The skin and subcutaneous tissue at both the probe insertion and exit sites were then anesthetized via local administration of approximately 1 ml 2% lidocaine (20 mg/ml) plus epinephrine 1:100,000 (10 μ g/ml) and sodium bicarbonate per site. The epinephrine produced a local cutaneous vasoconstriction to minimize bleeding and the sodium bicarbonate stabilized the pH to minimize subject discomfort during lidocaine administration. Care was taken to not introduce the lidocaine solution into the muscle itself, thereby avoiding any alterations in the intramuscular environment.

Exercise

Subjects exercised upright on a stationary cycle at 60% of $VO_{2\text{ peak}}$ for 60 min. Exercise of this intensity and duration produce sustained (~100 min) skeletal muscle hyperemia (Pricher *et al.*, 2004). During the 60 min of exercise, heart rate was measured via 5-lead electrocardiogram (Quinton Instruments, Bothell, WA) and arterial pressure was determined via manual auscultometry. Subjects drank 10 ml per kg body weight of

water to offset volume loss during exercise. During the sham study, the 60 minutes of cycling was replaced with 60 minutes of quiet rest.

Muscle microdialysis

Immediately following exercise, subjects were laid supine for insertion of muscle microdialysis probes. Four microdialysis probes were inserted approximately 2-3 cm apart in the vastus lateralis muscle of each subject's right leg. Prior to insertion, a sterile field was established and the entire lateral thigh region was cleansed with an alcohol-based antiseptic (ChloraPrep). A flexible 20-gauge needle was inserted in the vastus lateralis, in a direction parallel to muscle fiber orientation. The insertion and exit sites were approximately 9 cm apart. Immediately after needle insertion, a sterilized microdialysis probe was threaded through the needle lumen. The needle was then removed, leaving only the microdialysis probe in place. Placement of all microdialysis probes was complete within 20 minutes from the end of the exercise bout.

Following placement, each microdialysis probe was attached to a perfusion pump (CMA 102 Microdialysis pump, CMA, North Chelmsford, MA) and perfused at a rate of 5 μ L/min with a modified Ringer's solution containing 3.0 mM glucose, 0.5 mM lactate, 5 mM ethanol, and [6-³H] glucose (2000 dpm/10 μ l). Concentrations of glucose and lactate in the perfusion solution were verified using a clinical glucose analyzer (YSI 2300 Stat Plus Glucose & Lactate Analyzer, YSI Life Sciences). Due to its similar composition of interstitial fluid, this perfusate served as the control solution, thus minimizing osmotic differences across the probe membrane and potential draining of the interstitial space (Lonroth *et al.*, 1987). [6-³H] glucose (2000 dpm/10 μ l) was included in the perfusate to

serve as an internal reference marker for recovery of glucose (Scheller & Kolb, 1991; MacLean *et al.*, 2001). Ethanol readily diffuses through the semi-permeable membrane and is not metabolized by the muscle tissue (Lieber, 1977). Therefore as local capillary blood flow increases, more ethanol is removed from the interstitial space, the ethanol concentration gradient in the vicinity of the probe increases, and less ethanol is collected as out-flowing dialysate (Hickner *et al.*, 1991, 1992). Thus the ethanol outflow-inflow ratio was used as an inverse surrogate to determine relative local blood flow.

Two of the probes were perfused with the control solution plus combined 1 mM H₁-receptor antagonist pyrilamine maleate, and 3 mM H₂-receptor antagonist cimetidine, to block local H₁- and H₂-receptors. These doses were previously verified to produce H₁- and H₂-receptor blockade in skeletal muscle via muscle microdialysis (see Chapter III). Following the placement of the microdialysis probes, 15 minutes was allowed to verify probe function, set up collection of dialysate, and flush out probe dead space, after which time the distal tips of the microdialysis probes were placed in microcentrifuge tubes for dialysate collection. Non-porous tape was placed over the top of the microcentrifuge tubes to prevent evaporation of ethanol. The microcentrifuge tubes were replaced every 20 minutes, through 115 minutes following probe insertion, thus dialysate was collected from 35-55, 55-75, 75-95, 95-115, and 115-135 minutes postexercise. All microcentrifuge tubes were weighed before and after dialysate collection to verify fluid balance across the microdialysis probe membrane. Microcentrifuge tubes were sealed, placed in a refrigerator, and dialysate was analyzed within 48 hours of collection.

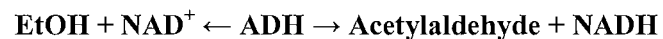
Oral glucose load

Forty minutes after microdialysis probe insertion, to induce an increase in blood and interstitial glucose concentrations, each subject consumed 0.1 oz per kg body weight (up to 10 oz maximum) Oral Glucose Tolerance Beverage (Trutol 100, NERL Diagnostics, East Providence, RI), which contained 10 g of glucose per oz. Therefore, subjects received an oral glucose dose of 1 g per kg body weight up to 100 g maximum.

Analyses

Ethanol assay

Ethanol analysis was performed in duplicate using a modified version of the enzymatic analysis described by Hickner *et al.* (Hickner *et al.*, 1992). The assay uses alcohol dehydrogenase to break down ethanol (EtOH) per the following reaction:



Briefly, 1 ml Glycine-Hydrazine Buffer with NAD, 50 μl Alcohol Dehydrogenase Solution, and 5 μl dialysate were combined in test tubes, covered, and incubated in a 25 $^{\circ}\text{C}$ bath for 75 minutes. Immediately following incubation, the contents of each test tube were transferred to a cuvette and read by a digital filter fluorometer (Barnstead-Turner Quantech) against a standard curve. Ethanol concentrations in the dialysate and perfusate were then used to determine the ethanol outflow-inflow ratio, thereby enabling qualification of local blood flow.

Probe recovery

[6-³H] glucose was included in the microdialysis perfusate to serve as an internal reference for probe glucose recovery (Scheller & Kolb, 1991; MacLean *et al.*, 2001). 10 µl samples of perfusate or dialysate were pipetted into a 5 ml scintillation vial, then combined with 3 ml of scintillation fluid (Ultima Gold, Shelton, CT). Scintillation vials were then vortexed and read by a scintillation counter (Beckman LS 6800). Probe recovery, per the internal reference method, was calculated as:

$$\text{Recovery (\%)} = (\text{Perfusate activity (cpm)} - \text{Dialysate activity (cpm)}) / \text{Perfusate activity (cpm)}$$

Assessment of interstitial glucose concentrations

Interstitial glucose concentrations were determined using the glucose concentrations from the perfusate and dialysate, as well as the probe recovery, using the internal reference method. The internal reference method uses the following calculation to derive interstitial glucose concentrations:

$$[\text{Glucose}]_{\text{interstitial}} = (([\text{glucose}]_{\text{dialysate}} - [\text{glucose}]_{\text{perfusate}}) / \text{recovery} + [\text{glucose}]_{\text{perfusate}})$$

Statistics

The results were analyzed with a Two-Way Repeated Measures ANOVA (effects of drug and time) with SAS Proc Mixed (SAS v9.1, SAS Institute, Cary, NC).

Differences were considered significant when $P \leq 0.05$. All values are reported as means \pm SE unless otherwise noted.

Results

Subject characteristics

Subject characteristics are presented in Table 1. $\text{VO}_{2\text{peak}}$ values were within the normal range for young, healthy subjects of sedentary to endurance trained status.

Table 1. Subject characteristics-Chapter IV.

	Exercise protocol	Sham protocol
n	7	7
Age (years)	23.1 ± 2.1	22.1 ± 2.3
Height (cm)	176.3 ± 6.9	173.8 ± 13.6
Weight (kg)	73.1 ± 9.7	76.3 ± 19.1
Body mass index (kg m^{-2})	23.5 ± 2.5	24.8 ± 3.1
$\text{VO}_{2\text{peak}}$ ($\text{ml kg}^{-1} \text{min}^{-1}$)	54.3 ± 14.6	
Workload at 60% of $\text{VO}_{2\text{peak}}$ (watts)	161.5 ± 31.8	
Baecke sport index (arbitrary units)	12.7 ± 3.2	
Index of physical activity ($\text{MET}\cdot\text{hr}\cdot\text{wk}$)	163.5 ± 57.4	

Values are means ± SD; $\text{VO}_{2\text{peak}}$, peak oxygen consumption; MET, metabolic equivalents

Exercise

During the exercise protocol, the goal was to have each subject exercise for 60 min at 60% $\text{VO}_{2\text{peak}}$. The percentage of heart rate reserve (heart rate reserve is defined as maximal heart rate achieved during VO_2 peak testing minus the resting supine heart rate) attained during exercise ($73.7 \pm 1.6\%$) was consistent with the target workload. Heart rate increased from 53.5 ± 3.9 beats min^{-1} during supine rest to 152.4 ± 1.6 beats min^{-1} during exercise (measured 30 min into exercise bout; $P < 0.05$). Mean arterial pressure increased from 80.3 ± 1.8 mmHg during supine rest to 90.3 ± 1.9 mmHg during exercise ($P < 0.05$). During the sham period, neither heart rate ($P = 0.134$) nor arterial pressure ($P = 0.319$) changed from pre-sham supine rest.

Hemodynamics

Mean arterial pressure reached a nadir 55 min following exercise (78.3 ± 1.9 mmHg; $P < 0.05$ vs preexercise), thereafter returning to values not different from preexercise (all $P \geq 0.15$). Heart rate remained elevated above preexercise levels throughout the postexercise period ($P \leq 0.013$, all time points). During the sham protocol, neither heart rate (all $P \geq 0.134$) nor mean arterial pressure (all $P \geq 0.310$) changed from pre-sham baseline at any time point.

Blood glucose

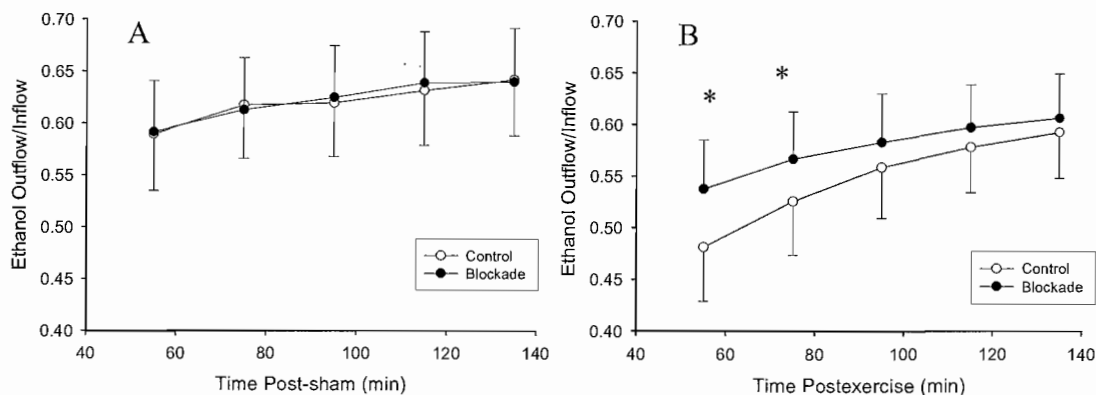
Preexercise blood glucose concentrations, obtained from heated hand veins, were 4.33 ± 0.14 mM in the sham protocol and 4.41 ± 0.10 mM in the exercise protocol ($P = 0.958$ vs. sham). During the sham period, blood glucose concentration was 4.67 ± 0.14 mM, while during exercise blood glucose concentration was 4.35 ± 0.21 mM. In both protocols, the oral glucose load induced a sharp increase in blood glucose levels, starting 95 minutes following sham or exercise (sham 5.80 ± 0.38 mM; exercise 5.67 ± 0.31 mM; both $P < 0.05$ vs. preexercise baseline). In both protocols blood glucose concentrations reached their peak 115 minutes following sham (7.44 ± 0.47 mM) or exercise (7.74 ± 0.46 mM).

Local blood flow

As illustrated in Figure 3, local blood flow in the vastus lateralis in both the sham and exercise protocol was assessed by ethanol outflow-inflow ratio, which is an inverse surrogate to flow in the region surrounding the microdialysis probe (Hickner *et al.*, 1991, 1992). In the sham protocol, there were no differences in ethanol outflow-inflow ratio

between the control and blockade sites at any time point ($P \geq 0.389$). In the postexercise condition, however, ethanol outflow-inflow ratios were higher in the blockade sites at 55 minutes (0.481 ± 0.05 control vs. 0.538 ± 0.04 blockade; $P = 0.030$) and 75 minutes (0.526 ± 0.05 control vs. 0.567 ± 0.05 blockade; $P = 0.043$) following exercise, but were not significantly different at the subsequent time points ($P \geq 0.168$).

Figure 3. Ethanol outflow-inflow ratios following sham (A) and exercise (B). Open circles denote control sites; filled circles denote combined blockade with pyrilamine and cimetidine. * $P < 0.05$ vs. control at same time point; $n = 7$ for each group.

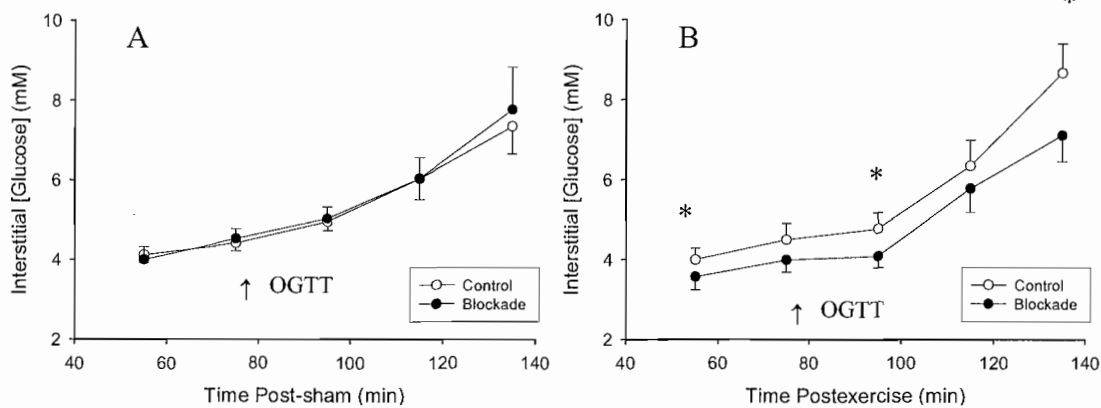


Interstitial glucose concentrations

The interstitial glucose concentrations, in both the sham and exercise protocol, are shown in Figure 4. In the sham protocol, there were no differences in interstitial glucose concentrations between the control and blockade probes at any time point (all $P \geq 0.507$). In contrast, postexercise interstitial glucose concentrations were higher in the control probes at 55 minutes (control 3.99 ± 0.29 vs. blockade 3.56 ± 0.33 mM; $P = 0.043$), 95 minutes (control 4.76 ± 0.40 vs. blockade 4.08 ± 0.29 mM; $P = 0.048$) and 135 minutes (control 8.66 ± 0.73 vs. blockade 7.10 ± 0.66 mM; $P = 0.014$) following exercise. On average, interstitial glucose concentrations were 0.748 mM higher in the control versus

the blockade sites. In both protocols, in the control and blockade sites, the oral glucose load administered at 80 minutes postexercise evoked a marked rise in interstitial glucose concentrations, starting 115 minutes following sham or exercise (both $P \leq 0.05$).

Figure 4. Interstitial glucose concentrations following sham (A) and exercise (B). Open circles denote control sites; filled circles denote combined blockade with pyrilamine and cimetidine. * $P < 0.05$ vs. control at same time point; $n = 7$ for each group.

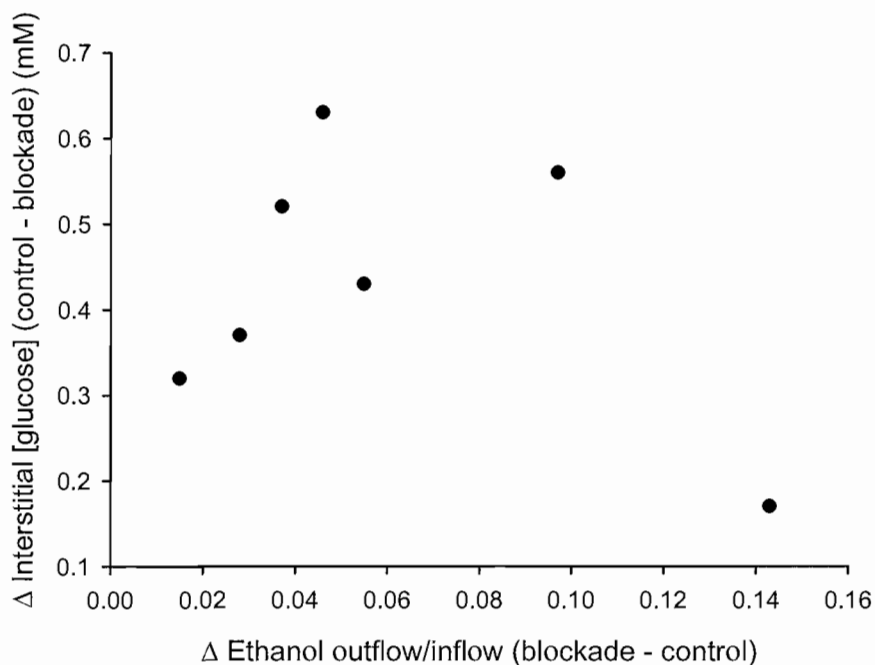


Ethanol outflow-inflow vs. interstitial glucose concentrations

Figure 5 illustrates the relationship between postexercise ethanol outflow-inflow ratios, expressed as the difference between blockade and control sites, and interstitial glucose concentrations, expressed as the difference between control and blockade sites 55 minutes postexercise. Although there is no notable positive correlation between the ethanol outflow-inflow and interstitial glucose concentrations, both the differences in ethanol outflow-inflow ratios and the differences in interstitial glucose concentrations are positive in all seven subjects. Moreover, it is interesting to note that, if the point representing the greatest difference in ethanol outflow-inflow ratios were deleted, a

moderate positive correlation ($r = 0.605$; $P < 0.05$) would exist between relative blood flow and interstitial glucose concentrations.

Figure 5. Relationship between ethanol outflow-inflow ratio and interstitial glucose concentrations following exercise. Outflow-inflow ratio expressed as blockade – control; Interstitial glucose concentration expressed as control – blockade; $n = 7$; 55 min postexercise time point represented.



Discussion

The goal of this study was to determine the effect of combined local H₁- and H₂-receptor blockade, administered via skeletal muscle microdialysis, on postexercise interstitial glucose concentrations. In agreement with our hypothesis, postexercise interstitial glucose concentrations were higher in the control versus the combined H₁- and H₂-receptor antagonist sites. This finding suggests a histaminergic component of postexercise interstitial glucose delivery in humans.

This is the first study to investigate the effect of local administration of H₁- and H₂-receptor antagonists on muscle blood flow and glucose regulation following exercise. Previous research has shown orally administered H₁- and H₂-receptor antagonists blunt the sustained skeletal muscle hyperemia following dynamic exercise (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). However, this route of delivery may evoke systemic effects and induce counter-regulatory hemodynamic responses that could impact factors independent of blood flow. An advantage of using skeletal muscle microdialysis for drug delivery lies in the ability of investigators to precisely target the region of interest while simultaneously measuring substances within the interstitium. While the results of the current investigation imply histaminergic skeletal muscle hyperemia facilitates interstitial glucose delivery, there are a number of alternative explanations for the reduced interstitial glucose concentrations in the face of local H₁- and H₂-receptor blockade.

Potential histaminergic mechanisms

Results from previous studies utilizing skeletal muscle microdialysis have consistently observed resting interstitial glucose concentrations to be on the order of 3.6 – 3.8 mM (Maggs *et al.*, 1995; Muller *et al.*, 1995; MacLean *et al.*, 1999). These interstitial glucose concentrations from prior investigations are in line with the resting interstitial glucose concentrations we found in the sham protocol prior to administration of the oral glucose load. Furthermore, in agreement with previous findings, we observed notably higher plasma glucose concentrations, as derived from arterialized samples, than those found in the interstitium (MacLean *et al.*, 1999). The glucose concentration

gradient that exists, between the arterial blood and both the interstitium and the venous blood, aids in delivery of glucose to the skeletal muscle cells, an effect that is enhanced when either arterial blood glucose is elevated and/or interstitial glucose concentrations are reduced. Furthermore, the discovery that interstitial glucose concentrations rise at the onset of exercise, despite elevated glucose uptake, lends support to the idea that glucose delivery is limited at rest (MacLean *et al.*, 1999). In fact, the aforementioned observation is in accordance with the results from the current investigation.

There are at least four feasible mechanisms by which local H₁- and H₂-receptors may mediate glucose delivery to the interstitium following exercise. The first and most likely means by which interstitial glucose delivery is enhanced is via a sustained elevation in local blood flow. This concurs with our current findings that ethanol outflow-inflow ratio, an inverse surrogate of muscle blood flow, was markedly increased through 75 minutes postexercise in the microdialysis blockade sites, while there was no difference in ethanol outflow-inflow ratios between control and blockade sites in the sham protocol. The ethanol outflow-inflow ratio is considered to represent nutritive blood flow – that is, flow through blood vessels supplying muscle cells to promote nutrient exchange (Newman *et al.*, 2002). Indeed, we found notably greater interstitial glucose concentrations in the control versus the blockade sites following exercise, both before and after the administration of the oral glucose load. Previous investigations have shown that rises in local blood flow induced by heating or pharmacological means, led to increased dialysate glucose concentrations, while reductions in local blood flow elicited the opposite effect (Hickner *et al.*, 1991, 1992). Our results indicate the physiological

elevation in postexercise blood flow has a similar effect on interstitial glucose concentrations.

The second potential mechanism by which local H₁- and H₂-receptors may mediate postexercise interstitial glucose delivery is by evoking changes in vascular permeability. If capillaries demonstrate a postexercise histaminergic increase in permeability, this would be expected to impact both clearance of ethanol and interstitial delivery of glucose. Histamine is known to evoke a rapid and transient increase in microvascular permeability (Majno & Palade, 1961; Killackey *et al.*, 1986; Hill *et al.*, 1997; van Hinsbergh & van Nieuw Amerongen, 2002). This enhanced permeability is mediated by H₁-receptors and may be reversed by administration of H₁-receptor antagonists (Niimi *et al.*, 1992). However, this effect has been found primarily in the post-capillary venules (Hill *et al.*, 1997; van Hinsbergh & van Nieuw Amerongen, 2002) and in studies on cultured human umbilical vein endothelial cells (Niimi *et al.*, 1992; Ikeda *et al.*, 1999), while evidence of H₁-receptor mediated increases in permeability at the level of the capillary is sparse. Moreover, there are notable differences in permeability responses between arterial and venular vessels, as histamine has been shown to evoke an H₂-receptor mediated reduction in vascular permeability in cultured bovine aortic endothelial cells (Ikeda *et al.*, 1999). Thus, while it is plausible histaminergic increases in capillary permeability play a role in glucose delivery to the interstitium following exercise; there is a lack of evidence to support that explanation at this time.

A third way local H₁- and H₂-receptors may promote postexercise interstitial glucose delivery is by supporting enhanced insulin-mediated capillary recruitment and

subsequent delivery of insulin to the interstitium. Studies using both the rat (Vincent *et al.*, 2004) and human model (Coggins *et al.*, 2001) have shown insulin increases capillary recruitment. Insulin binds primarily to insulin-like growth factor-I and insulin receptors in the vascular endothelium (Wang *et al.*, 2006), which stimulates nitric oxide synthesis to induce local vasodilation (Rattigan *et al.*, 2005). Inhibition of nitric oxide synthase has been shown to attenuate microvascular recruitment and limb glucose uptake, as measured by arterial-venous concentration differences, in response to insulin infusion (Vincent *et al.*, 2003). Insulin-mediated capillary recruitment likely promotes muscle glucose uptake via both an increase in surface area and a decrease in distance for diffusion to the skeletal muscle cells. Along these lines, skeletal muscle glucose uptake has been found to track the timeframe of interstitial insulin levels, which suggests insulin delivery to the interstitium is crucial in timely muscle glucose uptake (Miles *et al.*, 1995; Vincent *et al.*, 2005). This notion is supported by a recent study by Chiu and coworkers who found intramuscular injection of insulin resulted in an immediate rise in hindlimb glucose uptake in dogs (Chiu *et al.*, 2008). Thus, glucose delivery and subsequent uptake is reliant on delivery of insulin to the microvasculature, for promotion of capillary recruitment and timely diffusion into the interstitial space. Local H₁- and H₂-receptors may affect this process, by mediating local blood flow or trans-capillary movement of insulin to the interstitial space, thereby impacting skeletal muscle interstitial glucose delivery.

A fourth possible explanation for the results of the current investigation is that local administration of H₁- and H₂-receptor antagonists following exercise may enhance

local glucose uptake by the skeletal muscle cells. If this were the case, even in the face of comparable interstitial glucose delivery to the areas surrounding the control and blockade sites, the interstitial glucose in the region surrounding the blockade probes could be reduced. However, we were unable to find evidence to suggest either pyrilamine or cimetidine enhances skeletal muscle glucose uptake or GLUT4 translocation.

Furthermore, if there was an effect of H₁- and H₂-receptor blockade on glucose uptake by the skeletal muscle cells, considering the fact that interstitial glucose concentrations were not different between control and blockade sites during the sham protocol, this effect would be specific to the postexercise condition, which seems unlikely.

Methodological considerations

The variability in the outflow-inflow ratios and interstitial glucose concentrations in the current study are greater than we anticipated, resulting in some time points at which there are no differences between control and blockade sites. This can largely be attributed to a relatively small subject population, so if we were to increase the number of subjects, we would likely see significant differences across a greater range of time points in the exercise protocol. Nevertheless, the entire pattern of the relative responses to prior exercise between the control and blockade sites indicates substantially reduced local blood flow and interstitial glucose concentrations in the blockade versus control sites. Moreover, as illustrated in Figure 5, 55 minutes after exercise, ethanol outflow-inflow ratios were higher and interstitial glucose levels were lower in the blockade sites in every subject, which suggests that decreased local blood flow has an adverse effect on interstitial glucose delivery.

Microdialysis ethanol outflow-inflow ratio is not considered a quantitative measure, per se, of local blood flow (Radegran, 1999). Factors independent of blood flow, such as capillary permeability and lymph flow, may affect clearance of ethanol to some degree (Hickner *et al.*, 1995). In addition, initial trauma caused by probe insertion may affect ethanol clearance and dialysate glucose concentrations. However, ethanol outflow-inflow ratios have been shown to be sensitive to perturbations designed to increase and decrease local blood flow (Hickner *et al.*, 1991, 1992; Hickner *et al.*, 1995). Moreover, ethanol outflow-inflow ratios and dialysate glucose concentrations have been shown to be stable within 20 minutes of probe insertion (Hickner *et al.*, 1994). Our first dialysate collection period in the current study (35-55 min postexercise) started 15 minutes after probe insertion; therefore only one collection period was likely to be even minimally affected by residual trauma response. Thus, skeletal muscle microdialysis is an effective tool to assess relative changes in local blood flow, and has been found to be especially sensitive in the absence of skeletal muscle contraction and during lower blood flow conditions (Hickner *et al.*, 1995). Moreover, the current protocol was designed and implemented to minimize potential methodological concerns.

Perspectives

The current investigation provides evidence that postexercise skeletal muscle hyperemia influences interstitial glucose delivery and this effect is blunted by local administration of H₁- and H₂-receptor antagonists. This discovery may have implications regarding our understanding of recovery following dynamic exercise. Elevated blood flow to the previously active muscles may be crucial for timely postexercise glycogen

repletion, which may affect performance during subsequent bouts of exercise. These findings may also be relevant to populations which have reduced skeletal muscle perfusion, such as the aged, obese individuals and those suffering from diabetes. For instance, it is possible that by increasing glucose delivery, postexercise skeletal muscle hyperemia may be a key mechanism by which to circumvent insulin resistance in diabetes. These findings, therefore, have mechanistic and clinical relevance.

In conclusion, postexercise interstitial glucose concentrations, as determined by skeletal muscle microdialysis, were greater in the control versus the combined H₁- and H₂-receptor antagonist sites. This finding suggests postexercise delivery of glucose to the interstitial space of the previously active skeletal muscle is mediated, in part, by local H₁- and H₂-receptors in humans.

In the study described in Chapter IV of this dissertation, we concluded postexercise interstitial glucose concentrations, as determined by skeletal muscle microdialysis, were greater in the control versus the combined H₁- and H₂-receptor antagonist sites. This suggested glucose delivery to interstitial space was mediated, in part, by local H₁- and H₂-receptor-mediated skeletal muscle hyperemia. We then turned our attention to systemic postexercise glucose levels in the face of blockade, by examining the effect of oral H₁- and H₂-receptor antagonists on the glycemic response to an oral glucose load following exercise.

CHAPTER V

H₁- AND H₂-HISTAMINE RECEPTOR BLOCKADE AUGMENTS THE GLYCEMIC RESPONSE TO POSTEXERCISE ORAL GLUCOSE LOAD

Introduction

An acute bout of moderate-intensity dynamic exercise results in a sustained rise in skeletal muscle blood flow from that of pre-exercise levels (Halliwill, 2001; Pricher *et al.*, 2004). This postexercise skeletal muscle hyperemia is found in both men and women (Senitko *et al.*, 2002) and in both sedentary and endurance exercise trained individuals (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). Recent evidence suggests this postexercise skeletal muscle hyperemia is mediated by two (H₁ and H₂) histamine receptor subtypes (Lockwood *et al.*, 2005b; McCord *et al.*, 2006; McCord & Halliwill, 2006). While the mechanisms of postexercise hyperemia are becoming more apparent, it is unclear what purpose this increased blood flow might serve. Along these lines, Williams *et al.* investigated the relationship between leg blood flow and oxygen uptake following 60 minutes of moderate-intensity exercise. They found the time course of leg blood flow recovery did not match that of oxygen uptake, suggesting skeletal muscle hyperemia does not subserve oxygen delivery to the previously exercised skeletal muscle (Williams *et al.*, 2005). The function, therefore, of

this histamine-receptor-mediated hyperemia found after an acute bout of dynamic exercise has yet to be elucidated.

It is well established that skeletal muscle glucose uptake is enhanced, in an insulin-independent manner, during the first 90 minutes postexercise (Wasserman & Halseth, 1998; Richter *et al.*, 2001a; Henriksen, 2002). Much research has focused on the role of glucose transporters, primarily the GLUT4 isoform found in skeletal muscle, on glucose uptake during and immediately following exercise (Kennedy *et al.*, 1999; Thorell *et al.*, 1999). In contrast, the impact of postexercise hyperemia on glucose delivery has been sparsely explored. Several investigations in both animals (Grubb, 1977; Schultz, 1977) and humans (Hickner *et al.*, 1991; Baron *et al.*, 1994; Durham *et al.*, 2003) suggest increased limb blood flow enhances skeletal muscle glucose uptake. However, these prior studies have not examined the relationship between skeletal muscle blood flow and glucose kinetics following exercise. Therefore, the current investigation was designed to determine if postexercise skeletal muscle hyperemia influences glucose regulation via enhanced glucose kinetics following a bout of dynamic exercise. We tested the hypothesis that the glycemic response to an oral glucose load (OGTT) following exercise would be higher and more sustained with H₁- and H₂-receptor blockade versus control.

Methods

This study was approved by the Institutional Review Board of the University of Oregon and was conducted in accordance with the guidelines set forth by the Office for

Protection of Human Subjects of the University of Oregon. Each subject gave his or her written consent prior to participation in the study.

Subjects

Twenty healthy, non-smoking, normotensive subjects (13 men; 7 women), between the ages of 20 and 35, participated in this study. Twelve subjects (8 men; 4 women) participated in an exercise protocol and eight subjects (5 men; 3 women) participated in a sham (no exercise) protocol. For all study visits, subjects reported to the laboratory at least three hours post-prandial, having refrained from alcohol consumption and exercise for 24 hours and consumption of caffeine for 12 hours. Subjects were taking no medications with the exception of oral contraceptives. In addition, female subjects were studied during the early follicular phase of their menstrual cycle or during the placebo phase of the oral contraceptive cycle, to minimize the potential effects of reproductive hormones on cardiovascular and metabolic regulation.

Screening visit

Subjects participating in the exercise protocol initially visited the laboratory to perform a peak aerobic power test on a cycle ergometer, in addition to self-reporting activity levels on two questionnaires. Subjects performed an incremental cycle exercise test (Lode Excaliber, Groningen, The Netherlands) comprised of 1-minute workload increments to determine peak oxygen uptake ($VO_{2\text{ peak}}$). Specifically, after a 2-minute warm-up period of easy cycling (20–30 watts), workload was increased by 20, 25, or 30 watts every minute. Selection of the workload increment was based on self-reported subject activity levels, with the goal of producing exhaustion within 8–12 minutes.

Subjects were instructed to maintain 60-80 revolutions per minute to maximize muscular efficiency. Whole body oxygen uptake was measured via a mixing chamber (Parvomedics, Sandy, UT) integrated with a mass spectrometry system (Marquette MGA 1100, MA Tech Services, St. Louis, MO). Peak aerobic power was determined as either when subjects were unable to maintain 60 revolutions per minute, had obtained a respiratory exchange ratio of greater than 1.15, and/or had reached subjective exhaustion [rating of perceived exertion on the Borg (Borg, 1970) scale of 19–20] within the 8- to 12-minute period.

After resting for approximately 10 minutes, subjects returned to the cycle ergometer to determine the workload corresponding to a steady-state oxygen consumption of 60% of $\text{VO}_{2\text{ peak}}$. The workload was titrated, as needed, to reach a steady state oxygen consumption of 60% of $\text{VO}_{2\text{ peak}}$, which was usually attained within 5 minutes. This workload was used on the exercise study day for the 60-minute exercise bout, as this intensity and duration of exercise has been shown to evoke sustained (~100 minutes) postexercise skeletal muscle hyperemia (Pricher *et al.*, 2004).

Experimental protocol

For both the sham and exercise protocols, subjects reported for parallel experiments on 2 separate days. The order of experiments was randomized between a combined H₁- and H₂-receptor antagonist (fexofenadine and ranitidine) and a control day. H₁-receptor antagonism was produced via oral administration of 540 mg fexofenadine hydrochloride (Allegra; Aventis Pharmaceuticals Inc, Kansas City, MO). This oral dose of fexofenadine adequately and selectively blocks H₁-receptors, with a half-life of

approximately 12 hours and a time to peak concentration between 0.83 – 1.33 hours (Russell *et al.*, 1998). Fexofenadine is non-sedating, does not appear to cross the blood-brain barrier or have anti-muscarinic effects (Hardman JG, 2001). Oral H₁-receptor antagonism via 540 mg fexofenadine has been shown to attenuate elevations in postexercise femoral vascular conductance in humans (Lockwood *et al.*, 2005b).

H₂-receptor antagonism was produced via oral administration of 300 mg ranitidine hydrochloride (Zantac; Pfizer Consumer Healthcare, Morris Plains, NJ). This oral dose of ranitidine adequately and selectively blocks H₂-receptors, with a half-life of 2.6 hours and a time to peak concentration of 2.2 hours (Garg *et al.*, 1985). Ranitidine is non-sedating, does not appear to cross the blood-brain barrier or have any cardiovascular effects (Hardman JG, 2001). Oral H₂-receptor antagonism via 300 mg ranitidine has been shown to blunt elevations in postexercise femoral vascular conductance in humans (McCord *et al.*, 2006).

On each study day, subjects were laid in the supine position for instrumentation. An intravenous catheter was inserted retrogradely in the dorsal vein of the left hand to obtain blood samples. During the exercise protocol, subjects underwent a 60-min period of seated upright cycling at 60% VO_{2 peak}. Exercise of this intensity and duration produces a sustained (~100 min) postexercise skeletal muscle hyperemia (Pricher *et al.*, 2004). During exercise, subjects consumed 10 ml of water per kilogram of body weight to offset volume loss during exercise. During the sham study, the 60 minutes of cycling was replaced with 60 minutes of quiet rest. Immediately after exercise or sham, each subject consumed 0.1 oz per kg body weight (up to 10 oz maximum) Oral Glucose

Tolerance Beverage (Trutol 100, NERL Diagnostics, East Providence, RI), which contained 10 g of glucose per oz. Therefore, subjects received an oral glucose dose of 1 g per kg body weight up to 100 g maximum. Measurements were taken in the supine position prior to exercise or sham and through 120 minutes following the oral glucose tolerance test (OGTT). Pre-exercise and postexercise measurements included heart rate, arterial pressure, femoral blood flow, as well as collection of blood samples for determination of oxygen saturation and concentrations of glucose, insulin, C-peptide, and glucagon. During exercise, blood pressure and heart rate were measured every 15 minutes.

Measurements

Heart rate and arterial pressure

Heart rate was monitored throughout both protocols via 5-lead electrocardiogram (Quinton Instruments, Bothell, WA). Arterial pressure was measured with an automated oscillometric device (Dinamap Pro100 vital signs monitor, Critikon Inc, Tampa, FL) during resting conditions. Arterial pressure during exercise was determined via manual auscultometry.

Leg blood flow

Mean blood velocities and diameters of the common femoral artery were measured using a linear ultrasound probe (10MHz linear-array vascular probe, GE Vingmed System 5, Horton, Norway) placed distal to the inguinal ligament, approximately 2–3 cm proximal to the bifurcation. The entire width of the artery was insonated with an angle of 60 degrees and velocity measurements were taken

immediately before diameter measurements. Leg blood flow was calculated as artery cross-sectional area multiplied by femoral mean blood velocity, doubled to represent both legs, and reported as $\text{ml} \cdot \text{min}^{-1}$. Leg vascular conductance was calculated as flow for both legs/mean arterial pressure and expressed as $\text{ml} \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$.

Arterialized blood samples

“Arterialization” of venous blood samples was accomplished by placing each subject’s cannulated hand in a custom-made heating chamber (“hot-box”), which was flushed with air at 55°C . Actual skin temperature was raised to approximately 42°C , which is below the temperature that evokes sensations of pain. Heating the hand in this way induces the opening of arterial-venous anastomoses in the hand circulation, thereby allowing investigators to sample “arterialized” venous blood with higher and less variable oxygen saturations and higher glucose concentrations compared to non-arterialized blood samples (Morris *et al.*, 1997). Arterialization of venous blood samples was confirmed by immediate measurement of oxygen saturation in duplicate by a co-oximeter (Radiometer Copenhagen). Glucose concentrations of arterialized venous blood were measured in duplicate with a clinical glucose analyzer (YSI 2300 Stat Plus Glucose and Lactate Analyzer, YSI Life Sciences). Blood samples were collected pre-exercise, half-way through exercise, immediately postexercise, and 10, 20, 30, 45, 60, 90, and 120 minutes following oral glucose load. Samples were promptly placed on ice, centrifuged at 4°C , separated, and stored at -80°C until analyzed.

Blood hormone analysis

Analysis of insulin, glucagon, and C-peptide was conducted by the core lab at the Oregon Clinical and Translational Research Institute (OCTRI) by standard methods.

Estimation of metabolic clearance rate and insulin sensitivity

The MCR est and ISI est indices, developed by Stumvoll and co-workers (2000) use results from the OGTT to estimate metabolic clearance rate of glucose and insulin sensitivity. Using subject BMI, plasma glucose, and plasma insulin data, they have a high correlation ($r \geq 0.79$) with the euglycemic hyperinsulinemic clamp (Stumvoll *et al.*, 2000). They use the following equations:

$$\text{MCR est (OGTT)} = 18.8 - 0.271 \text{ BMI} - 0.0052 \times \text{I 120} - 0.27 \times \text{G 90}$$

$$\text{ISI est (OGTT)} = 0.226 - 0.0032 \times \text{BMI} - 0.0000645 \times \text{I 120} - 0.0037 \times \text{G 90}$$

where BMI = body mass index, I 120 = plasma insulin at 120 min OGTT, and G 90 = plasma glucose at 90 min OGTT.

Leg glucose delivery

Delivery of glucose to the legs was calculated as the product of arterialized glucose concentrations, derived from heated hand vein samples, and femoral blood flow per the following equation:

$$\text{Leg glucose delivery (mg min}^{-1}\text{)} = ((\text{Arterialized [glucose] (mg dL}^{-1}\text{)})/100) \times \text{Leg blood flow (ml min}^{-1}\text{)}$$

Statistics

The results were analyzed with a Two-Way Repeated Measures ANOVA (effects of drug and time) with SAS Proc Mixed (SAS v9.1, SAS Institute, Cary, NC).

Differences were considered significant when $P \leq 0.05$. All values are reported as means \pm SE unless otherwise noted.

Results

Subject characteristics

Subject characteristics are presented in Table 2. $VO_{2\text{peak}}$ values are within the normal range for young, healthy subjects of sedentary to endurance trained status.

Table 2. Subject characteristics-Chapter V.

	Exercise protocol	Sham protocol
N	12	8
Age (years)	24.3 \pm 3.6	24.2 \pm 5.3
Height (cm)	182.5 \pm 12.1	179.4 \pm 10.2
Weight (kg)	77.9 \pm 18.7	79.3 \pm 14.7
Body mass index (kg m ⁻²)	23.1 \pm 3.3	24.5 \pm 3.5
$VO_{2\text{peak}}$ (ml kg ⁻¹ min ⁻¹)	54.0 \pm 9.1	
Workload at 60% of $VO_{2\text{peak}}$ (watts)	180.5 \pm 54.9	
Baecke sport index (arbitrary units)	12.3 \pm 3.1	
Index of physical activity (MET·hr·wk)	168.7 \pm 61.2	

Values are means \pm SD; $VO_{2\text{peak}}$, peak oxygen consumption; MET, metabolic equivalents

Exercise

During the exercise protocol, the goal was to have each subject exercise for 60 minutes at 60% $VO_{2\text{peak}}$. The percentage of heart rate reserve (heart rate reserve is defined as maximal heart rate achieved during $VO_{2\text{peak}}$ testing minus the resting supine heart rate) attained during exercise (control 70.22 \pm 1.7%; blockade 69.82 \pm 2.3%; $P = 0.78$) was consistent with the target workload. Heart rate increased from 53.1 \pm 2.0 beats min⁻¹ during supine rest to 150.8 \pm 2.1 beats min⁻¹ during exercise (measured 30 min into exercise bout) on the control day and from 51.2 \pm 2.1 beats min⁻¹ during rest to 149.4 \pm

2.9 beats min^{-1} during exercise on the blockade day ($P < 0.05$ vs. rest on both days). Mean arterial pressure increased from 79.1 ± 1.1 mmHg during supine rest to 91.2 ± 1.9 mmHg during exercise on the control day and from 77.8 ± 1.5 mmHg during supine rest to 90.3 ± 1.7 mmHg during exercise on the blockade day (both $P < 0.05$ vs. rest on both days). During the sham protocol, pre-sham resting heart rate (control 53.8 ± 3.1 ; blockade 53.5 ± 3.6 beats min^{-1} ; $P = 0.898$) and mean arterial pressure (control 76.5 ± 2.0 ; blockade 74.9 ± 1.7 mmHg; $P = 0.232$) did not differ between control and blockade days. Moreover, during the sham period on both study days, neither heart rate (control 53.3 ± 3.3 ; blockade 55.0 ± 3.6 beats min^{-1} ; both $P \geq 0.391$ vs. baseline) nor arterial pressure (control 77.2 ± 2.7 ; blockade 76.2 ± 1.8 ; both $P \geq 0.072$ vs. baseline) changed from pre-sham supine rest.

Hemodynamics

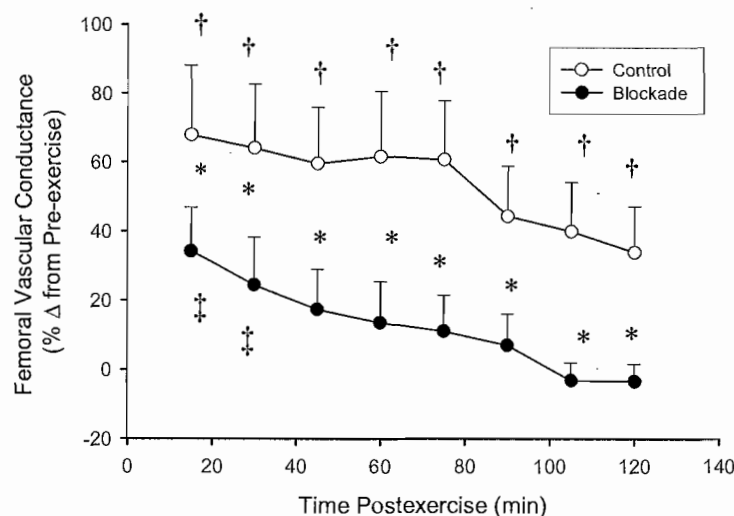
Table 3 shows the hemodynamic data derived from the exercise protocol. On both the control and blockade days, mean arterial pressure reached a nadir 45 minutes following exercise (control 71.9 ± 1.2 ; blockade 75.9 ± 1.6 mmHg; $P < 0.05$ vs. control). Heart rate ($P \geq 0.16$) and femoral blood flow ($P \geq 0.185$) were not different between control and blockade days at any time point. However, as illustrated by Figure 6, the sustained elevation in femoral vascular conductance during the control trials, expressed as percent change from pre-exercise, was significantly blunted in the blockade trials, through 120 minutes postexercise (all $P \leq 0.016$ vs. control). Furthermore, whereas femoral vascular conductance in the control condition remained $34.0 \pm 14.8\%$ above pre-exercise levels through 120 minutes postexercise ($P \leq 0.039$), femoral vascular

conductance in the blockade condition remained significantly elevated ($24.5 \pm 13.8\%$; $P = 0.027$) only through 30 minutes postexercise. During the sham protocol, heart rate ($P \geq 0.139$) and mean arterial pressure ($P \geq 0.088$) did not differ between the control and blockade days at any time point.

Table 3. Exercise protocol hemodynamics. $n = 12$ for heart rate and mean arterial pressure; $n = 8$ for femoral blood flow; $*P < 0.05$ vs. control at same time point. Values are means \pm SE.

Time	Heart rate (beats min^{-1})		Mean arterial pressure (mmHg)		Femoral blood flow (ml min^{-1})	
	Control	Blockade	Control	Blockade	Control	Blockade
Baseline	53.1 \pm 2.0	51.2 \pm 2.1	79.1 \pm 1.1	77.8 \pm 1.5	136.6 \pm 32.2	142.1 \pm 29.4
Exercise	150.8 \pm 2.1	149.4 \pm 2.9	91.2 \pm 1.9	90.3 \pm 1.7	N/A	N/A
Post-15	71 \pm 2.7	69.8 \pm 2.6	79.7 \pm 1.8	81.1 \pm 1.9	208.1 \pm 37.8	187.3 \pm 26.9
Post-30	64.6 \pm 2.3	66.2 \pm 2.7	75.5 \pm 1.4	*78.2 \pm 1.7	192.5 \pm 37.2	166.8 \pm 27.3
Post-45	62.9 \pm 2.2	65.4 \pm 2.8	71.9 \pm 1.2	*75.9 \pm 1.6	180.9 \pm 36.6	155.4 \pm 29.4
Post-60	62.4 \pm 2.0	64.1 \pm 2.6	72.5 \pm 1.2	*76.0 \pm 1.9	179.9 \pm 35.4	151.5 \pm 29.1
Post-75	60.8 \pm 2.1	62.5 \pm 2.6	73.6 \pm 1.0	76.0 \pm 1.7	180.9 \pm 33.8	147.9 \pm 27.3
Post-90	61.5 \pm 2.6	61.9 \pm 2.5	75.1 \pm 1.2	76.5 \pm 1.7	169.3 \pm 32.9	145.5 \pm 28.9
Post-105	61.0 \pm 2.3	61.8 \pm 2.4	75.6 \pm 1.5	77.2 \pm 2.0	164.3 \pm 32.7	138.7 \pm 30.5
Post-120	61.3 \pm 2.5	61.0 \pm 2.7	76.5 \pm 1.4	77.2 \pm 1.8	159.2 \pm 30.8	136.1 \pm 29.2

Figure 6. Percent change in femoral vascular conductance from pre-exercise baseline in response to 60 minutes of cycling at 60% of $\text{VO}_{2\text{peak}}$. Open circles denote control day; filled circles denote H_1 - and H_2 -receptor blockade (fexofenadine and ranitidine) day. $n = 8$. $*P < 0.05$ vs. control at same time point. $\dagger P < 0.05$ vs. pre-exercise control; $\ddagger P < 0.05$ vs. pre-exercise blockade.

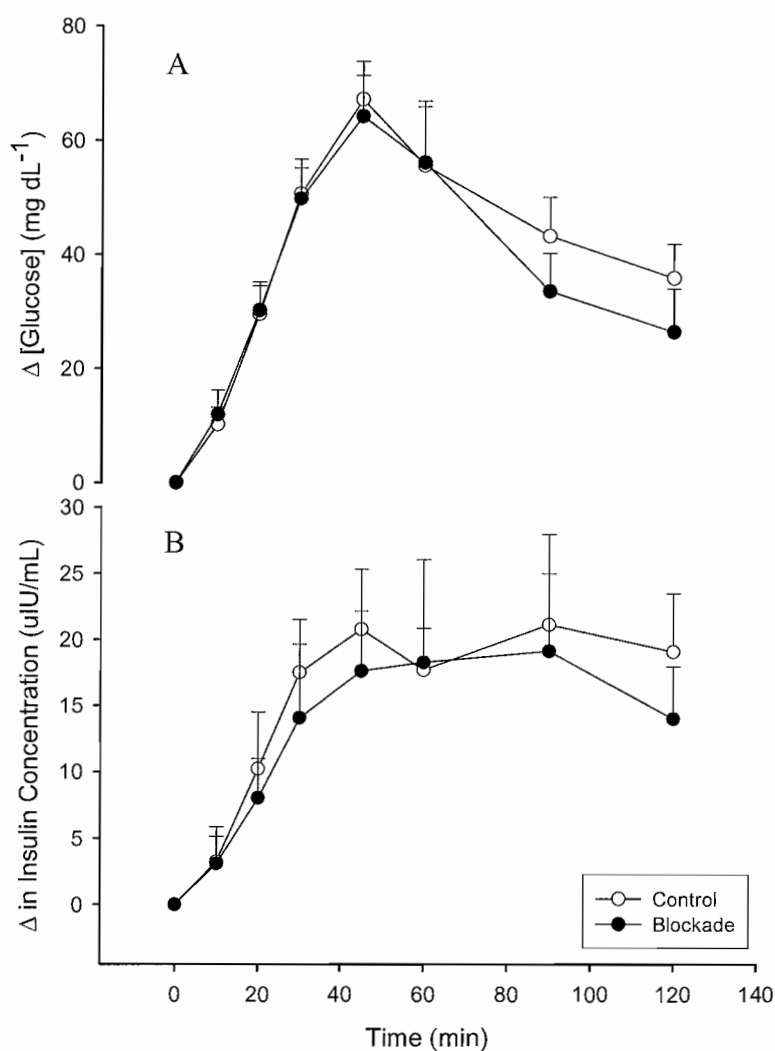


Glycemic and insulin response to oral glucose load

During the sham protocol, baseline blood glucose concentrations were not different between control (75.4 ± 1.7 mg dL⁻¹) and blockade (78.2 ± 1.3 mg dL⁻¹) days ($P = 0.255$). Blood glucose concentration during the sham period was 77.2 ± 2.0 mg dL⁻¹ on the control day and 80.0 ± 1.4 mg dL⁻¹ on the blockade day ($P = 0.316$ vs. control). Figure 7 shows the glycemic and insulin response, expressed as change from immediate post-sham baseline, to the oral glucose load during the sham protocol. Blood glucose concentration immediately before glucose load was 77.1 ± 2.1 mg dL⁻¹ on the control day and 77.6 ± 1.0 mg dL⁻¹ on the blockade day ($P = 0.829$ vs. control). During both the control ($\Delta 67.2 \pm 7.2$ mg dL⁻¹) and blockade ($\Delta 64.2 \pm 7.2$ mg dL⁻¹; $P = 0.751$ vs. control) days, blood glucose concentration peaked 45 minutes following the oral glucose load. At no time point during the sham protocol did the glycemic response differ between the control and blockade days ($P \geq 0.271$). During the sham protocol, baseline plasma insulin concentrations were not different between control (4.49 ± 1.2 μ IU mL⁻¹) and blockade (6.10 ± 1.5 μ IU mL⁻¹) days ($P = 0.072$). Plasma insulin concentration during the sham period was 4.61 ± 1.2 μ IU mL⁻¹ on the control day and 5.91 ± 1.7 μ IU mL⁻¹ on the blockade day ($P = 0.239$ vs. control). Plasma insulin concentration immediately before glucose load was 3.90 ± 0.95 μ IU mL⁻¹ on the control day and 4.60 ± 1.3 μ IU mL⁻¹ on the blockade day ($P = 0.190$ vs. control). As shown in Figure 7B, on both the control ($\Delta 21.1 \pm 3.9$ μ IU mL⁻¹) and blockade ($\Delta 19.1 \pm 8.8$ μ IU mL⁻¹; $P = 0.830$ vs. control) days, plasma insulin concentration reached its peak 90 minutes following the glucose

load. At no time point during the sham protocol did the plasma insulin response to oral glucose load differ between the control and blockade days ($P \geq 0.160$).

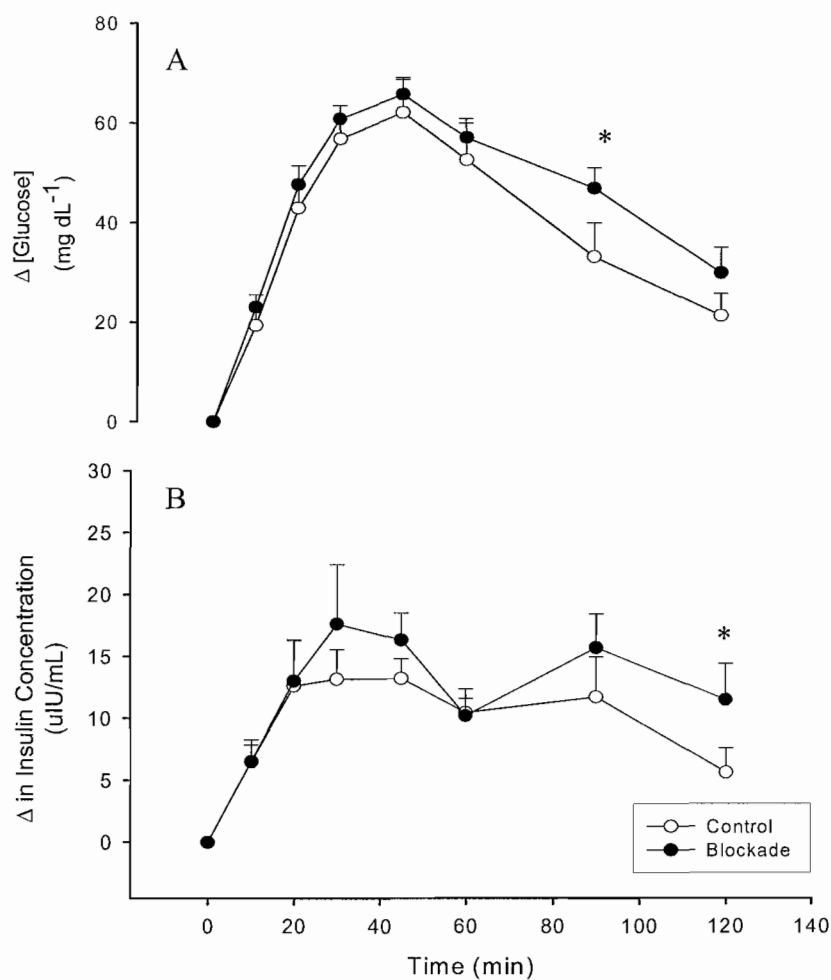
Figure 7. Sham protocol: change in (A) blood glucose and (B) plasma insulin in response to oral glucose load. Open circles denote control day; filled circles denote H_1 - and H_2 -receptor blockade (fexofenadine and ranitidine) day. $n = 8$.



During the exercise protocol, pre-exercise blood glucose concentrations were not different between control ($80.6 \pm 1.2 \text{ mg dL}^{-1}$) and blockade ($81.0 \pm 1.6 \text{ mg dL}^{-1}$) days ($P = 0.799$). Blood glucose concentration during exercise was $76.6 \pm 2.2 \text{ mg dL}^{-1}$ on the control day and $75.0 \pm 2.4 \text{ mg dL}^{-1}$ on the blockade day ($P = 0.282$ vs. control). Figure 8

shows the glycemic and insulin response, expressed as change from immediate postexercise baseline, to the oral glucose load during the exercise protocol. Blood glucose concentration immediately before glucose load was 79.6 ± 2.7 mg dL⁻¹ on the control day and 76.6 ± 2.3 mg dL⁻¹ on the blockade day ($P = 0.167$ vs. control). During both the control ($\Delta 62.1 \pm 6.7$ mg dL⁻¹) and blockade ($\Delta 65.7 \pm 3.4$ mg dL⁻¹; $P = 0.517$ vs. control) days, change in blood glucose concentration was greatest 45 minutes following glucose load. As illustrated by Figure 8A, 90 minutes following oral glucose load blood glucose concentration remained more elevated on the blockade ($\Delta 46.9 \pm 4.1$ mg dL⁻¹) than on the control day ($\Delta 33.1 \pm 6.8$ mg dL⁻¹; $P = 0.016$ vs. blockade). During the exercise protocol, pre-exercise plasma insulin concentrations were not different between control (3.58 ± 0.5 μ IU mL⁻¹) and blockade (3.84 ± 0.5 μ IU mL⁻¹) days ($P = 0.669$). Plasma insulin concentration during exercise was 1.82 ± 0.1 μ IU mL⁻¹ on the control day and 2.33 ± 0.3 μ IU mL⁻¹ on the blockade day ($P = 0.139$ vs. control). Plasma insulin concentration immediately before glucose load was 3.59 ± 0.6 μ IU mL⁻¹ on the control day and 3.65 ± 0.5 μ IU mL⁻¹ on the blockade day ($P = 0.939$ vs. control). As shown in Figure 8B, on both the control ($\Delta 13.2 \pm 2.4$ μ IU mL⁻¹) and blockade ($\Delta 17.6 \pm 4.8$ μ IU mL⁻¹; $P = 0.299$ vs. control) days, plasma insulin concentration peaked 30 minutes following the glucose load. As shown in Figure 8B, 120 minutes following the glucose load, plasma insulin concentration remained more elevated on the blockade day ($\Delta 11.5 \pm 2.9$ μ IU mL⁻¹) than on the control day ($\Delta 5.6 \pm 2.0$ μ IU mL⁻¹; $P = 0.047$ vs. blockade).

Figure 8. Exercise protocol: change in (A) blood glucose and (B) plasma insulin following postexercise oral glucose load. Open circles denote control day; filled circles denote H₁- and H₂-receptor blockade (fexofenadine and ranitidine) day. $n = 12$. * $P < 0.05$ vs. control at same time point.



Metabolic clearance rate and insulin sensitivity index

The estimated metabolic clearance rates of glucose and the insulin sensitivity indices in response to oral glucose load following both sham and exercise are shown in Figure 9. During the sham protocol, there was no difference between the estimated metabolic clearance rates on the control ($14.17 \pm 0.38 \text{ mg kg}^{-1} \text{ min}^{-1}$) and blockade days

($14.04 \pm 0.38 \text{ mg kg}^{-1} \text{ min}^{-1}$; $P = 0.453$ vs. control). Likewise, during the exercise protocol estimated metabolic clearance rates were not different between control ($14.45 \pm 0.26 \text{ mg kg}^{-1} \text{ min}^{-1}$) and blockade days ($14.3 \pm 0.25 \text{ mg kg}^{-1} \text{ min}^{-1}$; $P = 0.132$ vs. control).

The estimated insulin sensitivity during the sham protocol was not different between the control ($0.175 \pm 0.005 \text{ } \mu\text{mol kg}^{-1} \text{ min}^{-1} \text{ pM}^{-1}$) and blockade trials ($0.173 \pm 0.005 \text{ } \mu\text{mol kg}^{-1} \text{ min}^{-1} \text{ pM}^{-1}$; $P = 0.459$ vs. control). There were also no differences in estimated insulin sensitivity between the control ($0.178 \pm 0.003 \text{ } \mu\text{mol kg}^{-1} \text{ min}^{-1} \text{ pM}^{-1}$) and blockade ($0.176 \pm 0.003 \text{ } \mu\text{mol kg}^{-1} \text{ min}^{-1} \text{ pM}^{-1}$; $P = 0.182$ vs. control) days during the exercise protocol.

Leg glucose delivery

Estimated leg glucose delivery, both before and following postexercise oral glucose load, is shown in Figure 10. There was no difference in leg glucose delivery between the control ($108.7 \pm 26.4 \text{ mg min}^{-1}$) and blockade ($115.7 \pm 27.6 \text{ mg min}^{-1}$) days prior to exercise ($P = 0.556$). There was a trend towards enhanced leg glucose delivery on the control day at 30 minutes (control $264.4 \pm 56.6 \text{ mg min}^{-1}$; blockade $236.1 \pm 46.8 \text{ mg min}^{-1}$) and 60 min (control $225.7 \pm 45.2 \text{ mg min}^{-1}$; blockade $204.3 \pm 37.7 \text{ mg min}^{-1}$) postexercise, although differences in these values were not statistically significant at either time point (both $P = 0.385$). At 90 minutes postexercise, leg glucose delivery was $182.1 \pm 43.9 \text{ mg min}^{-1}$ on the control day and $183.9 \pm 39.3 \text{ mg min}^{-1}$ on the blockade day ($P = 0.933$).

Figure 9. Estimated (A) metabolic clearance rate of glucose and (B) Insulin sensitivity index to oral glucose load following sham and exercise. Open bars denote control day; filled bars denote H₁- and H₂-receptor blockade (fexofenadine and ranitidine) day. *n* = 8 for sham protocol; *n* = 12 for exercise protocol.

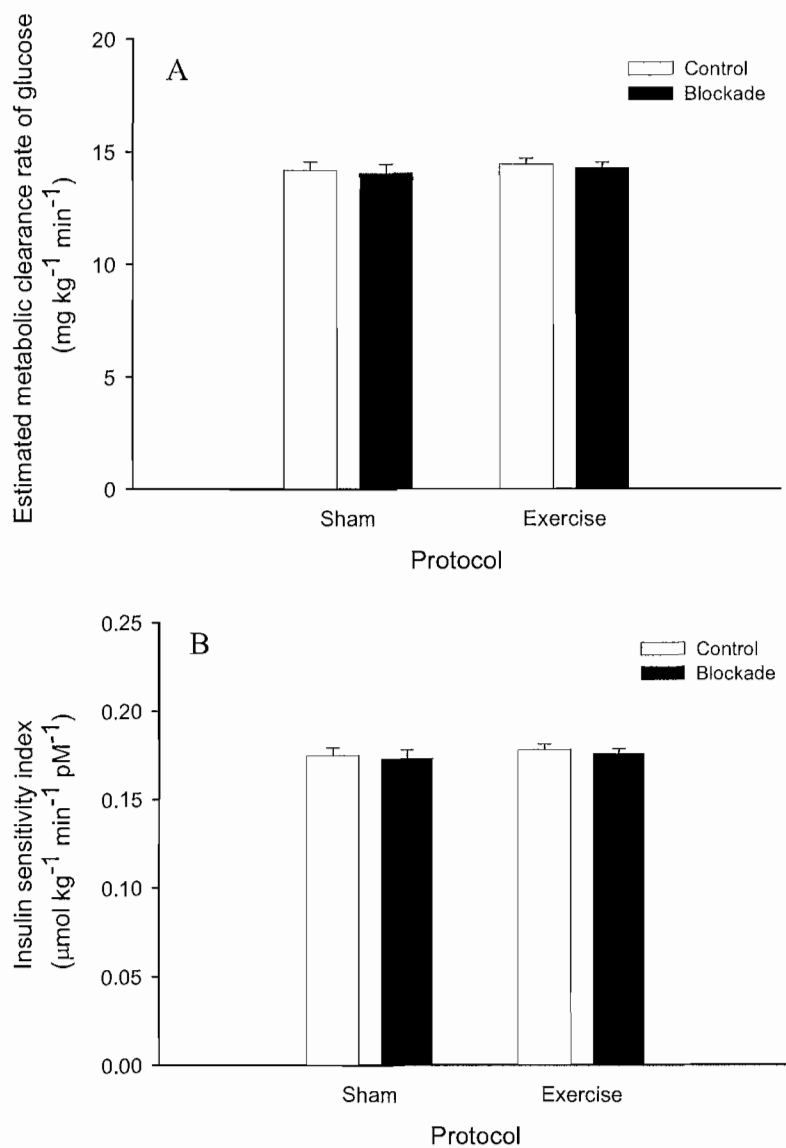
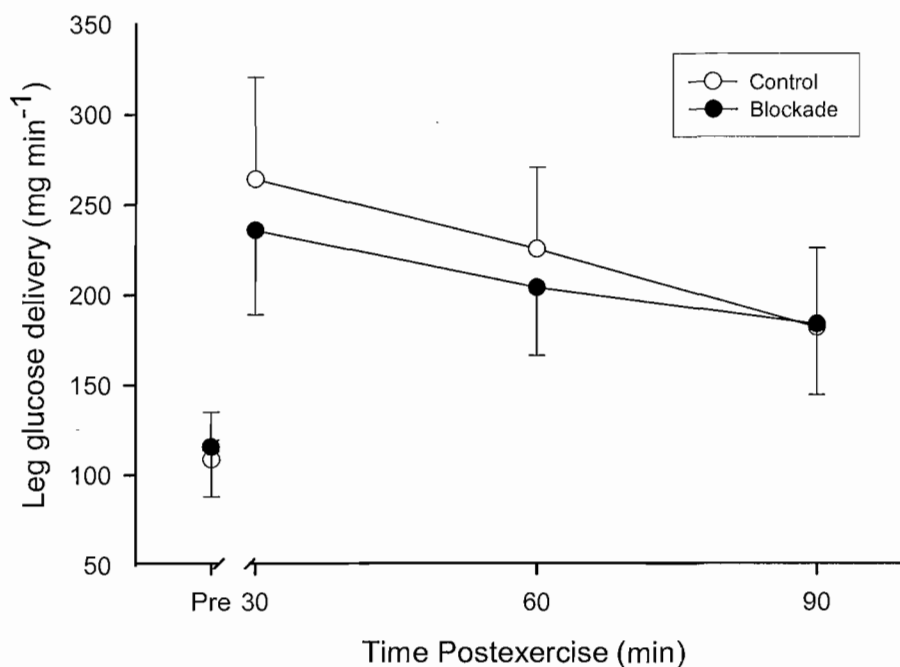


Figure 10. Leg glucose delivery before and after postexercise oral glucose load. Open circles denote control day; filled circles denote H₁- and H₂-receptor blockade (fexofenadine and ranitidine) day. *n* = 8.



Discussion

The goal of this study was to determine the effect of the combination of orally administered H₁- and H₂-receptor antagonists on glucose regulation following a postexercise oral glucose load. In agreement with our hypothesis, the glycemic response to postexercise oral glucose load was more sustained on the H₁- and H₂-receptor blockade versus the control day, suggesting a histaminergic effect on postexercise glucose kinetics.

The finding that the glycemic response to an oral glucose load is prolonged during H₁- and H₂-receptor antagonism suggests delivery of glucose to skeletal muscle cells is blunted in the blockade condition. It has recently been established that combined oral H₁- and H₂-receptor blockade attenuates postexercise hyperemia in previously active

skeletal muscles by approximately 80% (McCord & Halliwill, 2006) which is in line with the postexercise hyperemia found in the current study. While the current investigation does not allow for direct measurement of glucose delivery to the skeletal muscle cells *per se*, it implies glucose regulation is a function of skeletal muscle hyperemia following dynamic exercise.

It is natural to consider the delivery of glucose itself as a potential key function of postexercise hyperemia. However, perhaps equally crucial to the regulation of blood glucose is the delivery of insulin to the microvasculature, where it can impose both its hemodynamic and metabolic effects. Along these lines, physiological concentrations of insulin have been shown to evoke increased limb blood flow (Laakso *et al.*, 1990) and capillary recruitment (Coggins *et al.*, 2001) in humans. This insulin-mediated vasodilation and capillary recruitment is dependent upon nitric oxide, as nitric oxide synthase inhibition diminishes microvascular recruitment and, importantly, insulin-mediated skeletal muscle glucose uptake (Steinberg *et al.*, 1994; Vincent *et al.*, 2003). Movement of insulin from the central circulation to the skeletal muscle microvasculature and subsequently to the interstitium is required to initiate this capillary recruitment, therefore anything that aids insulin transport to the interstitium should accelerate muscle glucose uptake. Skeletal muscle glucose uptake tracks the timeframe of interstitial insulin levels better than plasma insulin levels, which suggests insulin delivery to the interstitium is crucial in timely muscle glucose uptake (Miles *et al.*, 1995; Vincent *et al.*, 2005). This notion is supported by a recent study by Chiu and coworkers who found

intramuscular injection of insulin resulted in an immediate rise in hindlimb glucose uptake in dogs (Chiu *et al.*, 2008).

Evidence suggests insulin-mediated capillary recruitment occurs quickly, while insulin-mediated increases in limb blood flow are slower, and delayed relative to muscle glucose uptake (Vincent *et al.*, 2004). Only about 30% of capillaries are perfused at rest (Honig *et al.*, 1982), which implies a flow limitation to skeletal muscle insulin and/or glucose delivery under resting conditions (Vincent *et al.*, 2005), as opposed to conditions in which capillary recruitment is increased. In fact, it has been suggested in situations where skeletal muscle glucose uptake is elevated, increases in limb blood flow will increase insulin delivery to facilitate further glucose delivery, especially when capillary recruitment is augmented (Clark *et al.*, 2003; Clerk *et al.*, 2004). Thus, as previously suggested, it seems insulin is not only a mediator of its own delivery (Vincent *et al.*, 2005) but it appears both insulin-mediated vasodilation and capillary recruitment are key regulators of muscle glucose uptake. Therefore, since insulin mediates glucose uptake via metabolic and vasculature actions, postexercise skeletal muscle hyperemia likely facilitates this process by enhancing delivery of insulin to the microvasculature when the stimulus for glucose uptake is high, such as following exercise. If this insulin delivery is attenuated by H₁- and H₂-receptor antagonists, that could help explain the prolonged glycemic response on the blockade days.

Another factor that may help explain the sustained glycemic response during H₁- and H₂-receptor blockade is the potential effect of H₁- and H₂-receptor antagonists on vascular permeability. If capillaries demonstrate a histaminergic increase in permeability

following exercise, diffusion of glucose to the muscle cells would be expected to decline during H₁- and H₂-receptor blockade, resulting in greater recirculation of glucose. Histamine evokes a rapid and transient increase in microvascular permeability (Majno & Palade, 1961; Killackey *et al.*, 1986; Hill *et al.*, 1997; van Hinsbergh & van Nieuw Amerongen, 2002) that is mediated by H₁-receptors and may be reversed by administration of H₁-receptor antagonists (Niimi *et al.*, 1992). While this effect has been typically observed in the post-capillary venules (Svensjo & Grega, 1986; van Hinsbergh & van Nieuw Amerongen, 2002) and in studies on cultured human umbilical vein endothelial cells (Niimi *et al.*, 1992; Ikeda *et al.*, 1999), there is a dearth of evidence H₁-receptors mediate increases in permeability at the level of the capillary. Furthermore, permeability responses to histamine differ between arterial and venular vessels, as histamine has been shown to evoke an H₂-receptor mediated reduction in vascular permeability in cultured bovine aortic endothelial cells (Ikeda *et al.*, 1999). Therefore, while histaminergic effects on capillary permeability may factor into the enhanced glycemic response to postexercise oral glucose load during H₁- and H₂-receptor blockade, there is currently limited evidence to support this notion.

A fourth possible explanation for the results of the current investigation is that orally administered H₁- and H₂-receptor antagonists following exercise may inhibit the transport of glucose into skeletal muscle cells. Blunted glucose transport would reduce the concentration gradient between the capillaries and the interstitial space, reducing diffusion of glucose and leading to the sustained elevation of blood glucose in response to oral glucose load. However, although histamine has been shown to stimulate glucose

transport in cultured cardiac endothelial cells in an H₁-receptor-dependent manner, this result was attributed to effects on endothelial permeability, as opposed to factors such as enhanced GLUT4 translocation (Thomas *et al.*, 1995). Furthermore we were unable to find compelling evidence of a histaminergic effect on skeletal muscle glucose transport or GLUT4 activity, so we cannot determine if this is a mechanism that factors into the results of our investigation.

If pancreatic insulin secretion were lessened by H₁- and H₂-receptor antagonists, this could help explain the prolonged elevation in blood glucose following postexercise oral glucose load. However, insulin release in response to intravenous glucose administration has been shown to be unaltered by H₁- and H₂-receptor antagonists (Pontiroli *et al.*, 1982) and H₂-receptor blockade had no effect on insulin secretion following oral glucose tolerance tests (Scarpignato *et al.*, 1981). Moreover, in the current investigation, the plasma insulin response to postexercise oral glucose load was equal or greater on the blockade day, which argues against blunted insulin secretion due to H₁- and H₂-receptor blockade.

An effect of H₁- and H₂-receptor antagonism on hepatic glucose uptake or release could contribute to the prolonged glycemic response to the postexercise oral glucose load during H₁- and H₂-receptor blockade. However, we were unable to find evidence of reduced hepatic glucose uptake or enhanced hepatic production during H₁- and H₂-receptor blockade. Moreover, the combination of postexercise sympathetic withdrawal (Halliwill *et al.*, 1996a) and consumption of an oral glucose beverage should suppress

glucogenesis, likely minimizing any impact of H₁- and H₂-receptor blockade in this scenario.

Finally, an impact of H₁- and H₂-receptor blockade on gastric emptying could help explain the sustained postexercise glycemic response in the current study. Previous investigations on the effect of oral H₂-receptor antagonists on gastric emptying have yielded equivocal results, as H₂-receptor blockade has been found to facilitate (Ohira *et al.*, 1993) and delay (Forrest *et al.*, 1976) gastric emptying in humans. However, in the current investigation, the sham protocol was undertaken to examine the effect of H₁- and H₂-receptor blockade on the glycemic response to oral glucose load in the absence of prior exercise. We found no difference in the glycemic or insulin response between the control and blockade days during the sham protocol. Combined with the nearly superimposable rise in blood glucose in response to the postexercise oral glucose load, this indicates any effects of H₁- and H₂-receptor blockade on gastric emptying in our study were minimal.

Methodological considerations

Although the oral glucose tolerance test (OGTT) has been widely used to assess glucose tolerance in a wide array of populations (Cobelli *et al.*, 2007), numerous factors associated with the OGTT may affect its reproducibility. These factors include variations in glucose absorption, neuro-hormonal interactions, splanchnic glucose uptake, and possible effects on incretin hormones, which are known to stimulate insulin secretion in response to glucose ingestion (Muniyappa *et al.*, 2008). However, when combined with various indices of insulin sensitivity and/or modeling techniques, the OGTT can be used

to estimate β -cell function and insulin sensitivity in humans. Furthermore, significant advantages of OGTT include its more physiological delivery of glucose, its relative ease of use, and reduced invasiveness, relative to the FSIVGT and euglycemic hyperinsulinemic clamp. Finally, as previously indicated, the results of our sham protocol suggest the combined H₁- and H₂-receptor blockade had no discernable effect on the glycemic response to OGTT in the absence of previous exercise.

As previously noted, oral administration of H₁- and H₂-receptor antagonists may evoke systemic effects and induce counter-regulatory responses. However, given the widespread use of fexofenadine and ranitidine to treat allergies and gastrointestinal disorders, the relevance of using oral blockade in the current study outweighed potential systemic effects.

An unexpected finding in the current investigation was the lack of difference in the estimated metabolic clearance rates of glucose and insulin sensitivity indices between the control and blockade days following postexercise oral glucose load. Given the sustained postexercise glycemic response and the equal or greater insulin response on the H₁- and H₂-receptor blockade day, we were anticipating that both the estimated metabolic clearance rate of glucose and insulin sensitivity index would be higher on the control day. Along these lines, although no statistically significant differences existed between the control and blockade days, there was a trend for enhanced leg glucose delivery under the control condition. However, the MCR est and ISI est indices, which are well correlated ($r \geq 0.79$) with the euglycemic hyperinsulinemic clamp (Stumvoll *et al.*, 2000) indicated no differences in metabolic clearance rate of glucose or insulin sensitivity between the

control and blockade trials. Therefore, to further investigate these parameters, we plan on using recently analyzed C-peptide samples in conjunction with the minimal model technique.

Perspectives

The results of the current investigation indicate commonly used medications may impact normal glucose regulation following exercise. Although the current investigation was conducted on healthy subjects, these findings reveal potential implications of H₁- and H₂-receptor antagonists for those who depend on exercise to regulate their blood glucose levels. This may be particularly relevant to populations which have reduced skeletal muscle perfusion, such as the aged, obese individuals and those suffering from diabetes. For example, it is possible that by mediating glucose regulation, postexercise hyperemia may be an important mechanism by which to circumvent insulin resistance in diabetes. Furthermore, effects of these widespread medications may confound exercise as a “treatment” for both the healthy population and those suffering from various diseases. Further research is warranted to investigate the mechanisms behind this apparent relationship between cardiovascular and metabolic regulation, including studies to determine if these findings extend to those with disease, such as those suffering from diabetes.

In conclusion, the glycemic response to postexercise oral glucose load was more sustained on the H₁- and H₂-receptor blockade versus control day. This finding suggests a histaminergic effect on postexercise glucose kinetics in humans.

CHAPTER VI

CONCLUSIONS

Please note: This chapter has been written in the general format of a brief, contemporary review as an initial draft for future submission to *Exercise and Sport Science Reviews*. Unlike reviews from *Exercise and Sport Science Reviews* however, I have cited unpublished findings and have referred to figures included within this dissertation.

Introduction

Over the past several years, there have been substantial advances in our understanding of blood flow regulation following dynamic exercise in humans. It has become clear that acute exercise induces a sustained rise in blood flow, with the vast majority of this blood being directed to skeletal muscle. The function of this skeletal muscle hyperemia, however, has only recently been investigated. Current work in our laboratory is focused on the relationship between skeletal muscle hyperemia and the well established increase in skeletal muscle glucose uptake following acute exercise. Therefore, the purpose of this review is to examine this emerging area of study, aimed at determining the impact of postexercise skeletal muscle hyperemia on glucose regulation in humans.

Regional changes in postexercise vascular conductance

Humans experience a sustained drop in systemic vascular resistance (Halliwill, 2001) following a bout of dynamic exercise. Postexercise reductions have been found in calf (Hara & Floras, 1992; Halliwill *et al.*, 2000) and forearm vascular resistance in both healthy individuals (Coats *et al.*, 1989) and those with mild to moderate hypertension (Cleroux *et al.*, 1992). Thus, vasodilation is found in both previously active and inactive regions following dynamic exercise.

Pricher *et al.* found that both postexercise splanchnic and renal vascular conductance was not different from preexercise levels, but elevations in leg vascular conductance were sustained for 100 minutes following exercise (Pricher *et al.*, 2004). In a companion study, Wilkins and colleagues found, despite an elevation in core body temperature that persisted for 90 minutes postexercise, cutaneous vascular conductance quickly returned to the level prior to the exercise bout (Wilkins *et al.*, 2004b). A recent investigation found overall cerebral blood flow to be unchanged and blood flow to some regions (e.g., insular cortex) to be reduced following exercise that evoked postexercise hypotension (Williamson *et al.*, 2004). Thus, it does not appear that vasodilation of the renal, splanchnic, cerebral, or cutaneous vasculature contributes to the rise in systemic vascular conductance following dynamic exercise. Rather, the primary contributors to increases in postexercise systemic vascular conductance seem to be elevations in blood flow to previously exercised and non-exercised skeletal muscles (skeletal muscle hyperemia).

Mechanisms of postexercise vasodilation

The sustained vasodilation observed in humans following dynamic exercise is mediated by both neural and vascular factors (Halliwill *et al.*, 1996a). Following an acute bout of dynamic exercise, there is a downward resetting of the arterial baroreflex, so muscle sympathetic nervous activity is attenuated for a given diastolic blood pressure (Halliwill *et al.*, 1996a). This resetting occurs at the level of the central nervous system, as opposed to changes in baroreceptors sensitivity, *per se* (Halliwill *et al.*, 1996b).

Reduced vasoconstrictor responses to given levels of sympathetic outflow also contribute to elevated vascular conductance following exercise (Halliwill *et al.*, 1996a). This blunted transduction of sympathetic outflow into vasoconstriction most likely results from inhibition of pre-synaptic neurotransmitter release, rather than the effects of local vasodilators on the vasculature following exercise (Hara & Floras, 1992; Halliwill *et al.*, 1996a; Halliwill *et al.*, 2003).

Recently it was discovered that ingestion of the H₁-receptor antagonist fexofenadine hydrochloride attenuates elevations in femoral vascular conductance following exercise and the effect is most pronounced during the first 30 minutes following exercise (Lockwood *et al.*, 2005b). McCord and coworkers investigated the role of H₂-receptors in postexercise vasodilation and found the H₂-receptor antagonist ranitidine blunted increases in femoral and brachial vascular conductance following exercise, with the effect most pronounced from 60-90 minutes postexercise (McCord *et al.*, 2006). Subsequently, when combined H₁- and H₂-receptor antagonists were ingested by both sedentary and endurance trained individuals, the hyperemia seen through 90

minutes postexercise in the control condition was reduced by 80% (McCord & Halliwill, 2006). Thus it has become clear H_1 - and H_2 -receptors play a key role in mediating postexercise hyperemia in both trained and untrained men and women.

Function of postexercise skeletal muscle hyperemia?

Although our understanding of the mechanisms underlying postexercise skeletal muscle hyperemia has evolved over the last few years, the function of this sustained elevation in blood flow has been elusive. In examining the relationship between leg blood flow and oxygen uptake following exercise, Williams et al. found the time course of leg blood flow recovery did not match that of oxygen uptake, suggesting the skeletal muscle hyperemia does not subserve oxygen delivery to the previously exercised muscle (Williams *et al.*, 2005).

It is possible that postexercise skeletal muscle hyperemia plays a role in glucose regulation, as skeletal muscle glucose uptake is augmented, in an insulin-independent manner, the first 90 minutes postexercise (Wasserman & Halseth, 1998). The glucose taken up following exercise contributes to glycogen repletion in humans (Casey *et al.*, 2000) and the rate of glycogen synthesis is typically highest the first 30 minutes following exercise (Price *et al.*, 1994). Furthermore, as illustrated by studies employing single-legged exercise in humans (Bergstrom & Hultman, 1966; Richter *et al.*, 1989), glucose uptake and glycogen repletion occur preferentially in the previously exercised skeletal muscle. Investigations into the effect of blood flow manipulation on glucose delivery have suggested increased limb blood flow enhances skeletal muscle glucose uptake (Hickner *et al.*, 1991; Baron *et al.*, 1994; Durham *et al.*, 2003). However, while

mechanisms surrounding both postexercise skeletal muscle hyperemia and postexercise glucose regulation have been investigated, there is a dearth of information about the relationship between these factors.

Effect of local H₁- and H₂-receptor blockade on postexercise interstitial glucose

Recently, we set out to determine the effect of local H₁- and H₂-receptor blockade, administered via skeletal muscle microdialysis, on postexercise interstitial glucose concentrations (Chapter IV). Skeletal muscle microdialysis is an elegant technique that allows continuous delivery of drugs to the interstitial space via a semi-permeable membrane, while simultaneously monitoring concentrations of various substances within the interstitium (Hickner, 2000). In addition, whereas orally administered H₁- and H₂-receptor antagonists may evoke systemic effects and induce counter-regulatory responses, muscle microdialysis allows targeted delivery of drugs and the ability to isolate a specific region from which to sample.

We predicted postexercise interstitial glucose concentrations, as determined by muscle microdialysis, would be higher in the control versus the combined H₁- and H₂-receptor antagonist sites. Immediately following 60 minutes of moderate intensity cycling exercise, subjects were placed supine and four muscle microdialysis fibers were placed into the vastus lateralis. Two probes were randomly perfused with a physiological control solution and two probes were perfused with the same solution with the addition of a combination of 1 mM H₁-receptor antagonist pyrilamine maleate and 3 mM H₂-receptor antagonist cimetidine, to block local H₁- and H₂-receptors. All four probes also contained ethanol and tritiated glucose, which served as an internal reference marker to

determine probe recovery. Ethanol readily diffuses through the probe membrane and is not metabolized by the muscle, so the ratio of ethanol recovered in the outflowing dialysate to the inflowing perfusate (outflow-inflow ratio) is an inverse surrogate of local blood flow.

Starting 15 minutes after probe insertion, dialysate was collected in microcentrifuge tubes which were replaced every 20 minutes, through 135 minutes postexercise (100 minutes post fiber insertion) and subjects consumed an oral glucose tolerance beverage half way through collection of dialysate. As illustrated in Chapter IV, Figure 3B, the ethanol outflow-inflow ratio was lower at 55 and 75 minutes postexercise in the control sites than in the blockade sites, indicating a greater relative local blood flow in the control sites. As seen in Figure 4B, interstitial glucose concentrations were also higher in the control sites at 55, 95 and 135 minutes postexercise. Taken together, these results indicate postexercise delivery of glucose to the interstitial space of the previously active skeletal muscle is mediated, in part, by local H₁- and H₂-receptors.

Effect of oral H₁- and H₂-receptor blockade on postexercise glycemic response

In addition to studying glucose delivery at the level of the skeletal muscle, we examined the effects of H₁- and H₂-receptor antagonism on systemic glucose concentrations following exercise (Chapter V). We tested the hypothesis that the glycemic response to an oral glucose load following exercise would be more sustained with oral H₁- and H₂-receptor blockade versus control. Subjects underwent oral glucose tolerance tests after 60 minutes of moderate intensity exercise on both a control and blockade day, when they received a combination of H₁- and H₂-receptor antagonists

(Allegra and Zantac). In agreement with previous research, the postexercise hyperemia, as assessed by changes in femoral vascular conductance, was markedly reduced on the blockade day. Moreover, on the blockade day the glycemic response to postexercise oral glucose load was greater 90 minutes postexercise. This sustained glycemic response during blockade suggests a histaminergic effect on postexercise glucose regulation.

Proposed interactions between postexercise hyperemia and glucose regulation

Illustration 2 shows the proposed relationship between blood flow and glucose regulation following exercise. As shown in the illustration, when the H₁- and H₂-receptor antagonists (represented by the encircled b) bind to the H₁-receptors, located on the vascular endothelium or H₂-receptors, located on the vascular smooth muscle of the resistance vessel, the normal histaminergic dilation (presumably mediated by histamine, represented by the encircled h) is diminished. This results in reduced blood flow and thus reduced glucose delivery to the capillaries, where diffusion of nutrients occurs. When interpreting the results of the two previous studies, it is likely postexercise skeletal muscle hyperemia plays a significant role in glucose delivery, as local blockade reduces interstitial glucose concentration and oral blockade augments arterialized blood glucose concentration.

It is intuitive to consider the delivery of glucose itself as a potential key purpose of postexercise hyperemia. However, perhaps just as important to the regulation of blood glucose is the delivery of insulin to the microvasculature, where it can impose both its hemodynamic and metabolic effects. Along these lines, physiological concentrations of insulin have been shown to evoke increased capillary recruitment in humans (Coggins *et*

al., 2001). This insulin-mediated capillary recruitment is dependent upon nitric oxide, as nitric oxide synthase inhibition attenuates microvascular recruitment and, importantly, insulin-mediated skeletal muscle glucose uptake (Vincent *et al.*, 2003). Transport of insulin from the central circulation to the skeletal muscle microvasculature is required to initiate this capillary recruitment; therefore anything that promotes insulin transport to the interstitium should accelerate muscle glucose uptake. Skeletal muscle glucose uptake tracks the timeframe of interstitial insulin levels better than plasma insulin levels, which suggests insulin delivery to the interstitium is crucial in timely muscle glucose uptake (Vincent *et al.*, 2005). This notion is supported by a recent study by Chiu and coworkers who found intramuscular injection of insulin resulted in an immediate rise in hindlimb glucose uptake in dogs (Chiu *et al.*, 2008).

Evidence suggests insulin-mediated capillary recruitment occurs quickly, while insulin-mediated increases in limb blood flow are slower, and delayed relative to muscle glucose uptake (Vincent *et al.*, 2004). Only about 30% of capillaries are perfused at rest (Honig *et al.*, 1982), which implies a flow limitation to skeletal muscle insulin and/or glucose delivery under resting conditions (Vincent *et al.*, 2005), as opposed to conditions in which capillary recruitment is increased. In fact, it has been suggested in situations where skeletal muscle glucose uptake is elevated, increases in limb blood flow will increase insulin delivery to facilitate further glucose delivery, especially when capillary recruitment is augmented (Clerk *et al.*, 2004). In Illustration 2 the dashed lines branching off of the arteriole represent unrecruited capillaries, as a result of attenuated insulin delivery to the microvasculature during H₁- and H₂-receptor blockade.

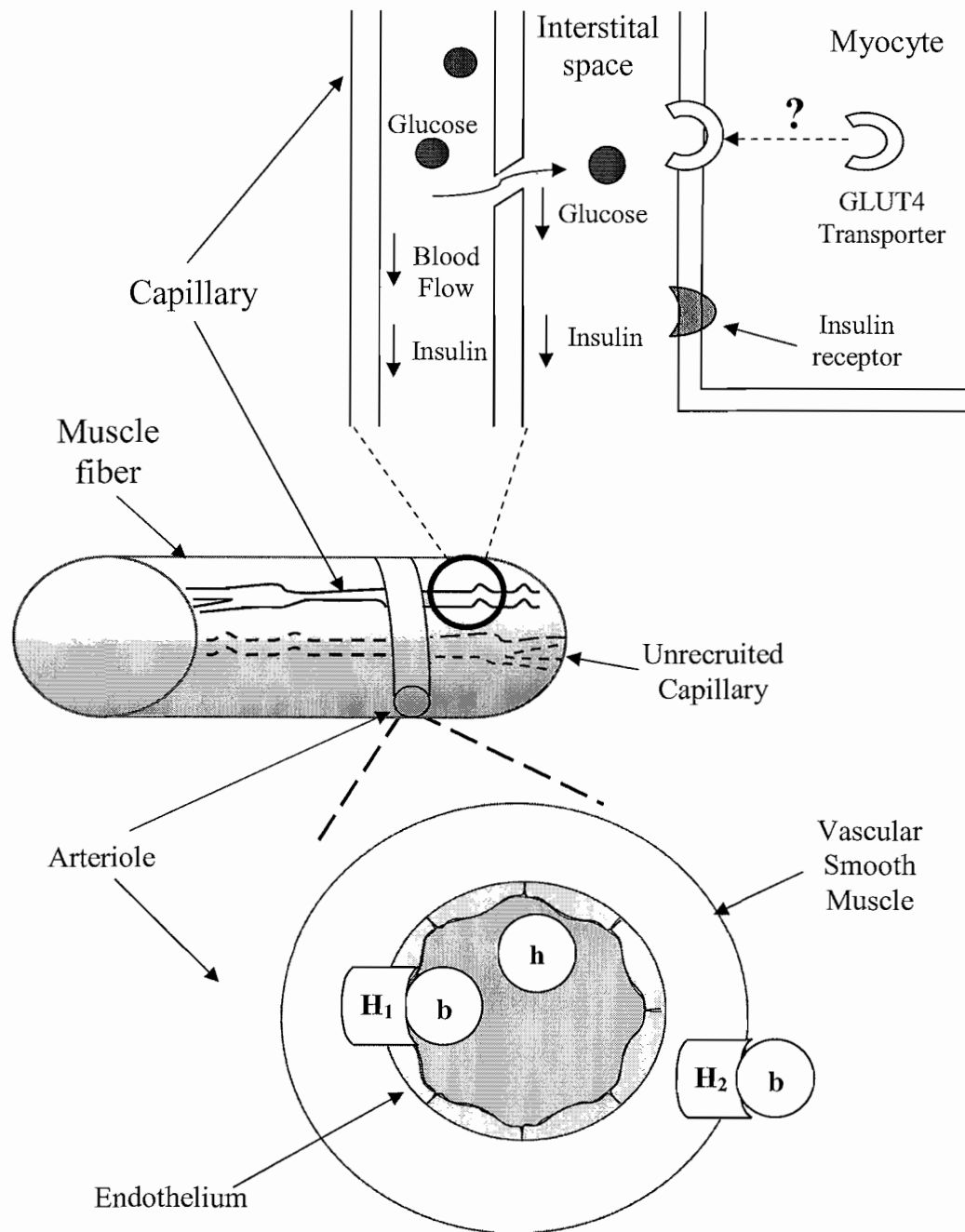


Illustration 2. Proposed interactions between postexercise hyperemia and glucose regulation. The encircled b's represent H₁- and H₂-receptor antagonists. The encircled h represents histamine.

In addition to reduced capillary recruitment, blunted insulin delivery will adversely impact GLUT4 translocation to the skeletal muscle cell surface, thereby inhibiting glucose transport into the myocyte. Thus, as previously suggested, it seems insulin is not only a mediator of its own delivery (Vincent *et al.*, 2005) but it appears both insulin-mediated vasodilation and capillary recruitment are key regulators of muscle glucose uptake. Therefore, since insulin mediates glucose uptake via metabolic and vasculature actions, postexercise skeletal muscle hyperemia likely facilitates this process by enhancing delivery of insulin to the microvasculature when the stimulus for glucose uptake is high, such as following exercise. If this insulin delivery is hindered by H₁- and H₂-receptor antagonists, that could help explain both the prolonged glycemic response and the reduced interstitial glucose concentrations in the face of H₁- and H₂-receptor blockade.

Another factor that may help explain both the sustained glycemic response during oral H₁- and H₂-receptor blockade and the reduced interstitial glucose concentrations during local H₁- and H₂-receptor blockade is the potential effect of H₁- and H₂-receptor antagonists on vascular permeability. If capillaries demonstrate a histaminergic increase in permeability following exercise, diffusion of glucose to the muscle cells would be expected to decline during H₁- and H₂-receptor blockade, resulting in less delivery and greater recirculation of glucose. Histamine evokes a rapid and transient increase in microvascular permeability (van Hinsbergh & van Nieuw Amerongen, 2002) that is mediated by H₁-receptors and may be reversed by administration of H₁-receptor antagonists (Niimi *et al.*, 1992). While this effect has been typically observed in the post-

capillary venules (Svensjo & Grega, 1986) and in studies on cultured human umbilical vein endothelial cells (Niimi *et al.*, 1992; Ikeda *et al.*, 1999), there is a lack of compelling evidence H₁-receptors mediate increases in permeability at the level of the capillary. Furthermore, permeability responses to histamine differ between arterial and venular vessels, as histamine has been shown to evoke an H₂-receptor mediated reduction in vascular permeability in cultured bovine aortic endothelial cells (Ikeda *et al.*, 1999). Therefore, while histaminergic effects on capillary permeability may play a role in postexercise glucose kinetics, there is currently limited evidence to lend credence to this notion.

Future directions

Although the recent investigations discussed in this review have shed some light on interactions between skeletal muscle hyperemia and glucose regulation following exercise, there are still a myriad of questions to be answered. There is much to learn about the events downstream of glucose transport, as studies utilizing muscle biopsy or magnetic resonance spectroscopy to examine how postexercise skeletal muscle glycogen concentrations are affected by H₁- and H₂-receptor antagonists are warranted. Studies utilizing the triple-tracer method or investigations measuring glucose uptake across the leg following exercise will allow investigators to see the relationship between blood flow and glucose regulation in a new light. Furthermore, studying how postexercise blood flow and glucose regulation impacts fat metabolism is highly relevant, given the rise in obesity in the United States.

In addition to employing cutting-edge techniques to probe these questions, varying interventions and populations may be studied. For example, how might different durations and intensities or even modes of exercise, such as resistance training, affect the relationship between postexercise blood flow and glucose regulation? Would this relationship be different in trained versus untrained individuals or change in response to chronic training? Moreover, how might this relationship change in those who may already have compromised blood flow or glucose regulation, such as overweight individuals, or those suffering from diabetes? This becomes a particularly compelling question, given the nearly epidemic rise in the diagnoses of diabetes in the United States. There seems to be no shortage of questions to ask and studies to conduct in this exciting area of research.

APPENDIX A

ETHANOL ASSAY PROCEDURES

This procedure is derived from Robert Hickner's original work on the Ethanol Perfusion Technique for use with microdialysis and by correspondence with Hickner. It uses alcohol dehydrogenase to break down ethanol (EtOH) by the chemical reaction:



Preparation of Ethanol / Ringer's Perfusate for use with Microdialysis:

500 ml of Ringer's Solutions with ethanol, lactate, and glucose (3 mmolar dextrose, 0.5 mmolar lactate, 5 mmolar ethanol)

You will need the following:

- Ringer's Injection, 500 ml (Solution) (Hypoxia Storage)
- Lactated Ringer's Injection, 500 ml (Hypoxia Storage)
- Ringer's Injection with 5% Dextrose, 500 ml (Hypoxia Storage)
- Ethanol, 200 Proof, 1 Gallon Container (Flammables Cabinet)
- 1 - 1 ml Monoject Tuberculin Syringe w/ Detachable Needle (IV Cart)
- 1 - 60 ml Monoject Syringe (IV Cart)
- 1 - 20 ml Monoject Syringe (IV Cart)
- 2 - 10 ml Monoject Syringe (IV Cart)
- 3 - 16 Gauge x 38.1 mm (1 ½ inch) Needles (IV Cart)
- 1 - 25 Gauge x 50.8 mm (2 inch) Needle (IV Cart)
- 1 - Electronic Balance (reads to .0001 g)

-It is important that this solution remain sterile. Wear gloves and use only sterile equipment.

-Find the appropriate amount of ethanol to add to the Ringer's Solution using the equation:

$$\text{Desired molar concentration EtOH (46.068 g / 1 mole)} * (1 \text{ L} / 1000 \text{ ml}) * 500.15 \text{ ml} = \\ \text{Desired \# of grams EtOH per 500.15 ml of solution.}$$

$$\text{Example: } 0.005 \text{ mole/L EtOH (46.068 g EtOH / 1 mole)} * (1 \text{ L} / 1000 \text{ ml}) * 500.15 \text{ ml} = \\ \underline{0.1152 \text{ g per 500.15 ml of solution}}$$

-Taking the Ringer's Injection draw out 24 ml using the 60 ml syringe and discard.

- Draw out 15 ml of Lactated Ringer's Injection using the 20 ml syringe and set aside.
- Draw out 9 ml of Ringer's Injection with 5% Dextrose using the 10 ml syringe and set aside.
- Using the other 10 ml syringe w/ 25 gauge needle draw up 1-2 ml of ethanol.
- Remove the needle from the 10 ml syringe.
- From the 10 ml syringe draw up approximately 0.1 ml of ethanol using the 1 ml tuberculin syringe.
- Remove the air bubbles from the 1 ml syringe and push out the ethanol used to flush the needle and syringe.
- Mass the flushed syringe and needle on the balance and tare.
- Draw approximately 0.14 ml of ethanol into the syringe and mass. You need 0.1152 g of ethanol and 0.14 ml should be close to this. (For a 5 mmolar solution 500.15 ml in volume you need 0.1152 g of ethanol and ethanol has a density of 0.789 g/ml so; $0.1152 \text{ g} (1 \text{ ml} / 0.789 \text{ g}) = 0.146 \text{ ml}$)
- Inject the ethanol into the Ringer's Injection then add the 15 ml of Lactated Ringer's Injection and 9 ml of Ringer's Injection with 5% Dextrose.
- This solution is then ready for use.

Preparation of Ethanol Assay for use with Microdialysis:

140 ml of Glycine-Hydrazine Buffer w/ NAD (**G-H B w/ NAD**) @ pH 9.0 (Glycine (22.0 mmolar), Sodium Pyrophosphate Decahydrate (74.6 mmolar), Hydrazine Sulfate (50.0 mmolar), and NAD (0.54 mmolar))

100 ml of Ammonium Sulfate Buffer (**ASB**) (Ammonium Sulfate (22 mmolar))

1 ml Alcohol Dehydrogenase (ADH) Solution (**ADS**) (1 ml Ammonium Sulfate Buffer, 1.1 mg ADH)

You will need the following:

- 1 vial (50 mg) β -nicotinamide adenine dinucleotide (NAD^+) (Freezer)
- 4.6463 g Sodium Pyrophosphate Decahydrate ($\text{Na}_4\text{O}_7 \cdot 10 \text{ H}_2\text{O}$) (Chem. Cabinet)
- 0.2306 g Glycine ($\text{C}_2\text{H}_5\text{NO}_2$) (Chem. Cabinet)
- 0.9084 g Hydrazine Sulfate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{SO}_4$) (Chem. Cabinet)
- 0.2907 g Ammonium Sulfate ($(\text{NH}_4)_2\text{SO}_4$) (Chem. Cabinet)
- 0.0011 g Alcohol Dehydrogenase (lyophilized powder, 314 units/mg) (Sub 80 Freezer)
- 50 ml 25% w/v NaOH (prepare from 50 % w/v in the flammables cabinet)
- 250 ml Deionized Water
- 1 - 200 ml Beaker
- 1 - 150 ml Beaker
- 1 - 10 ml Screw Top Vial
- 1 - 200 ml Screw Top Glass Container

- 1 - Rainin 1000 L Pipette
- 1 - Box Rainin 1000 L Pipette Tips
- 1 - Electronic Balance (reads to .0001 g)
- 1 - Stir Bar
- 2 - Magnetic Stirrers
- 1 - Electronic pH meter (preferably with a long probe)
- 1 - Ring Stand
- 1 - Ring Stand Clamp

For the G-H B w/ NAD:

- Remember that you are handling chemicals that can splash and spill. Wear gloves and goggles. Mind the warning labels on the chemicals used.
- Calibrate the pH meter that will be used to track the pH of this solution (refer to manual for instructions).
- Add 100 ml of DI water to a 200 ml beaker along with the stir bar.
- Set the 200 ml beaker on the stir plate and set up the pH probe in the ring stand clamp such that it is submerged in the water but does not touch the stir bar or the beaker surfaces.
- Set up the pH meter for continuous monitoring or begin by running a baseline reading to make sure the probe is working properly and the pH of the water is around 7.
- Mass out the Glycine (0.2306 g), Hydrazine Sulfate (0.9084 g), and Sodium Pyrophosphate (4.6464 g) using weigh boats and the electronic balance. Be cautious when handling the Hydrazine Sulfate as it is toxic.
- Start the magnetic stirrer and add the chemicals.
- Add an additional 30 ml of DI water to the 200 ml beaker. Check the pH.
- Add dropwise the 25% w/v NaOH while monitoring the pH. When the pH reaches a value of 9.0 stop (**Note:** it may be necessary to use a higher % w/v NaOH solution to achieve a pH of 9.0 without going over 140 ml).
- Fill the beaker with DI water to the 135 ml mark.
- Remove the stir bar from the 200 ml beaker as it takes up approximately 1.3 ml of volume. Use a scoopula or stir rod for the final adjustments. (**Note:** the pH will change very slowly here. Be sure to monitor constantly and stir well to ensure that you have not gone over pH 9.0)
- Adjust the pH to 9.0 if it is lower and then remove the pH probe. The pH should not change significantly when filling the beaker with DI water to the 140 ml mark.
- Check the pH of the solution. It should be at or very near 9.0.
- If the solution is for immediate use, mass out and add the NAD. Check the pH again.
- If the solution is for later use do not add the NAD, wait until the solution is to be used then add the NAD.**
- Pour the contents of the beaker into the 200 ml screw top glass container.
- The prepared solution w/ NAD has a shelf life of approximately 1 ½ -2 weeks under refrigeration. Adjust the volume of the solution prepared to what is expected to be used during this period.

For ASB:

- Remember that you are handling chemicals that can splash and spill. Wear gloves and goggles. Mind the warning labels on the chemicals used.
- Add 50 ml of DI water to a 150 ml beaker along with the stir bar.
- Mass out the Ammonium Sulfate (0.2907 g).
- Turn on the magnetic stirrer and place the Ammonium Sulfate in the 150 ml beaker.
- After the solution is mixed add DI water to the 150 ml beaker to the 100 ml mark.
- Pour this solution into a storage vessel and place under refrigeration.
- The Ammonium Sulfate Buffer is stable at room temperature indefinitely and 100 ml should be good for 100 enzyme preparations. Each enzyme preparation should then be good for approximately 20 sample assays, so a 100 ml preparation of Ammonium Sulfate Buffer should be enough for approximately 2000 sample assays.

For ADS:

-The solution should be made during or just before the ethanol assay procedure due to its short shelf life.

- Weight out 1.1 mg of ADH (this amount is based on a total activity of approximately 332 units of ADH per 1 ml of solution and as lyophilized ADH activity of 314 units/mg) into the small screw top vial (*can also use a 1.5 ml microcentrifuge tube*).
- Add 1 ml of the Ammonium Sulfate Buffer using the Rainin 1000 L Pipette to the vial.
- Mix by twisting the vial along the vertical axis. Do not shake or invert as this will cause the ADH crystals to stick to the vial walls. The final product should be a slightly discolored liquid.
- The **ADS has a life of 90 minutes when stored between 0 and 25 degrees Celsius.** This vial is good for approximately 20 sample assays.

Ethanol Assay Procedure:

You will need the following:

- Dialysate Samples
- Glycine-Hydrazine Buffer w/ NAD (G-H B w/ NAD)
- Alcohol Dehydrogenase Solution (ADS)
- Plastic Test Tubes w/ Stoppers (glass tubes may be used)
- Plastic 1.5 ml Fluorometer Cuvettes (clear on all sides)
- 1 Box Rainin 1000 L Pipette Tips (blue box)
- 1 Box Rainin 200 L Pipette Tips (green box)
- 1 Box Rainin 20 L Pipette Tips (red box)
- 1 Rainin 1000 L Pipette
- 1 Rainin 200 L Pipette
- 1 Rainin 20 L Pipette

- 1 Rectangular, Heat-Safe Container w/ at least 1000 ml capacity
- 1 Hot Plate w/ Magnetic Stirrer
- 1 Magnetic Stirrer
- 1 Thermometer, Centigrade
- 1 Ring Stand
- 1 Ring Stand Clamp
- 1 Test Tube Rack
- 1 Fluorometer (or a spectrometer capable of reading absorbance at 340 nm)
- 1 Sharpie Marker

- Remember that you are handling chemicals that can splash and spill. Wear gloves and goggles. Mind the warning labels on the chemicals used.
 - Procure the needed supplies and arrange them on the counter in the way that seems most appropriate for you to perform the procedure that follows.
 - Allow the Glycine-Hydrazine Buffer w/ NAD and Ammonium Sulfate Buffer to warm to room temperature before use. Also make sure to use the magnetic stirrer to mix these solutions thoroughly particularly the G-H B.
 - To prepare the water bath, fill the rectangular container ~ half way with warm water, add a stir bar, and place on the hot plate.
 - Position the ring stand and clamp such that it can hold the thermometer in the bath container without it contacting the container's surface or the stir bar.
 - Set the hot plate to between LOW and 2. The water temperature should be 37 degrees Celsius. Add cold water to the bath if it gets above this and turn off the hot plate.
 - Turning on the stirrer will ensure even heating of the water.
 - Label the test tubes and arrange them in the rack.
 - To each test tube add 2 ml of the Glycine-Hydrazine Buffer w/ NAD using the 1000 L pipette.
- If not already done, prepare the ADH solution.
- Add 50 microliters of ADH solution to each test tube using the 200 L pipette.
 - Stopper the tube and invert to mix contents.
 - Add 5 microliters of the appropriate dialysate to each test tube using the 20 L pipette and stopper the tube. The alcohol content of the dialysate is extremely low so it is very important the tubes be sealed to prevent the sample from evaporating. (**Note:** when using such small volumes make sure to puncture the surface of the sample liquid and visually confirm that a sample was obtained. Surface tension can keep you from drawing a sample. *Depress, submerge, release*)
 - Mix the contents by inverting the test tube several times.
 - Place the test tubes, within the test tube rack, in the bath so that they remain vertical and the solution is submerged in the water but the top of the test tubes are not. You may lay a flat object of sufficient weight on top of the tubes w/in the rack, to prevent them from floating
 - Incubate the test tubes at 37 degrees Celsius for 25 minutes (25 degrees Celsius for 75 minutes also works) making sure to keep the temperature stable.

- During this time label the cuvettes you will be using. It is also a good idea to wipe down the outer surface of the cuvettes with an alcohol swab to remove fingerprints and any film that may interfere with the fluorometry readings.
- It is important that you don't shake the tubes as air bubbles and shock affect the ADH.
- During this procedure the sample preparations may have to be staggered depending on the number of samples and the measurement technique being used.
- Once the incubation period is over remove the test tubes and invert one last time.
- Pour the contents of each test tube into the appropriately labeled cuvette.
- Take the fluorometric or spectroscopic reading following the protocol appropriate for the device used.
- If using fluorometry, use a 360 nm excitation filter & a 460 nm emission filter
- Using UV spectroscopy the absorbance of NADH (a product of the break down of ethanol by ADH) at 340 nm will increase with increased alcohol concentration.
- For either fluorometry or spectroscopy, readings can be referenced to a standard curve created based on known concentrations of ethanol
- Therefore, we have included mixing procedures for ethanol standards, used in creating a standard curve, below. In addition, although we haven't used the calculations on the following page, we have included them for your information.

Ethanol standard mixing procedures

- Ethanol stock solution: Mix 5.8 μ L 200 proof ethanol with 10 ml DI water for [10 mM] ethanol. This will serve as the highest point on the standard curve. Keep covered to avoid evaporation of ethanol.
- Perform 2 subsequent 10-fold dilutions (e.g., mix 1 ml stock solution with 9 ml DI water to obtain 1 mM solution) to attain the remaining ethanol concentrations for standard curve:
 - 1 mM
 - 0.1 mM

Calculation methods:

To calculate the amount of ethanol in a given sample there are two methods which are outlined in the instruction sheet for there NAD-ADH Reagent Multiple Test Vials.

- The first method uses a 0.08% standard to calculate the ethanol concentration of a sample using the formula:

$$\text{Alcohol (mg/dl)} = A_{340 \text{ Sample}} / A_{340 \text{ Standard}} * \text{Concentration of Ethanol Standard (mg/dl)}$$

So if a sample has an absorbance at 340 nm of 0.3492 and the 0.08% standard (convert to mg/dl by multiplying w/v by 1000) has an absorbance of 0.2136 then the equation would be:

$$\text{Alcohol (mg/dl)} = (0.3492 / 0.2136) * (0.08\% \text{ w/v} * 1000) = 130.79 \text{ mg/dl}$$

To convert the answer from mg/dl to mmole/l multiply by 0.217 so:

$$\mathbf{130.79 \text{ mg/dl} * 0.217 = 28.4 \text{ mmole/l}}$$

□ The other method uses the absorbance of the sample only and uses the following formula:

$$\text{Alcohol (mg/dl)} = A_{340} * [(A * B * C) / (D * E * F * G)]$$

A = Total reaction volume (ml)

B = Molecular weight of ethanol (46.068)

C = Conversion of ml to dl (100)

D = Millimolar absorbtivity of NADH at 340 nm (6.22)

E = Volume of sample (ml)

F = Light path of cuvet (cm) (usually 1 cm)

G = Conversion of ml to liter (1000)

So for an A_{340} of 0.3492 using the above technique;

$$A = 1.052 \text{ ml}$$

$$B = 46.068$$

$$C = 100$$

$$D = 6.22$$

$$E = 0.002 \text{ ml}$$

$$F = 1 \text{ cm}$$

$$G = 1000$$

So;

$$\text{Alcohol (mg/dl)} = 0.3492 * [(1.052 * 46.068 * 100) / (6.22 * 0.002 * 1 * 1000)] = \mathbf{136 \text{ mg/dl}}$$

APPENDIX B**INFORMED CONSENT FORM - IRB# C2-116-08F****TITLE: Pilot study for microdialysis in skeletal muscle****INVESTIGATOR: Dr. J. R. Halliwill and Colleagues****APPROVED BY INSTITUTIONAL REVIEW BOARD: November 8, 2007**

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this study being done?

We are performing this pilot study which involves use of an experimental procedure to develop our ability to recover substances out of skeletal muscle. We have used this technique in skin and we are now adapting this method to use in skeletal muscle.

What will happen in the study?

1. You will arrive at Dr. Halliwill's laboratory in room 166 in Esslinger Hall at the University of Oregon for a one-day experiment. This experiment will take approximately six hours. You will meet with one of the investigators of the study to discuss the project, to see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. You will need to wear a t-shirt and refrain from eating for one hour prior to arrival. In addition, you will need to refrain from consuming caffeine (for example, coffee, tea, energy drinks like Red Bull, or soda like Coke, which contain caffeine) or medications (except oral contraceptives) for 12 hours prior to the study and abstain from alcohol or exercise for 24 hours prior to the study. If you use oral contraceptives, you should take this when you normally do so.
2. During the study visit, your heart rate will be monitored by electrocardiogram electrodes placed on your skin. Your blood pressure will be measured at periodic intervals by the inflation of a blood pressure cuff around your arm.
3. You will have 4-5 small probes (these are called "microdialysis fibers") placed into the vastus lateralis (outer thigh) muscle of your leg. First, the area of skin where the

probes will enter and exit will be numbed with a local anesthetic. Then a small needle will be placed through the skin and through the muscle, exiting back out about 2-3 inches from where it entered your skin and muscle. The small probes will be passed through the needle, and then the needle will be withdrawn, leaving the small probes passing through your muscle. The two probes will be separated by about 1 inch. These will remain in place throughout the rest of the study.

4. We will need to wait about 1-2 hours after the small probes are placed in your muscle to let the insertion trauma (redness of your skin around the small probes) to go away. During this time we will infuse a solution into the probes but you should not feel anything with this infusion.

5. After the insertion trauma passes, we will put small doses of several drugs through the small probes in your muscle. The drugs you may receive include the following:

a. Ethanol alcohol. This is used to determine how much blood flow muscle receives. You should not feel anything when this drug infuses into your muscle.

b. Histamine. This substance is naturally produced by your body and will cause blood vessels to open in the local area of muscles around the probe. You should not feel anything when this drug infuses into your muscle. However, it is possible you may feel an itching sensation combined with redness to your skin where the probe is located approximately 30 minutes after the infusion starts.

c. Compound 48/80. This is a substance that releases histamine, which is naturally produced by your body, which will cause blood vessels in that local area of muscles to open. You should not feel anything when this drug infuses into your muscles. However, it is possible you may feel an itching sensation combined with redness to your skin where the probe is located approximately 30 minutes after the infusion starts.

d. Cromolyn (a common ingredient in over the counter allergy remedies). This drug blocks the release of histamine. You should not feel anything when this drug infuses into your muscle.

e. Pyrilamine (a common ingredient in over the counter cold remedies). This drug blocks the effects of histamine and will prevent blood vessels in muscle from opening up if histamine is present. You should not feel anything when this drug infuses into your muscle.

f. Cimetidine (brand name Tagament). This drug blocks the effects of histamine and will prevent blood vessels in muscle from opening up if histamine is present. You should not feel anything when this drug infuses into your muscle.

g. Sodium nitroprusside. This drug will cause blood vessels in the local area of muscle to open up. You should not feel anything when this drug infuses into your muscle.

6. After the study, we will remove the flexible probes and a bandage will be placed over that area of skin. You will then be sent home.

7. You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit study. Some examples of discomfort include shortness of breath, light-headedness, and nausea.

How long will I be in the study?

You will be in the study for one day. The study will last six hours. You will need to refrain from eating for one hour prior to the study.

What are the risks of the study?

1. Muscle Microdialysis: There may be some discomfort during the insertion of the small probes into your muscle. We will use a local anesthetic to numb skin where the probes will be inserted to minimize this discomfort, but you may feel pressure or a dull ache in the muscle as the needle moves through the muscle. The infusions through the fiber should not be painful, and there should only be minor swelling at the sites. At the end of the study, the fibers will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study, but you may feel some muscle soreness for several days. You will be given some ibuprofen (e.g. Advil or Motrin) to relieve some of the initial soreness. Although the small probes are sterile, there is a slight risk of infection at the sites where the probes were placed. You will be instructed on how to keep the area clean for a day or two following the study and will need to inform the researchers immediately if you have any redness or swelling in the area. The researchers will also ask you to return to the lab the day after the study visit so they can see how the sites are healing.
2. Infusions: We will be infusing very small doses of each drug and only into a very small area of your muscle. You will not have any systemic (whole body) effects of these drugs in the doses given in this study. There is a minimal risk of an allergic reaction to these drugs.

May I participate if I am pregnant or breast-feeding?

This study may be harmful to an unborn or breast-fed child. There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child in a woman who takes part in this study. Breast-feeding mothers are not able to take part in this study. Women who can still become pregnant must have a negative pregnancy test no more than 24 hours before taking part in the protocol orientation trial on the initial visit. If the pregnancy test is positive (meaning that you are pregnant), you will not be able to take part in the study. There is no cost for the pregnancy test.

Are there benefits to taking part in this study?

This study will not make your health better. The muscle dialysate fluid samples are not being collected for diagnostic purposes. The results will not be reviewed by a physician and no action will be taken if a laboratory result falls outside of the normal range.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not pay for any tests or procedures that are done just for this research study. You will get \$60 for participating in this study. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the study has ended, the amount of money you receive will be pro-rated at a rate of \$10 per hour of the study you complete.

Who can answer my questions?

You may talk to Dr. John Halliwill at any time about any question you have on this study. You may contact Dr. Halliwill by calling the Department of Human Physiology at (541) 346-5425.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with The University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel	Office for Protection of Human Subjects
Office of the President	University of Oregon
University of Oregon	Eugene, OR 97403
Eugene, OR 97403	(541) 346-2510
(541) 346-3082	

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be \$100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive \$500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a “subject identification number”. The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Halliwill’s office. The list of names will be destroyed when study results are published or 24 months after your participation, whichever comes first. All blood samples will be destroyed when study results are published or 24 months after your participation, whichever comes first.

The Office for Responsible Conduct of Research and/or authorized representatives of the Food and Drug Administration (FDA) or National Institute of Health may need to review records of individual subjects. As a result, they may see your name; but they are bound by rules of confidentiality not to reveal your identity to others.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact the Office for Protection of Human Subjects, 5237 University of Oregon, Eugene, OR 97403, 541/346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

(Date)

(Signature of Participant)

(Date)

(Signature of Individual Obtaining Consent)

APPENDIX C**INFORMED CONSENT FORM - IRB# C2-294-08F-3****TITLE: Interstitial glucose concentrations following exercise****INVESTIGATOR: Dr. J. R. Halliwill and Colleagues****APPROVED BY INSTITUTIONAL REVIEW BOARD: June 7, 2007**

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

When humans exercise, there is an increase in blood flow to the skeletal muscle. This increase in blood flow lasts for several hours after exercise but we do not understand all the mechanisms that cause this increase in blood flow or why it happens. The purpose of this study is to provide more information on what causes blood flow increases to occur and to determine whether or not this blood flow is involved in metabolism.

What will happen in the study?**Initial visit**

1. You will arrive at Dr. Halliwill's laboratory in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately two hours. You will meet with one of the investigators of the study to discuss the project, to see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. You will be given a physical activity questionnaire, which will allow investigators to determine your activity level. The questionnaire will take approximately 15 minutes to complete. You will need to wear a t-shirt and refrain from eating for two hours prior to arrival. In addition, you will need to refrain from consuming caffeine (for example, coffee, tea, red bull, coke, etc.) or medications (except oral contraceptives) for 12 hours prior to the study and abstain from alcohol or exercise for 24 hours prior to the study. If you use oral contraceptives, you should take this when you normally do so.

2. If you are a woman who can still become pregnant, you will be asked to undergo a pregnancy test during the initial visit (and again during the study visit). For this test, you will be asked to collect a sample of urine in the women's restroom near the physiology lab. If the test is "positive," indicating that you are pregnant, you will not be allowed to participate and will be advised to see your physician or the University of Oregon Health Center.
3. Graded Exercise Test: You will pedal on an exercise bicycle while wearing a mouth piece, nose clip, and electrocardiogram electrodes (heart rhythm monitor). If you are a woman, the adhesive patches used for this test will be placed on you by one of the women in the lab. After a 5-minute warm-up, you will be asked to maintain a selected pedaling rate as pedaling resistance (work) is increased every minute until you reach your maximum exercise capacity. This is to measure your overall aerobic fitness level. It normally takes 10 to 15 minutes for people to reach their maximum effort. The total time for this test (including placement of ECG electrodes, warm-up, exercise, and cool-down) is approximately one hour. This session will serve to familiarize you with the procedures to be used on the study visit. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study visit.
4. You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit study. Some examples of discomfort include shortness of breath, light-headedness, and nausea.
5. This session will serve to familiarize you with the procedures to be used on the study day. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study day. At this time you will be reminded not to donate blood or to participate in other research studies where they will be drawing blood for 8 weeks.

Study visit

1. You will then return to Dr. Halliwill's laboratory to participate in the two study visits, one of which will be between 7 and 10 days after the initial visit and the other will be between 7 and 10 days after that. These two testing sessions will take approximately five hours each. You will need to wear a t-shirt, shorts, and refrain from eating at least two hours prior to arrival.
2. If you are a woman who can still become pregnant, you will be asked to undergo a pregnancy test during the study visit.
3. During the study visit, your heart rate will be monitored by electrocardiogram electrodes placed on your skin. If you are a woman, the adhesive patches used for this test will be placed on you by one of the women in the lab. Your blood pressure will be measured at periodic intervals by the inflation of a blood pressure cuff around your arm.
4. During the study visit, you will periodically breathe small amounts of acetylene gas mixed with air through a mouthpiece. Acetylene gas is an inert gas that is not harmful in any way to people and it is not flammable in the concentrations that are

- used in the lab. This is used to study how much work the heart is performing. Periodically, a small probe (ultrasound-Doppler probe) will be held over an artery at your groin-hip intersection. The ultrasound-Doppler probe uses ultrasound waves to measure blood flow in the artery.
5. Venous Catheter: You will have one small flexible needle (“intravenous catheter”) placed into a vein in the back of your hand. The skin will be sterilized before this procedure. This catheter will remain in your skin throughout the five hour study. During the study, this hand will be placed in a warming chamber to heat the skin of your hand to about 108°F which will feel warm but should not cause discomfort. Several blood samples will be collected from the catheter during the five hour study. In total, 150 ml will be withdrawn during the visit. This is approximately five ounces (a little more than half a cup) and about a third of the amount withdrawn when you volunteer to “give blood” for Lane Memorial Blood Bank or similar blood donation programs. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin.
 6. Bicycle exercise session: You will be asked to pedal on a bicycle at a moderate rate for one hour on one of the study visits. On the other study visit, you will not exercise but will sit and rest for a few minutes.
 7. Muscle Microdialysis: You will have 4-5 small probes (these are called “microdialysis fibers”) placed into the vastus lateralis (outer thigh) muscle of your leg. This will be done after you have exercised (or rested). First, the area of skin where the probes will enter and exit will be numbed with a local anesthetic (lidocaine/xylocaine with epinephrine). Then a small needle will be placed through the skin and through the muscle, exiting back out about 2-3 inches from where it entered your skin and muscle. The small probes will be passed through the needle, and then the needle will be withdrawn, leaving the small probes passing through your muscle. This process of inserting a small needle through the skin and muscle to place a probe will be repeated 4 or 5 times, once for each of the probes that is placed. The probes will be separated by about 1 inch. These will remain in place throughout the rest of the study. There may be some discomfort during the insertion of the small probes into your muscle. We will use a local anesthetic to numb skin where the probes will be inserted to minimize this discomfort, but you may feel pressure or a dull ache in the muscle as the needle moves through the muscle. The infusions through the fiber should not be painful, and there should only be minor swelling at the sites.
 8. Microdialysis infusions: We will put small doses of several drugs through the small probes in your muscle. The drugs you may receive include the following:
 - a. Ethanol alcohol. This is used to determine how much blood flow muscle receives. You should not feel anything when this drug infuses into your muscle.
 - b. Pyrilamine (a common ingredient in over the counter cold remedies). This drug blocks the effects of histamine and will prevent blood vessels in the muscle from opening up if histamine is present. You should not feel anything when this drug infuses into your muscle.

- c. Cimetidine (brand name Tagament). This drug blocks the effects of histamine and will prevent blood vessels in the muscle from opening up if histamine is present. You should not feel anything when this drug infuses into your muscle.
 - d. Radioactive nuclide. Tritiated glucose is a radioactive labeled form of glucose, a sugar. It is being used to help measure the levels of glucose in your muscle. You should not feel anything when this drug infuses into your muscle.
9. Oral Glucose Tolerance Test: You will be given a flavored concentrated sugar-water beverage to drink. You will be asked to drink up to 10 ounces.
 10. At the end of the study visit, the fibers will be withdrawn from your leg and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study, but you may feel some muscle soreness for several days. You will be given some ibuprofen (e.g. Advil or Motrin) to relieve some of the initial soreness. Although the small probes are sterile, there is a slight risk of infection at the sites where the probes were placed. You will be instructed on how to keep the area clean for a day or two following the study and will need to inform the researchers immediately if you have any redness or swelling in the area. The researchers will also ask you to return to the lab the day after the study visit so they can see how the sites are healing.
 11. You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit or the study visit. Some examples of discomfort include shortness of breath, light-headedness, and nausea.

How long will I be in the study?

You will be in the study for three days (initial visit and two study visits). The initial visit will last two hours. The study visits will last five hours each. You will need to refrain from eating for two hours prior to the study visits.

What are the risks of the study?

1. Graded exercise testing: There is some minor discomfort associated with exercise testing, including temporary fatigue, shortness of breath, and muscle soreness. These sensations resolve within minutes after the test is completed. There is the possibility of some residual muscle soreness in the few days following the exercise test. There is also the risk of a heart attack or death during an exercise test. The risk of a complication requiring hospitalization is about 1 incident in 1000. The risk of a heart attack during or immediately after an exercise test is less than 1 incident in 2500. The risk of death during or immediately after an exercise test is less than 1 incident in 10,000. In the unlikely case of a life-threatening heart rhythm, the laboratory is equipped with an Automatic Electronic Defibrillator that is located in the same room where the study is taking place. Dr. Halliwill has up to date Advanced Cardiac Life

- Support (ACLS) training. In the event of an emergency, the Department of Public Safety (6-6666) will be called in order to activate the emergency medical system (i.e., 911) and will direct an ambulance to the correct location.
2. Intravenous catheters: There may be some discomfort during the insertion of the small flexible needle into your vein. Once the needle is in place, the pain should subside. Blood sampling through the needle should not be painful, and there should only be minor swelling at the site. At the end of the study, the needle will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the needle is sterile, there is a slight risk of infection at the site where the needle was placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. The most common complications of inserting a small needle into a vein are a small bruise and pain at the site of the needle location which may last several days after removal of the catheter.
 3. Blood withdrawal: In total, 150 ml will be withdrawn during the study visit. This is approximately five ounces (a little more than half a cup) and about a third of the amount withdrawn when you volunteer to "give blood" for Lane Memorial Blood Bank or similar blood donation programs. You will not be allowed to donate blood for 8 weeks before the study, or for 8 weeks after the study.
 4. Muscle Microdialysis: There may be some discomfort during the insertion of the small probes into your muscle. We will use a local anesthetic to numb skin where the probes will be inserted to minimize this discomfort, but you may feel pressure or a dull ache in the muscle as the needle moves through the muscle. The infusions through the fiber should not be painful, and there should only be minor swelling at the sites. At the end of the study, the fibers will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study, but you may feel some muscle soreness for several days. You will be given some ibuprofen (e.g. Advil or Motrin) to relieve some of the initial soreness. Although the small probes are sterile, there is a slight risk of infection at the sites where the probes were placed. You will be instructed on how to keep the area clean for a day or two following the study and will need to inform the researchers immediately if you have any redness or swelling in the area. The researchers will also ask you to return to the lab the day after the study visit so they can see how the sites are healing.
 5. Infusions: We will be infusing very small doses of each drug and only into a very small area of your muscle. You will not have any systemic (whole body) effects of these drugs in the doses given in this study. There is a minimal risk of an allergic reaction to these drugs.
 6. Radioactive nuclide: This research study involves exposure to radiation from tritiated glucose, a radioactive labeled form of glucose. This radiation exposure is not necessary for your medical care and is for research purposes only. The total amount of radiation that you will receive in this study is equivalent to a uniform whole body exposure of less than 1 day of exposure to natural background radiation. For comparison, the amount of radiation you would receive from a chest X-ray is

equivalent to 5 days of exposure and the amount of radiation you would receive from a dental X-ray is equivalent to 11 days of exposure. This use involves minimal risk and is necessary to obtain the research information desired.

7. Oral Glucose Tolerance Test: Drinking the concentrated sugar-water beverage can lead to nausea, vomiting, diarrhea, or fainting in some individuals. You should notify the investigator immediately if you feel any significant discomfort after drinking this beverage.

May I participate if I am pregnant or breast-feeding?

This study may be harmful to an unborn or breast-fed child. There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child in a woman who takes part in this study. Breast-feeding mothers are not able to take part in this study. Women who can still become pregnant must have a negative pregnancy test no more than 24 hours before taking part in the graded exercise test on the initial visit and each of the two study visits. If the pregnancy test is positive (meaning that you are pregnant), you will not be able to take part in the study. There is no cost for the pregnancy test.

Are there benefits to taking part in this study?

This study will not make your health better. Blood and fluid samples collected from microdialysis are not being collected for diagnostic purposes. The results will not be reviewed by a physician and no action will be taken if a laboratory result falls outside of the normal range.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not need to pay for any tests or procedures that are done just for this research study. You will get \$120 for participating in this study. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the study has ended, the amount of money you receive will be pro-rated at a rate of \$10 per hour of the study you complete.

Who can answer my questions?

You may talk to Dr. John Halliwill at any time about any question you have on this study. You may contact Dr. Halliwill by calling the Department of Human Physiology at (541) 346-5425.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with the University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel	Office of Human Subjects Compliance
Office of the President	University of Oregon
University of Oregon	Eugene, OR 97403
Eugene, OR 97403	(541) 346-2510
(541) 346-3082	

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be \$100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive \$500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a "subject identification number". The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Halliwill's office area.

The Office for Responsible Conduct of Research and/or authorized representatives of the Food and Drug Administration (FDA) or National Institute of Health (NIH) may need to review records of individual subjects. As a result, they may see your name, but they are bound by rules of confidentiality not to reveal your identity to others.

The list of names will be destroyed when study results are published or 24 months after your participation, whichever comes first. All blood and muscle dialysate fluid samples will be destroyed when study results are published or 24 months after your participation, whichever comes first. Other information may be stored by the researchers indefinitely.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Office for Protection of Human Subjects, 5237 University of Oregon, Eugene, OR 97403, (541) 346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

(Date)

(Signature of Participant)

(Date)

(Signature of Individual Obtaining Consent)

APPENDIX D**INFORMED CONSENT FORM - IRB# A548-07F**

TITLE: Short-term benefits of exercise on cardiovascular-metabolic integration in humans (Protocol 3)

INVESTIGATOR: Dr. J. R. Halliwill and Colleagues

APPROVED BY INSTITUTIONAL REVIEW BOARD: February 6, 2008

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign the form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

Why is this research study being done?

When humans exercise, there is an increase in blood flow to the skeletal muscle. This increase in blood flow lasts for several hours after exercise but we do not understand all the mechanisms that cause this increase in blood flow or why it happens. The purpose of this study is to provide more information on what causes blood flow increases to occur and to determine whether or not this blood flow is involved in metabolism.

What will happen in the study?

Initial visit

1. You will arrive at Dr. Halliwill's laboratory in Esslinger Hall at the University of Oregon for an initial visit. This initial visit will take approximately two hours. You will meet with one of the investigators of the study to discuss the project, to see the laboratory, and to read this form. Your height and weight and resting blood pressure will be measured, and you will be asked questions about your health history. You will be given a physical activity questionnaire, which will allow investigators to determine your activity level. The questionnaire will take approximately 15 minutes to complete. You will need to wear a t-shirt and refrain from eating for one hour prior to arrival. In addition, you will need to refrain from consuming caffeine (for example, coffee, tea, red bull, coke, etc.) or medications (except oral contraceptives) for 12 hours prior to the study and abstain from alcohol or exercise for 24 hours prior

- to the study. If you use oral contraceptives, you should take this when you normally do so.
2. If you are a woman who can still become pregnant, you will be asked to undergo a pregnancy test during the initial visit (and again during the study visits). For this test, you will be asked to collect a sample of urine in the women's restroom near the physiology lab. If the test is "positive," indicating that you are pregnant, you will not be allowed to participate and will be advised to see your physician or the University of Oregon Health Center.
 3. Graded Exercise Test: You will pedal on an exercise bicycle while wearing a mouth piece, nose clip, and electrocardiogram electrodes (heart rhythm monitor). If you are a woman, the adhesive patches used for this test will be placed on you by one of the women in the lab. After a 5-minute warm-up, you will be asked to maintain a selected pedaling rate as pedaling resistance (work) is increased every minute until you reach your maximum exercise capacity. This is to measure your overall aerobic fitness level. It normally takes 10 to 15 minutes for people to reach their maximum effort. The total time for this test (including placement of ECG electrodes, warm-up, exercise, and cool-down) is approximately one hour. This session will serve to familiarize you with the procedures to be used on the study visits. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study visits.
 4. You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit study. Some examples of discomfort include shortness of breath, light-headedness, and nausea.
 5. This session will serve to familiarize you with the procedures to be used on the study day. It will also establish your maximal exercise tolerance on a bike and therefore will be used to establish the appropriate workload for the exercise session on the study day. At this time you will be reminded not to donate blood or to participate in other research studies where they will be drawing blood for 8 weeks.

Study visits

1. You will then return to Dr. Halliwill's laboratory to participate in the two study visits, one of which will be between 7 and 10 days after the initial visit and the other will be between 7 and 10 days after that. These two testing sessions will take approximately six hours each. You will need to wear a t-shirt, shorts, and refrain from eating at least three hours prior to arrival.
2. If you are a woman who can still become pregnant, you will be asked to undergo a pregnancy test during the study visits.
3. On one of the study visits you will be given a 540 mg dose of fexofenadine hydrochloride (brand name Allegra) and a 300 mg dose of ranitidine hydrochloride (brand name Zantac).
4. During the study visits, your heart rate will be monitored by electrocardiogram electrodes placed on your skin. If you are a woman, the adhesive patches used for this test will be placed on you by one of the women in the lab. Your blood pressure

- will be measured at periodic intervals by the inflation of a blood pressure cuff around your arm.
5. During the study visits, you will periodically breathe small amounts of acetylene gas mixed with air through a mouthpiece. Acetylene gas is an inert gas that is not harmful in any way to people and it is not flammable in the concentrations that are used in the lab. This is used to study how much work the heart is performing. A small probe (laser-Doppler probe) will be placed over an area of skin on your forearm and another on your thigh. The laser-Doppler probe uses light to measure skin blood flow in these areas, and is taped in place. Periodically, a small probe (ultrasound-Doppler probe) will be held over an artery at your elbow and an artery at your groin-hip intersection. The ultrasound-Doppler probe uses ultrasound waves to measure blood flow in these arteries.
 6. Venous Catheter: You will have one small flexible needle (“intravenous catheter”) placed into a vein in the back of your hand. The skin will be sterilized before this procedure. This catheter will remain in your skin throughout the six hour study. During the study, this hand will be placed in a warming chamber to heat the skin of your hand to about 108°F which will feel warm but should not cause discomfort. Several blood samples will be collected from the catheter during the six hour study. In total, 300 ml will be withdrawn during over the two visits. This is approximately ten ounces (a little more than a cup) and about two-thirds of the amount withdrawn when you volunteer to “give blood” for Lane Memorial Blood Bank or similar blood donation programs. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin.
 7. Bicycle exercise session: During the study visits, you will pedal on a bicycle at a moderate rate for 1 hour.
 8. Glucose Tolerance Tests: To see how your body handles glucose (sugar), you will be given a “glucose tolerance test.” The test will be given either orally or intravenously. For an oral test, you will be given a flavored concentrated sugar-water beverage to drink. You will be asked to drink up to 10 ounces. For an intravenous test, the researchers will inject a similar amount of sterile concentrated sugar-water into a catheter in a vein in your arm over the course of 2-4 minutes. The researchers will decide which type of test you will receive.
 9. Local heating: Towards the end of the study visit, we will warm the skin around the laser-Doppler probes with small heating devices. We will heat the skin in these areas to about 108°F for a period of 40 minutes. This will feel warm but should not cause discomfort.
 10. After the study, we will remove the flexible needle in your vein and a bandage will be placed over that area of skin as well. You will then be sent home.
 11. You should notify the investigator immediately if you feel any significant discomfort or concern about your well-being at any time during the initial visit or the study visit. Some examples of discomfort include shortness of breath, light-headedness, and nausea.

How long will I be in the study?

You will be in the study for three days (initial visit and two study visits). The initial visit will last two hours. The study visits will last six hours. You will need to refrain from eating for three hours prior to the study visits.

What are the risks of the study?

1. Graded exercise testing: There is some minor discomfort associated with exercise testing, including temporary fatigue, shortness of breath, and muscle soreness. These sensations resolve within minutes after the test is completed. There is the possibility of some residual muscle soreness in the few days following the exercise test. There is also the risk of a heart attack or death during an exercise test. The risk of a complication requiring hospitalization is about 1 incident in 1000. The risk of a heart attack during or immediately after an exercise test is less than 1 incident in 2500. The risk of death during or immediately after an exercise test is less than 1 incident in 10,000. In the unlikely case of a life-threatening heart rhythm, the laboratory is equipped with an Automatic Electronic Defibrillator that is located in the same room where the study is taking place. Dr. Halliwill has up to date Advanced Cardiac Life Support (ACLS) training. In the event of an emergency, the Department of Public Safety (6-6666) will be called in order to activate the emergency medical system (i.e., 911) and will direct an ambulance to the correct location.
2. Intravenous catheters: There may be some discomfort during the insertion of the small flexible needle into your vein. Once the needle is in place, the pain should subside. Blood sampling through the needle should not be painful, and there should only be minor swelling at the site. At the end of the study, the needle will be withdrawn and a sterile dressing will be applied. Any swelling or redness after the study should be gone a few hours after completion of the study. Although the needle is sterile, there is a slight risk of infection at the site where the needle was placed in your skin. You will be instructed how to keep the area clean for a day or two following the study. The most common complications of inserting a small needle into a vein are a small bruise and pain at the site of the needle location which may last several days after removal of the catheter. There is a possibility of a blood clot forming in the vein used for the infusion and this could make the vein painful and swollen; however, it is extremely unlikely that this would result in a serious health risk to you.
3. Fexofenadine Hydrochloride: If you are allergic to fexofenadine hydrochloride (brand name Allegra) you will not be allowed to participate in the study. On one of the study visits you will be given a 540 mg dose of fexofenadine hydrochloride. A dose of 180 mg fexofenadine hydrochloride is commonly prescribed. Doses up to 690 mg twice daily for a month showed no adverse effects in experimental studies.
4. Ranitidine Hydrochloride: If you are allergic to ranitidine hydrochloride (brand name Zantac) you will not be allowed to participate in the study. On one of the study visits you will be given a 300 mg dose of ranitidine hydrochloride. A dose of 300 mg

ranitidine hydrochloride is commonly prescribed. Studies have shown that doses as high as 400 mg in a single day have no adverse effects.

5. Blood withdrawal: In total, 300 ml will be withdrawn during over the two visits. This is approximately ten ounces (a little more than a cup) and about two-thirds of the amount withdrawn when you volunteer to "give blood" for Lane Memorial Blood Bank or similar blood donation programs. You will not be allowed to donate blood for 8 weeks before the study, or for 8 weeks after the study.
6. Oral Glucose Tolerance Test: This test transiently raises blood sugar levels. Drinking the concentrated sugar-water beverage can lead to nausea, vomiting, diarrhea, or fainting in some individuals. You should notify the investigator immediately if you feel any significant discomfort after drinking this beverage.
7. Intravenous Glucose Tolerance Test: This test transiently raises blood sugar levels. Having an injection of concentrated sugar-water can lead to generalized flushing, mild shoulder pain, or whole body warming. These symptoms generally subside within 10 minutes. The test can also produce mild swelling at the site of injection. You should notify the investigator immediately if you feel any significant discomfort after the injection.

May I participate if I am pregnant or breast-feeding?

This study may be harmful to an unborn or breast-fed child. There is not enough medical information to know what the risks might be to a breast-fed infant or to an unborn child in a woman who takes part in this study. Breast-feeding mothers are not able to take part in this study. Women who can still become pregnant must have a negative pregnancy test no more than 24 hours before taking part in the graded exercise test on the initial visit. If the pregnancy test is positive (meaning that you are pregnant), you will not be able to take part in the study. There is no cost for the pregnancy test.

Are there benefits to taking part in this study?

This study will not make your health better. Blood samples are not being collected for diagnostic purposes. The results will not be reviewed by a physician and no action will be taken if a laboratory result falls outside of the normal range.

What other choices do I have if I don't take part in this study?

This study is only being done to gather information. You may choose not to take part in this study.

What are the costs of tests and procedures?

You will not need to pay for any tests or procedures that are done just for this research study. You will get \$140 for participating in this study. This money is for the inconvenience and time you spent in this study. If you start the study but stop before the

study has ended, the amount of money you receive will be pro-rated at a rate of \$10 per hour of the study you complete.

Who can answer my questions?

You may talk to Dr. John Halliwill at any time about any question you have on this study. You may contact Dr. Halliwill by calling the Department of Human Physiology at (541) 346-5425.

What are my rights if I take part in this study?

Taking part in this research study is your decision. You do not have to take part in this study, but if you do, you can stop at any time. Your decision whether or not to participate will not affect your relationship with the University of Oregon.

You do not waive any liability rights for personal injury by signing this form. All forms of medical diagnosis and treatment whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop medical complications from participating in this study.

The investigators may stop you from taking part in this study at any time if it is in your best interest, if you do not follow the study rules, or if the study is stopped.

If you are physically injured because of the project, you and your insurance company will have to pay your doctor bills. If you are a UO student or employee and are covered by a UO medical plan, that plan might have terms that apply to your injury.

If you experience harm because of the project, you can ask the State of Oregon to pay you. If you have been harmed, there are two University representatives you need to contact. Here are their addresses and phone numbers:

General Counsel	Office of Human Subjects Compliance
Office of the President	University of Oregon
University of Oregon	Eugene, OR 97403
Eugene, OR 97403	(541) 346-2510
(541) 346-3082	

A law called the Oregon Tort Claims Act limits the amount of money you can receive from the State of Oregon if you are harmed. The most you could receive would be

\$100,000, no matter how badly you are harmed. If other people are also harmed by the project, all of you together could only receive \$500,000.

What about confidentiality?

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission. Subject identities will be kept confidential by assigning you a “subject identification number”. The names associated with each subject identification number will be kept in a locked file cabinet in Dr. Halliwill’s office area.

The Office for Responsible Conduct of Research and/or authorized representatives of the Food and Drug Administration (FDA) or National Institute of Health (NIH) may need to review records of individual subjects. As a result, they may see your name, but they are bound by rules of confidentiality not to reveal your identity to others.

The list of names will be destroyed when study results are published or 24 months after your participation, whichever comes first. All blood and muscle dialysate fluid samples will be destroyed when study results are published or 24 months after your participation, whichever comes first. Other information may be stored by the researchers indefinitely.

I have had an opportunity to have my questions answered. I have been given a copy of this form. I agree to take part in this study.

If you have questions regarding your rights as a research subject, contact Office for Protection of Human Subjects, 5237 University of Oregon, Eugene, OR 97403, (541) 346-2510.

Your signature indicates that you have read and understand the information provided above, that you willingly agree to participate, that you may withdraw your consent at any time and discontinue participation without penalty, that you will receive a copy of this form, and that you are not waiving any legal claims, rights or remedies.

(Date)

(Signature of Participant)

(Date)

(Signature of Individual Obtaining Consent)

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