

**Effects of Altering the Sequence of a Combined Aerobic and Resistance Exercise Session on
Energy Expenditure and Metabolism**

by

Megan Joy Bedbrook

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Despite the known benefits of performing aerobic and resistance exercise independently, the metabolic effects of performing aerobic and resistance exercise in succession, remain unclear. Several studies suggest that the alteration of exercise sequence may influence carbohydrate and lipid oxidation and energy expenditure during exercise and in recovery. High intensity resistance exercise performed prior to a bout of aerobic exercise has been shown to augment fat oxidation during the subsequent bout of aerobic exercise. Changes in hormone and metabolite concentrations from prior resistance exercise could potentially influence substrate selection and energy expenditure in a subsequent bout of aerobic exercise. However, an exercise session whereby aerobic exercise is followed by a bout of resistance exercise has yet to be evaluated to determine the metabolic effects (specifically, the differences in substrate selection for energy provision) when exercise sequence is altered. It was hypothesized that when resistance exercise was performed prior to a bout of aerobic exercise, sympathetic nervous system activity would be elevated, leading to an increase in non-esterified fatty acid (NEFA) and glycerol concentrations and resultant increase in lipid oxidation during the aerobic portion of the exercise compared to the opposite sequence. It was also hypothesized that during recovery there would be an increased reliance on fat oxidation for energy provision with a resistance-aerobic exercise sequence compared to an aerobic-resistance exercise sequence. Additionally, the differences in metabolite concentrations and respiratory parameters between two identical bouts of aerobic exercise performed on separate days (~1 week apart) were measured and it was hypothesized that day-to-day variability would be non-significant ($p > 0.05$). Plasma glucose, lactate, NEFA, glycerol, insulin, C-peptide, glucagon, epinephrine and norepinephrine concentrations in addition to oxygen consumption (VO_2) and respiratory exchange ratio (RER)

were measured in nine healthy, recreationally active males that participated in 3 different, randomized exercise trials (Trial A: aerobic exercise; Trial AR: aerobic exercise followed by a bout of resistance exercise; Trial RA: resistance exercise followed by an aerobic exercise bout). The aerobic exercise bout was performed at 60% $\text{VO}_{2\text{max}}$ for 30 min while the resistance exercise bout consisted of 5 exercises (overhead squat, chest press, triceps extension, shoulder press, and dead-lift) performed for 3 sets of 8 repetitions at 70% 1-RM. Contrary to the primary hypothesis, NEFA concentrations and lipid oxidation rates were similar for the aerobic exercise bout of both the AR and RA trials. During recovery, lipid oxidation was elevated immediately post-exercise in the RA trial compared to the AR trial, however there were no differences between trials by 15 min post-exercise. Furthermore, only epinephrine, and not norepinephrine, concentrations were significantly higher after aerobic exercise in the RA trial compared to the AR trial. VO_2 and energy expenditure values were similar for the duration of the 30 min recovery. These results suggest that while exercise sequence may influence carbohydrate and lipid oxidation immediately post exercise, substrate selection and utilization are similar during aerobic exercise bouts irrespective of the sequence in which aerobic and resistance exercise are performed. Thus, when resistance exercise is performed prior to aerobic exercise, compared to the opposite sequence, overall energy provision is not altered at the volume and intensity of exercise performed in this study.

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1.0 Introduction to the Regulation of Substrate Metabolism in Whole Body and in Skeletal Muscle

1.1 The Importance of Regulation of Substrate Metabolism in Mixed Aerobic-Resistance Exercise: An Overview

It is well established that exercise stimulates increased carbohydrate and lipid oxidation in skeletal muscle during exercise and in post-exercise recovery (DeFronzo et al, 1981; Melby et al, 1993; Romijn et al, 1993b; Romijn et al, 1995; Petitt et al, 2003; van Loon et al, 2003; Durham et al, 2004; Dreyer et al, 2008; Singhal et al, 2009). While these studies investigated substrate utilization in aerobic and resistance exercise independently, there is increasing interest in evaluating substrate metabolism during mixed aerobic-resistance exercise sessions (Goto et al, 2007; Kang et al, 2009). Investigating combined aerobic-resistance exercise sessions will advance our understanding of substrate metabolism and energy expenditure, and may be applied to weight loss and maintenance programs. Goto et al (2007) found that resistance exercise administered prior to an aerobic bout of exercise resulted in a greater increase in lipolysis during the subsequent aerobic activity compared to aerobic activity alone. Similarly, participants in a study by Kang et al (2009) completed resistance exercise of varying intensities (i.e. high versus low intensities) prior to engaging in a bout of aerobic exercise. The results of this study demonstrated that a preceding bout of high intensity—but not low intensity— resistance exercise augmented lipid oxidation during a subsequent bout of aerobic exercise. However, the opposite exercise sequence (i.e. aerobic followed by resistance exercise) has yet to be evaluated to determine the metabolic effects of exercise sequence on substrate selection for energy provision.

Changes in hormone and metabolite concentrations from prior exercise (e.g. increased catecholamines and decreased glucose resulting from a bout of resistance exercise) could potentially influence substrate selection and energy expenditure in a subsequent bout of exercise (Goto et al, 2007). When aerobic and resistance exercise are performed in succession, a more thorough understanding is needed of: 1) the potential differences in carbohydrate and lipid oxidation during and post-exercise when the sequence of aerobic and resistance exercise is altered, and 2) the potential mechanisms for determining the type and proportion of substrate involved in energy provision during different sequences of exercise. The knowledge gained from this area of research can be applied to design exercise programs that promote weight loss and weight maintenance in healthy, and possibly disease populations.

1.2 Regulation of Whole Body Substrate Metabolism: General Effects of Rest, Exercise and Dietary Influences

Insulin, glucagon and catecholamines (i.e. epinephrine and norepinephrine) have important roles in oxidizing substrates for energy provision (Bitensky et al, 1968; DeFronzo et al, 1981; Jiang and Zhang, 2003). To maintain energy provision (i.e. during exercise or following an overnight fast), low plasma insulin concentrations result in a reduced inhibition of intracellular lipases, which increase adipose tissue lipolysis and non-esterified fatty acid (NEFA) mobilization into systemic circulation (Goto et al, 2007b). Increased NEFA mobilization enhances NEFA availability for skeletal muscle uptake, and subsequent oxidation. Skeletal muscle and other lean tissues, not only rely on fatty acids for energy but also on plasma glucose as an energy substrate (Johnson et al, 2010). Thus, to maintain glucose homeostasis during periods of high-energy demands and/or fasting, liver glycogenolysis and gluconeogenesis

produce glucose, which is released into circulation (Wahren et al, 1971). During a fasted state, healthy individuals exhibit low insulin concentrations, and glucagon primarily regulates plasma glucose production through gluconeogenesis (Exton et al, 1967; Lavoie et al, 1997). High glucagon concentrations signal an increase in energy demand and stimulate gluconeogenesis and glycogenolysis in liver (Exton et al, 1968; Petersen et al, 2004). In skeletal muscle, epinephrine stimulation enhances intramuscular glycogen utilization, glycolysis and carbohydrate oxidation rates (Febbraio et al, 1998). However, during fasting conditions, epinephrine circulates in low concentrations and may not be a primary contributor to glucose homeostasis at rest (Näveri et al, 1985).

In contrast, substrate selection is altered with exercise compared to resting and fasting conditions. ATP demands are elevated and are accompanied by increased catecholamine and glucagon concentrations, as well as reduced insulin concentrations (Gyntelberg et al, 1977, Näveri et al, 1985; Goto et al, 2007b). These signals ultimately result in greater glucose production and NEFA mobilization to match these energy demands. Greater substrate availability primarily occurs through increased lipolysis in adipose tissue, glycogenolysis in skeletal muscle and gluconeogenesis in the liver (Exton et al, 1972b). During low intensity exercise, when insulin levels are reduced, plasma NEFAs are the primary energy substrate oxidized by skeletal muscle (Romijn et al, 1993b). However, the greater rates of lipid oxidation occur during moderate intensity exercise (~50-75% VO_{2max}) (Romijn et al, 1993b). Despite the high reliance on lipid metabolism for energy provision, glucose uptake is still elevated for the duration of an acute bout of moderate intensity exercise. Of note, the higher the intensity of exercise, the greater the inhibition of NEFA mobilization (Romijn et al, 1993; Romijn et al, 1995), and consequently, carbohydrate oxidation becomes the dominant substrate for ATP

production (van Loon et al, 2001). Epinephrine is also elevated in direct proportion to exercise intensity, which would inhibit NEFA mobilization and promote carbohydrate oxidation at high intensity exercise. Glucagon would also stimulate liver glycogenolysis and gluconeogenesis as well as skeletal muscle glycogenolysis and glycolysis during high intensity exercise (Petersen et al, 2004; Perry et al, 2008).

Food intake at rest can also influence substrate oxidation. A high fat meal at rest will increase circulating NEFAs as well as a reliance on NEFAs as energy substrate (Griffiths et al, 1994). The presence of insulin following a high fat meal will enhance translocation of CD 36, a fatty acid transporter, to the skeletal muscle membrane for fatty acid uptake (Schwenk et al, 2010). Concomitantly, glucagon concentration is reduced, attenuating glycogenolysis in the liver and skeletal muscle, as well as liver gluconeogenesis (Jiang and Zhang, 2003). In adipose tissue, decreased glucagon and increased insulin concentrations reduce NEFA mobilization and promote triacylglycerol (TAG) storage.

Conversely, a high carbohydrate meal will increase plasma glucose concentrations, stimulating glucose uptake and providing more substrate for skeletal muscle glycolysis or for glycogen storage (Acheson et al, 1988). Increased concentrations of plasma glucose lead to the release of insulin from the pancreas and subsequent binding of insulin to receptors on skeletal muscle, liver and adipose tissue. Insulin signaling promotes glucose uptake through increased translocation of GLUT 4 to the plasma membrane (James et al, 1994); this ultimately promotes increased glycogen synthesis in the liver and skeletal muscle. Contraction-induced translocation of GLUT4 and CD36 to the plasma membrane (Schwenk et al, 2010) will promote carbohydrate and lipid uptake for oxidation when the availability of these respective substrates is elevated during exercise. However, during an acute bout of steady-state exercise, Watt et al (2002) found

that carbohydrate ingestion (1gram/kg body weight) 75 min prior to aerobic exercise did not affect oxidative metabolism. Thus, a meal ingested prior to an overnight fast will not likely influence substrate oxidation during exercise.

1.3 Regulation of Substrate Metabolism in Skeletal Muscle during Exercise

ATP homeostasis is maintained through precise coordination of energy-regulating hormones, especially insulin, glucagon and catecholamines. Additional signals regulate pathways for carbohydrate and lipid metabolism in muscle including increased cellular AMP: ATP ratio, low plasma glucose and increased intracellular calcium concentrations, which predominantly occur during exercise. Rises in intramuscular calcium concentrations stimulate the AMPK pathway (Abbott et al, 2009), which is part of a signaling cascade that increases the translocation of GLUT 4 to the plasma membrane and ultimately increases glucose availability within skeletal muscle (Kurth-Kraczek et al, 1999; Ojuka et al, 2002). GLUT 4 is the main glucose transporter isoform for skeletal muscle glucose uptake (Gaster et al, 2000); it is regulated by muscle contraction (i.e. increases in calcium concentration which stimulate AMPK) as well as by plasma insulin. Binding of insulin to the insulin receptor (IR) induces autophosphorylation of the receptor as well as phosphorylation of the insulin receptor substrate (IRS-1) (Lizcano and Alessi, 2002; Figure 1). Activated IRS-1 subsequently associates with other enzymes in the insulin-signaling cascade to ultimately activate Akt (a serine kinase; also referred to as Protein Kinase B), which then increases GLUT 4 translocation to the plasma membrane (Lizcano and Alessi, 2002; Figure 1). However, insulin concentrations are normally low during exercise, and thus, GLUT4 translocation is primarily stimulated by contraction during exercise (Kennedy et al, 1999).

Once glucose is inside the cell, hexokinase transfers an inorganic phosphate from ATP to glucose, forming glucose-6-phosphate (G6P). This reaction is irreversible, ensuring that once glucose is taken up via GLUT 4, it becomes trapped within the skeletal muscle thus maintaining a high concentration gradient for glucose to continue entering the cell. Hexokinase production is up-regulated when the AMP:ATP ratio rises during moderate-high intensity exercise (>50-60% $\text{VO}_{2\text{max}}$), signaling a need for increased energy provision (Ojuka et al, 2000).

There are additional key points of regulation in glycolysis, including phosphofructokinase (PFK) and pyruvate kinase (PK), that are sensitive to energy changes (i.e. AMP: ATP ratio) and/or hormones such as epinephrine and glucagon. Similar to hexokinase, a higher AMP: ATP ratio (Stanley and Connett, 1991) and higher plasma glucagon concentrations activate PFK, increasing glycolytic rate. Epinephrine also indirectly increases PFK activity through AMPK signaling (Michiels, 2004). Therefore, with high intensity exercise, plasma glucagon and epinephrine concentrations rise to a greater extent and further increase PFK activity to favor carbohydrate oxidation compared to lower intensity exercise. PK converts phosphoenolpyruvate formed during glycolysis to pyruvate (Figure 1). This enzyme is also energy-sensitive such that during exercise when AMP levels rise and ATP concentrations drop, PK activity is elevated (Smart and Pritchard, 1982).

Once pyruvate is formed, it has two fates: it can enter the tricarboxylic acid cycle (TCA cycle) via pyruvate dehydrogenase (PDH) or be converted to lactate by lactate dehydrogenase. At rest and during low-to-moderate intensity exercise, pyruvate is predominantly regulated by PDH (Stephens et al, 2010). PDH irreversibly converts pyruvate into acetyl-CoA, which can be oxidized in the TCA cycle (Figure 1). The PDH complex is primarily regulated by 2 enzymes: PDH phosphatase, which promotes the conversion of the PDH complex to its active form

(PDHa) and PDH kinase, which converts PDH to its inactive form (PDHb). At rest, the PDH complex is found as PDHb (Kiilerich et al, 2008). With the onset of exercise, Ca^{2+} and a rise in pyruvate activate PDH phosphatase to convert PDHb to PDHa, increasing carbohydrate oxidation. Insulin similarly increases expression of PDH phosphatase (Wang et al, 2009); with an increase in PDH activity, glucose oxidation is enhanced.

Acetyl-CoA, the end product of the PDH reaction also functions as an allosteric inhibitor of PDH (Exton, 1972). Acetyl-CoA can be derived from PDHa as well as through beta-oxidation of fatty acids. Therefore, in situations where NEFA concentration is increased (i.e. in a fasted state or low-moderate intensity and/or prolonged exercise), a rise in acetyl-CoA inhibits the conversion of pyruvate to acetyl-CoA, reducing the rate of glycogenolysis and glycolysis. Overall, there is a decline in the contribution of carbohydrate oxidation and a concomitant increase in lipid oxidation for the production of ATP (Kiilerich et al, 2010). PDH activity, and thus carbohydrate oxidation, is thought to be additionally attenuated since there is increased expression of pyruvate dehydrogenase kinase 4 (PDK4), which would imply increased PDK4 activity to phosphorylate PDHa to PDHb (Ehrenborg and Kelly, 2009) (Figure 1). PDK4 is therefore another crucial enzyme, secondary to PDH, which regulates the transition from carbohydrate to lipid metabolism (Krämer et al, 2007). PDK4 activity is increased by higher ratios of ATP: ADP and NAD: NADH, and is inversely correlated with increased exercise intensity.

The AMPK signaling pathway is also known to promote beta-oxidation indirectly through attenuation of lipid synthesis (Brusq et al, 2006). Stimulation of the AMPK pathway phosphorylates acetyl-CoA carboxylase (ACC) (Krämer et al, 2007), reducing the conversion of acetyl-CoA to malonyl-CoA and ultimately limiting the amount of substrate available for TAG

synthesis (Krämer et al, 2007). Since malonyl-CoA is also an inhibitor of carnitine palmitoyl transferase 1 (CPT1) (McGarry and Foster, 1980), a reduction of malonyl-CoA removes inhibition on CPT1, allowing the enzyme to transfer NEFAs into the mitochondria for beta-oxidation (Ehrenborg and Kelly, 2009; Sugden et al, 2010), increasing overall skeletal muscle lipid oxidation.

AMPK, which is activated by a rise in AMP, also indirectly activates PGC1-alpha, allowing PGC1-alpha to co-activate PPAR-delta (Finck and Kelly, 2006; Ehrenborg and Krook, 2009), the primary isoform regulating lipid oxidation in response to fasting and exercise conditions in skeletal muscle (Krämer et al, 2007; Sugden et al, 2010). Increases in PPAR-delta have been associated with increased oxidative enzymes (Wang et al 2004), suggesting that beta-oxidation would be elevated when PPAR-delta is increased. While there are other fatty acid transporters, such as fatty acid binding protein (FABP), CD 36 appears to be the primary fatty acid transporter responsible for NEFA uptake into skeletal muscle (Bonen et al, 1999).

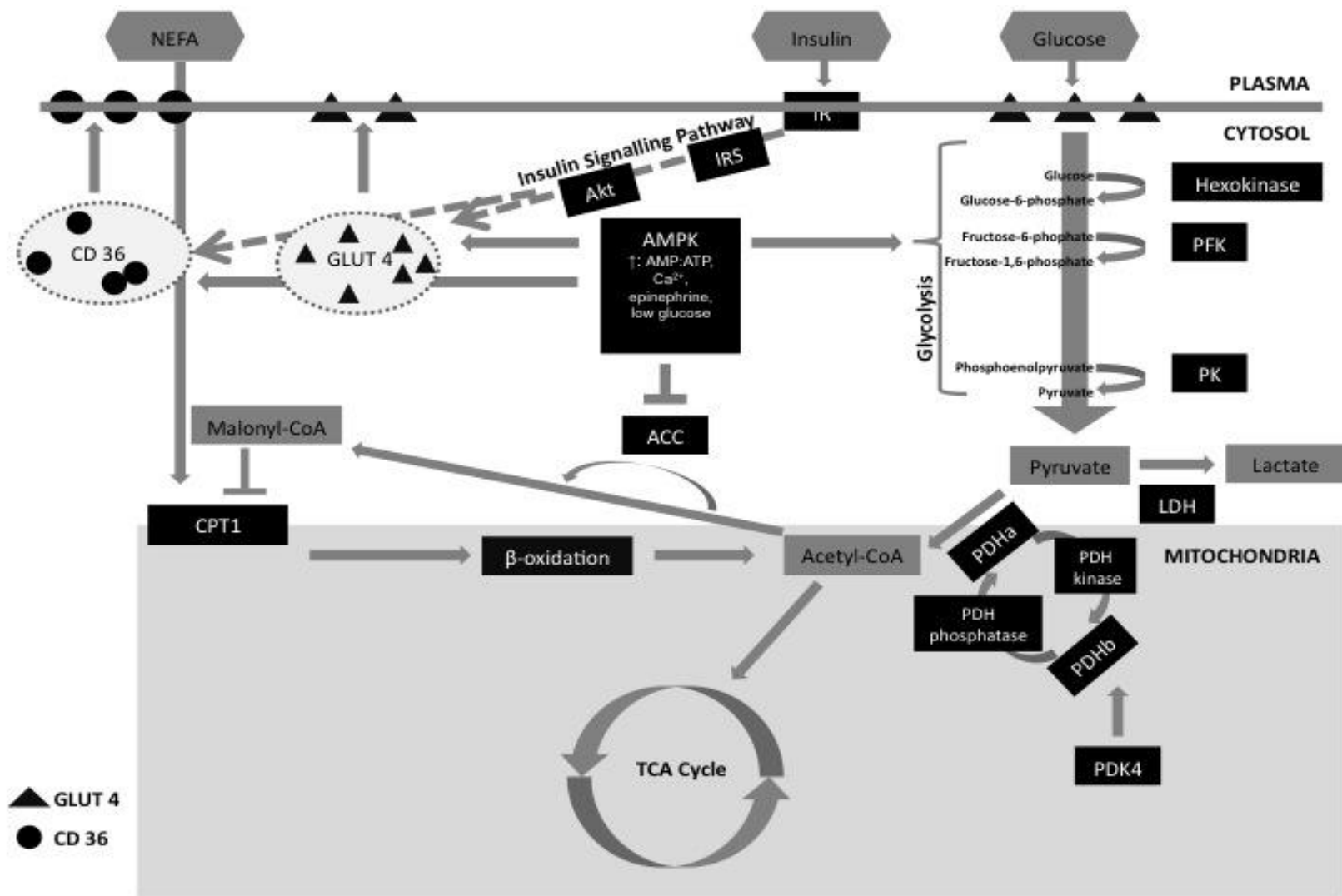


Figure 1. Schematic of a Skeletal Muscle Cell: Regulation of Glucose and Lipid Oxidation

2.0 Substrate Metabolism under Different Exercise Conditions

There are several points of regulation in glycolysis and oxidative metabolism in skeletal muscle. While the majority of work has focused on aerobic exercise metabolism, there is little work to understand the effects of resistance exercise on energy-producing pathways and substrate utilization. Although there is even less work that has investigated combined aerobic and resistance exercise, it is important to understand the potential effects on substrate oxidation and energy expenditure when the sequence in which aerobic and resistance exercise bouts are performed, is altered.

2.1 *Aerobic Exercise and Substrate Metabolism*

With the onset of muscle contraction at the initiation of aerobic exercise, there is a rise in epinephrine, intramuscular calcium concentrations and increased production of AMP and ADP through ATP utilization (Kahn et al, 2005; Goto et al, 2007b; Lee-Young et al, 2009).

Epinephrine and calcium have a stimulating effect on glycogen breakdown. At low intensities, the influx of Ca^{2+} also activates several key enzymes (eg. hexokinase and PDH phosphatase) (Koval et al, 1998; Mourtzakis et al, 2006) that increase pyruvate production and potentially carbohydrate oxidation.

Carbohydrates, from muscle glycogen and plasma glucose, contribute the majority of energy provision throughout an entire bout of high intensity aerobic exercise. Romijn et al (2000) compared carbohydrate and lipid oxidation rates in endurance trained women and found that while lipid oxidation was reduced from $43.1 \pm 3.5 \mu\text{mol/kg/min}$ at 65% $\text{VO}_{2\text{max}}$ to $30.1 \pm 3.6 \mu\text{mol/kg/min}$ at 85% $\text{VO}_{2\text{max}}$, the rate of carbohydrate oxidation increased from 106.5 ± 11.5

$\mu\text{mol/kg/min}$ at 65% $\text{VO}_{2\text{max}}$ to $232.1 \pm 15.1 \mu\text{mol/kg/min}$ at 85% $\text{VO}_{2\text{max}}$. Despite increased epinephrine and norepinephrine concentrations with increasing exercise intensities, TAG oxidation remains stimulated in muscle and adipose tissue (Hagström-Toft et al, 1998; Quish et al, 2005), but relative carbohydrate contribution to total energy provision is greater than that of fatty acids. AMPK activity is increased with more frequent muscle contraction (from working at higher exercise intensities where ATP utilization and Ca^{2+} concentration are increased) (Raney and Turcotte, 2006). Activation of the AMPK pathway increases the amount of GLUT4 translocated to the plasma membrane (Schwenk et al, 2010), which facilitates glucose uptake and ultimately carbohydrate oxidation. In fact, skeletal muscle glucose uptake increased approximately ~10-fold compared to rest in a 1-legged knee extensor incremental exercise study (Mourtzakis et al, 2008). Intramuscular glycogen stores (Romijn et al, 1993b; Romijn et al, 1995) also contribute to glucose availability for energy provision. In sync with increased glucose uptake, higher glycolytic enzyme activity (i.e. hexokinase, PFK1 and PK) is also stimulated by rises in AMP: ATP ratio, enhancing glucose oxidation at higher intensities of aerobic exercise compared to lower intensities (Romijn et al, 1993b; Lee-Young et al, 2009).

Carbohydrate oxidation contributes most appreciably to energy provision only at the initiation of low to moderate intensity exercise (Mourtzakis et al, 2006). Romijn et al (1993b) found that plasma NEFAs are the primary energy substrate oxidized by muscle during low (25% $\text{VO}_{2\text{max}}$) and moderate intensity (65% $\text{VO}_{2\text{max}}$) exercise. Lipid oxidation is favored in other acute exercise studies of moderate-intensity where catecholamine concentrations are elevated in combination with lower plasma insulin concentrations (Savard et al, 1987; Stich et al, 2000; Stallknecht et al, 2001; Mourtzakis et al, 2006). Ca^{2+} released during muscle contraction activates the AMPK pathway, which increases ACC inhibition and ultimately reduces malonyl-

CoA production (Brusq et al, 2006). With reduced production of malonyl-CoA, a CPT1 inhibitor (McGarry and Foster, 1980), NEFAs can enter the mitochondria for beta-oxidation and thus support energy provision.

During a moderate intensity exercise bout that lasts more than 30 minutes, there is a shift from carbohydrate to lipid oxidation (Romijn et al, 1993b). Goto et al (2007b) found that cycling at 60% VO_{2max} for two 30-min bouts resulted in a significant decrease in plasma glucose within the first 30 minutes of exercise, which continued to decline for the duration of the exercise. Since the decline in glucose was observed within the first 30 min, it is likely that pyruvate production was reduced, as a result of decreased AMP and ADP concentrations, which would reduce PDH activity and promote acetyl-CoA production via beta-oxidation.

Increased beta-oxidation increases acetyl-CoA concentrations, providing energy substrate for the TCA cycle as well as covalently reducing PDH activity and ultimately reducing glucose oxidation (Stephens et al, 2010). Elevated NEFA and glycerol concentrations were evidenced by Goto et al (2007b) during moderate intensity exercise, which implied that lipolysis was elevated to meet the energy demand of the exercising muscles. NEFA uptake would likely be enhanced with AMPK-induced translocation of CD36 to the plasma membrane. AMPK activation occurs during moderate intensity exercise when signals such as plasma insulin concentration are reduced and intracellular Ca^{2+} concentrations are elevated compared to rest or during low intensity exercise (Raney and Turcotte, 2006). AMPK inhibits ACC activity leading to a reduction in malonyl-CoA production and removal of the inhibition on CPT1 (Brusq et al, 2006). With CPT1 activated, fatty-acyl CoA can enter the mitochondria for beta-oxidation. While these changes occur during an acute bout of moderate intensity exercise, post-exercise substrate selection and oxidation still require further investigation.

2.2 *Resistance Exercise and Substrate Metabolism*

Resistance exercise is generally performed at a moderate to high intensity (70% 1RM to 100% 10 RM) (Melby et al, 1993; Petitt et al, 2003; Ormsbee et al, 2007; Singhal et al, 2009) and thus relies on the high-energy phosphate system and carbohydrate metabolism (i.e. intramuscular glycogen stores) for energy provision instead of fatty acid oxidation (Robergs et al, 1991; Roy and Tarnopolsky, 1998; Dreyer et al, 2008). Durham et al (2004) were the first to report that muscle glucose uptake was elevated 5-fold from pre-exercise rates when subjects performed 45 minutes of lower body resistance exercise at 70-80% 1RM (the protocol included 8 sets of 10 repetitions at 70% 1RM followed by 8 sets of 8 repetitions at 80% 1RM). Resistance exercise has also been confirmed as a potent stimulator of glucose uptake both during and after exercise (Biolo et al, 1999; Dreyer et al, 2008). One potential mechanism for augmented glucose uptake in resistance exercise is a threefold increase in blood flow, which enhances glucose extraction in the muscle (Durham et al, 2004).

In addition to muscle contraction, insulin concentrations rise slightly during resistance exercise (Dreyer et al, 2008), increasing translocation of GLUT 4 to the sarcolemma for greater glucose uptake during moderate-to-high intensity resistance exercise. Generally, the pathways that are stimulated at this intensity are similar to those activated during high intensity aerobic exercise. The AMPK pathway is activated as the AMP: ATP ratio increases; sequentially, PFK is phosphorylated in skeletal muscle resulting in glycolysis. The concomitant rise in catecholamine concentrations that also occurs with resistance exercise stimulates the muscular and extra-muscular tissues to contribute substrate for energy provision. In skeletal muscle, the rise in epinephrine along with elevated Ca^{2+} concentrations, activate glycogenolysis. High AMP concentrations from ATP utilization increase the rate of glycolytic enzymes (ex. hexokinase,

PFK1 and PK), which maintain a steady production of pyruvate. High epinephrine concentrations also increase cAMP concentrations within the liver (Cherrington and Exton, 1976) and result in increased rates of gluconeogenesis; glucose is released into circulation for delivery to exercising muscles and ultimately, for skeletal muscle glycolysis. Other tissues, such as adipose tissue, also contribute to energy provision during resistance exercise, albeit to a relatively smaller extent (Ormsbee et al, 2007). Ormsbee et al (2007) measured increased rates of lipolysis in adipose tissue during low-moderate intensity resistance exercise. This increase in NEFA availability would likely be followed with an increase in fatty acid uptake by skeletal muscle via CD36 for beta-oxidation.

2.3 Substrate Metabolism with Altered Aerobic and Resistance Exercise Sequence in a Single Exercise Session

Moderate intensity aerobic and resistance exercise independently promote increased skeletal muscle glucose uptake and rely on intramuscular glycogen stores (with relatively moderate contributions from lipid oxidation) for energy provision (Wahren et al, 1971; DeFronzo et al, 1981; Romijn et al, 1993b; Romijn et al, 1995; Roy and Tarnopolsky, 1998). From a practical perspective, resistance and aerobic exercise are prescribed to individuals to reap the benefits of both strength and cardiovascular training. These two exercises are either performed within the same exercise session or on separate days. It has been shown that the metabolic effects of resistance exercise persist for up to 15 hours post exercise (Melby et al, 1993; Petitt et al, 2003; Singhal et al, 2009) and for 24 hours after an acute bout of aerobic exercise (Magkos et al, 2007). However, there is limited work that examines the metabolic effects of performing resistance and aerobic exercise within the same session and whether there

are different metabolic effects based on the sequence in which these exercises are performed. When resistance exercise is performed immediately prior to aerobic exercise, elevated epinephrine concentrations and circulating NEFAs are documented immediately after the cessation of the resistance exercise (Goto et al, 2007). This would increase NEFA availability as a substrate for energy provision at the onset of aerobic exercise.

As such, lipid oxidation is augmented during the subsequent aerobic bout (Goto et al, 2007; Kang et al, 2009). Elevated catecholamine concentrations immediately following the resistance exercise (prior to aerobic exercise) are associated with increased circulating NEFAs (Goto et al, 2007); this is likely attributed to increased mobilization of NEFAs from adipose tissue into whole-body circulation. Thus, NEFA availability and/or uptake would likely be enhanced at the onset of aerobic exercise following a bout of resistance (Goto et al, 2007) or aerobic exercise (Goto et al, 2007b) compared to performing aerobic exercise without any preceding exercise (Goto et al, 2007). During resistance exercise, plasma insulin is elevated (Dreyer et al 2008), which would potentially stimulate translocation of CD36 to facilitate NEFA uptake into skeletal muscle (Schwenk et al 2010). With the increased availability of NEFA in muscle, more substrate would be expected to be available for CPT1 to transport into the mitochondria for beta-oxidation.

This elevation in beta-oxidation following resistance exercise supports increased lipid oxidation at the baseline of a subsequent bout of moderate intensity aerobic exercise. Continuous muscle contraction during this subsequent bout of aerobic exercise would maintain a heightened intracellular calcium concentration, further enabling the AMPK pathway to indirectly remove the inhibition of ACC on CPT1, ultimately maintaining lipid oxidation. Increased acetyl-CoA attributed to a rise in NEFA availability inhibits PDH activity to reduce carbohydrate

contribution to the TCA cycle, and ultimately, carbohydrate oxidation would be reduced in skeletal muscle (Stephens et al, 2010). Therefore, it is necessary to evaluate the changes in blood metabolites and energy-related parameters that occur when a bout of resistance exercise is performed prior to an aerobic exercise bout. The observation of alterations in substrate selection and utilization with alternate exercise sequences will provide the foundation for investigating mechanisms associated with these changes in carbohydrate and lipid metabolism. It is evident that fat oxidation is enhanced when resistance exercise is followed by aerobic exercise; however, the mechanisms for enhanced lipid oxidation remain unclear.

The opposite sequence of aerobic exercise preceding a resistance exercise bout has not been assessed to make comparisons with the alternate exercise sequence (resistance followed by aerobic exercise). With moderate intensity aerobic exercise lasting at least 30 min, NEFA mobilization occurs in concert with elevated epinephrine and reduced plasma insulin concentrations (Goto et al, 2007b). Overall, less carbohydrate is oxidized during aerobic exercise compared to resistance exercise. Thus, at the onset of resistance exercise immediately following an aerobic exercise bout, NEFA uptake via CD 36 translocation would likely be reduced due to reduced plasma insulin concentrations, as compared to the reverse sequence. Since plasma glucose concentration is maintained (Goto et al, 2007) while plasma insulin concentration is reduced, carbohydrate oxidation will be the primary contributor to energy provision and thus additional uptake of NEFA for energy provision is not expected at the onset of resistance exercise. Thus, the baseline of a resistance bout of exercise preceded by an aerobic bout of exercise would likely not be different from rest and therefore not provide an advantage for enhanced substrate oxidation and/or energy expenditure.

Further investigation is needed to compare two different sequences (i.e. resistance followed by aerobic exercise and vice versa), with identical work volumes, to reveal any differences between the net changes in energy provision and substrate selection. This comparison will also provide greater insight into the mechanisms that influence substrate selection and utilization.

2.4 Post-Exercise Recovery and Substrate Metabolism

During recovery from a bout of aerobic exercise, fat oxidation continues to be elevated to a greater extent than carbohydrate oxidation (van Loon et al, 2003). Sustained elevation of NEFAs (Bahr et al, 1991; Kiens and Richter, 1998; Goto et al, 2007b; Henderson et al, 2007; Magkos et al, 2007) persist for up to 24 hours after cessation of aerobic exercise (Magkos et al, 2007), which suggests increased mobilization of fatty acids from adipose tissue and/or reduced uptake of NEFAs by skeletal muscle. Plasma glycerol concentrations decline (Goto et al, 2007b) suggesting there may be depressed mobilization of fatty acids from adipose tissue and/or increased uptake of glycerol by liver for gluconeogenesis, which would be accompanied by a rise in glucagon. Epinephrine and norepinephrine are reduced in concert with increased insulin levels up to 30 minutes after cessation of aerobic activity (Goto et al, 2007b). Taken together, increased mobilization of fatty acids from adipose tissue likely occurs despite decreases in glycerol. The decline in glycerol is likely explained by a greater rate of uptake by liver relative to its release from adipose tissue. With the redistribution of blood flow following exercise, more blood flow is likely re-distributed to the liver (Schoemaker et al, 1998), while there is a reduced flow to the skeletal muscles (compared to increased blood flow during exercise (Durham et al, 2004)), which may enhance glycerol uptake by the liver. This in turn, would also explain the

elevated plasma NEFA concentrations that are reported (Goto et al, 2007b) as the rate of skeletal muscle uptake would likely be attenuated in relation to the reduced blood flow following exercise. However, the rise in insulin post-exercise would potentially continue to facilitate NEFA uptake via translocation of CD 36, as elevated catecholamine concentrations immediately post-exercise promote NEFA mobilization from adipose tissue.

During recovery from aerobic exercise, respiratory exchange ratio (RER) decreases, suggesting an increased reliance on fat oxidation post-exercise (Kuo et al, 2005). It is thought that fat oxidation will provide the energy required for glycogen re-synthesis via glycogen synthase (Bruce et al, 2001). Glycogen synthesis is controlled by substrate, covalent and allosteric modification. In particular, altered concentrations of glucose, G6P, insulin and glycogen have significant implications on glycogen synthesis (Graham and Shearer, 2002). Low glycogen content can itself stimulate glycogen synthase activity. Increased insulin concentrations, which are observed post-exercise, also activate glycogen synthesis, and facilitate GLUT4 translocation for glucose uptake. Once inside the muscle cell, glucose requires ATP to produce G6P using hexokinase; G6P ultimately becomes the key substrate and regulator of glycogen synthesis. To maintain adequate glucose supply for increased glycogen synthesis post-exercise (Wojtaszewski et al, 2003), lactate from the preceding exercise is a precursor for liver gluconeogenesis.

With increased glycogen re-synthesis following exercise, carbohydrate oxidation is simultaneously reduced (Richter et al, 1989; van Loon et al, 2003). Lower skeletal muscle AMP:ATP concentrations as well as lower intracellular Ca^{2+} and plasma epinephrine concentrations reduce glycolytic and glycogenolytic enzyme activity as well as AMPK stimulation of glycogenolysis. Thus, less pyruvate is being produced, resulting in reduced PDH activity;

instead, acetyl-CoA production is derived from beta-oxidation (Mourtzakis et al, 2006). Ultimately, during recovery from aerobic exercise, carbohydrate oxidation is reduced as glycogen synthesis is a priority post-exercise and lipid oxidation is elevated to provide ATP for glycogen synthesis (van Loon et al, 2003).

Similarly, during recovery from a bout of moderate-to-high intensity resistance exercise, there is a switch in substrate contribution from carbohydrate oxidation to increased lipid oxidation that remains elevated for up to 15 hours post- resistance exercise (Melby et al, 1993; Pettitt et al, 2003; Singhal et al, 2009). Signals that activate the AMPK pathway through lower intracellular ATP concentration (i.e. muscle contraction) are reduced; thus, skeletal muscle shifts from substrate oxidation to synthesis to replenish glycogen stores. Therefore, although glucose uptake in exercising muscles remains elevated above pre-exercise values for at least 2 hours post exercise, whole body glucose oxidation is significantly depressed during recovery (Dreyer et al, 2008) as glycogen is re-synthesized. While there are a few studies that have investigated the regulation of substrate metabolism following a bout of resistance exercise, understanding the interactions between lipid and carbohydrate utilization following resistance exercise warrants further evaluation.

Aerobic and resistance exercise independently promote increased lipid oxidation during recovery (Melby et al, 1993; Kiens and Richter, 1998; Votruba et al, 2002; van Loon et al, 2003; Ormsbee et al, 2009). Thus, an exercise session, which includes both aerobic and resistance exercise, would be expected to also result in increases in lipid oxidation (relative to carbohydrate oxidation) immediately post-exercise. This would likely be indicated with a lower post-exercise RER in addition to elevated plasma NEFA concentrations. However, to date, there are no studies that have examined substrate selection and subsequent oxidation during post-exercise recovery

from a combined aerobic and resistance exercise session. It is unknown if the sequence in which aerobic and resistance exercise performed in the same session was altered (i.e. aerobic preceding resistance exercise versus resistance preceding aerobic exercise) would be most reflective of the second of the two exercises performed in the exercise session. That is, in an exercise session where a bout of resistance exercise is followed by a bout of aerobic exercise, would post-exercise substrate oxidation follow the same trends observed in recovery as would be observed from an independent bout of aerobic exercise? And, would the magnitude of post-exercise substrate oxidation be different depending on the type of exercise performed initially in the exercise sequence?

It is a primary aim of this thesis to evaluate the effect of varying exercise sequence in a combined aerobic and resistance exercise session on energy and hormonal metabolism during the exercise as well as during recovery.

3.0 Objectives

1. Examine whether the order in which aerobic and resistance exercise are performed in a single, combined exercise session, where the total amount of work is identical, influences carbohydrate and lipid oxidation differently during the individual aerobic and resistance exercise bouts in an aerobic-resistance versus a resistance-aerobic exercise sequence.
2. Investigate the potential mechanisms for metabolic alterations in lipid and carbohydrate oxidation depending on the sequence of aerobic and resistance exercise in a single exercise session.
3. Quantify the differences in energy expenditure that occur during and up to 30 minutes post-exercise, when the exercise sequence is altered.
4. Assess the variability of blood metabolite measures and energy-related parameters when identical bouts of aerobic exercise are performed on two separate days to confirm that the differences observed between sessions is not related to day-to-day metabolic variability.

4.0 Hypotheses

1. The aerobic exercise bout performed in a resistance-aerobic exercise sequence will display increased NEFA and glycerol concentrations as well as lower plasma glucose concentrations and RER values. These changes in metabolic and energy-related parameters will correspond with greater contribution of lipid oxidation to energy provision, compared to a bout of aerobic exercise performed in an aerobic-resistance exercise sequence.
2. A resistance-aerobic exercise sequence will be associated with a heightened sympathetic nervous system response characterized by higher concentrations of epinephrine, norepinephrine and glucagon during the exercise session and 30 minutes post-exercise, compared to an aerobic-resistance exercise sequence.
3. During recovery, a resistance-aerobic exercise sequence will reduce plasma insulin concentrations, and maintain plasma glucose concentrations, while NEFA and glycerol concentrations will be elevated. In comparison, an aerobic-resistance exercise sequence will demonstrate reduced NEFA and glycerol concentrations, consistent with decreased mobilization of lipids from adipose tissue. RER will be lower for a resistance-aerobic exercise sequence as opposed to the alternate sequence indicating increased reliance on fat oxidation for energy provision when resistance exercise is performed prior to a bout of aerobic exercise.

4. Energy expenditure will be greater during the aerobic exercise bout as well as during recovery in a resistance-aerobic exercise sequence compared to an aerobic-resistance exercise sequence. Although the amount of work performed in each exercise session will be identical, performance of resistance exercise prior to aerobic exercise will result in greater fatigue of skeletal muscle which will reduce the mechanical efficiency of the exercising muscle and thus, more energy will be expended as muscle fiber recruitment increases to maintain the same work output (ie. cycling at 60% $\text{VO}_{2\text{max}}$ for 30 min) as in the aerobic-resistance exercise sequence. Consequently, more glycogen, although not measured in this study, will be used for energy provision and a larger energy expenditure will be incurred during recovery from a resistance-aerobic exercise sequence as lipid oxidation is increased to a greater extent than with the opposite sequence to provide ATP for glycogen re-synthesis.

5. Blood metabolite values and energy-related parameters (i.e. VO_2 , energy expenditure and RER) will not be significantly different when measured for two identical bouts of aerobic exercise that are performed on separate days. Since the aerobic exercise is performed under the same conditions, the hormonal response to cycling should activate regulatory enzymes (albeit not measured in this study) to the same extent and thus produce similar rates of carbohydrate and lipid oxidation as well as energy expenditure.

5.0 Study Design and Methods

5.1 General Study Design

Nine healthy, recreationally active males were recruited to participate in this study. This study comprised of an initial assessment to measure maximal oxygen consumption (VO_{2max}), body composition, resting energy expenditure and maximal aerobic and strength capacity. The initial assessment was followed by three exercise trials that were comprised of: 1) a single bout of aerobic exercise (30 min at 60% VO_{2max}), 2) aerobic exercise (30 min at 60% VO_{2max}) followed by a 5 min rest and then a bout of resistance exercise (30 min at 70% predicted 1-RM), and 3) resistance exercise (30 min at 70% predicted 1-RM) followed by 5 min of rest and then a bout of aerobic exercise (30 min at 60% VO_{2max}). The single aerobic bout (trial#1) was succeeded by an additional 30 min bout of aerobic exercise at the same intensity; however, only the first, single aerobic bout will be described and analyzed for the objectives of this thesis. All trials were separated by one week to minimize any lingering metabolic effects of previous exercise trials (Melby et al, 1993; Petitt et al, 2003; Magkos et al, 2007; Singhal et al, 2009); however, trials were not separated by greater than 1 week to maintain similar physical conditioning for each trial. The study received ethics clearance by the Office of Research Ethics at the University of Waterloo.

5.2 Participants

Eligible participants for this study were not previously diagnosed with any cardiovascular or respiratory disease or any condition that would prevent participation in exercise. In addition,

all participants engaged in less than 3 hours of structured physical activity each week. Maintaining similar activity levels and focusing on healthy males minimized disease-related effects, training effects and sex-related hormone fluctuations that could potentially influence the results of the study. Participants had a mean age of 25 ± 2 years (Range: 21 to 27 years). The average height, weight and body mass index (BMI) were 181.78 ± 6.26 cm (173.0 to 192.4 cm), 78.82 ± 14.54 kg (59.3 to 102.6 kg), and 23.91 ± 4.50 kg/m² (16.96 to 30.74 kg/m²), respectively.

5.3 *Initial Assessment*

Participants arrived for the initial assessment after an overnight fast. Participants were asked to avoid strenuous physical activity for 48 hours, consumption of caffeine for 24 hours, and alcohol consumption 24 hours; this minimized variability and confounding effects on metabolic measures and maintained consistent hydration. Participants were also asked to drive or use the bus to arrive to the laboratory to reduce any activity before the resting metabolic rate (RMR) assessment. During the initial assessment, RMR, body composition, aerobic and strength exercise capacity were determined in this respective order.

RMR determination involved the participant lying supine on a bed in a quiet room for a total of 50 minutes. Oxygen consumption (VO₂) was measured continuously using a Rudolph facemask and Vmax breath-by-breath system (Vmax; SensorMedics, Yorba Linda, CA). The data were reviewed and a five-minute period of steady-state oxygen consumption with minimal fluctuations in VO₂ values and low corresponding respiratory exchange ratio values was selected from the 50 minutes of collected data for resting metabolic rate determination (Matarese, 1997).

The Weir equation (Weir, 1949) is a validated and frequently used tool to calculate resting energy expenditure (kcal/min) and it was utilized in this study:

$$\text{Resting energy expenditure (kcal/min)} = (3.941 \cdot \text{VO}_2 \cdot 1000 + 1.106 \cdot \text{VCO}_2 \cdot 1000) \cdot 1.44$$

Participants remained lying in a supine position on the bed for an evaluation of body composition using bioelectrical impedance analysis (BIA; RJL-101S; RJL Systems, Clinton Twp, MI). To minimize the chance of a false measure of increased lean tissue mass, removal of jewelry and voiding prior to BIA measurement is recommended. Voiding was not performed in this study. However, a pilot study was conducted on 5 subjects who were tested with a full bladder and re-tested with an empty bladder, and showed no statistical difference between the 2 tests (Appendix 2). Two electrodes were placed on the dorsal surface of the participant's right hand and two electrodes on the participant's right foot. A low alternating current (50 KHz) was passed between electrodes on the hand and the electrodes on the foot. This produced a resistance and reactance value, which were input into an equation that was previously validated against dual-energy x-ray absorptiometry (DXA) in healthy males aged 18 to 94 years (Kyle et al, 2001):

$$\text{Fat free mass (kg)} = -4.104 + 0.518 \cdot \text{height}^2 / \text{resistance} + 0.231 \cdot \text{weight} + 0.130 \cdot \text{reactance} + 4.229$$

To evaluate aerobic capacity, an incremental exercise test was performed on a cycle ergometer (Ergometrics 800; Ergoline, Deutschland, Germany) to fatigue. Briefly, heart rate was measured continuously using an electrocardiogram (EK 10; Spacelabs Burdick, Issaquah, Washington) and blood pressure was measured at rest and every two minutes during the exercise test using a manual sphygmomanometer. VO_2 was also measured continuously using the Rudolph facemask and Vmax breath-by-breath system. Each participant started the test by pedaling at 50 Watts at a rate of 60 revolutions per minute (rpm). Rating of perceived exertion

(Borg, 1982) and blood pressure were recorded every 2 minutes. Pedaling resistance was increased by approximately 25 to 50 Watts every 2 minutes, depending on the increase in rating of perceived exertion between increments. The primary endpoint for this test was the participant's inability to maintain an rpm of 60. Secondary endpoints included an RER value greater than 1.15 and a systolic blood pressure greater than 220 mmHg. VO_{2max} was calculated using the average of the highest 3 values in the final work stage, and this was then used to calculate 60% VO_2 max—the intensity at which the aerobic portion of each exercise trial was performed. The University of Waterloo Office of Research Ethics Standard Operating Procedure 212 describes the incremental exercise test protocol in detail (Appendix 1).

Following five minutes of rest, participants began the muscular strength evaluation. Since participants were untrained, a multiple repetition-maximum (RM) test was chosen to avoid injury while still challenging specific muscle groups to achieve close to a 1-RM. Based on the protocol from Kang et al (2009), exercises that involved large muscle groups were chosen to elicit the greatest metabolic response to a single resistance session. In sequential order, the resistance exercises that each participant performed were: overhead squat, chest press, triceps extension, shoulder press, and dead lift. For each exercise, the participant performed a warm-up of 10 repetitions of the exercise at 50% of his estimated 1-RM. The participant was then given 1-2 minutes to rest before completing 8-10 repetitions at 70% of his estimated 1-RM. After another 1-2 minute rest, the participant completed his third set of 3-5 repetitions at 80 to 90% of his estimated 1-RM. The total repetitions and weight that was used during the last set for each exercise predicted maximal strength using the following equation from O'Conner et al (1989):

$$\text{Predicted 1-RM (lbs)} = \text{load lifted (lbs)} / (1 - 0.025 * \text{number of repetitions completed})$$

This equation assumes that a 1-RM represents 100% of the participant's maximal strength and for every additional repetition that is completed, the load that is able to be moved will decrease by 2.5%. Seventy percent of the predicted maximal strength for each exercise was then determined for the resistance bout of each exercise trial.

5.4 *Exercise Trials*

Based on the Consolidated Standards of Reporting Trials statement, the order of completion for the three exercise trials was pre-determined using simple, unblocked randomization derived by a computerized random number generator (www.randomization.com); this minimized the potential for training effects from a prior exercise trial to influence the results of the subsequent trial. Trials were denoted as follows: aerobic (A), aerobic-resistance (AR) and resistance-aerobic (RA). Table 1 further describes the exercise sequence for each of the three trials. In addition, each session was performed after an overnight fast, and although not evaluated in this study, subjects were instructed to maintain the same diet for 2 days prior to each trial. This was to avoid any confounding effects of diet on carbohydrate and lipid oxidation for energy provision. Water was provided ad libitum during the entire exercise trial. Similar to the initial assessment, participants were asked to avoid strenuous physical activity for 48 hours, consumption of caffeine for 24 hours, and alcohol consumption 24 hours prior to each exercise trial to minimize variability in metabolic measures.

Table 1. Exercise sequence for each trial

Denotation of Trial	First exercise bout	Second exercise bout
<i>A</i>	Aerobic	n/a
<i>AR</i>	Aerobic	Resistance
<i>RA</i>	Resistance	Aerobic

At the beginning of each trial, participants rested on a bed for approximately 5 minutes while a catheter was inserted into the antecubital vein of the participant's preferred arm. Electrodes for electrocardiogram measures and a Rudolph facemask were fitted to continuously measure heart rate, VO_2 and carbon dioxide output (VCO_2), respectively, for the entire duration of each trial. To ensure consistency in data analysis between participants, the last 10 minutes of respiratory data collected for both the first and the second bout of exercise during each trial was analyzed.

During the A trial, participants performed 30 minutes of cycling on a stationary cycle ergometer at 60% VO_{2max} . The AR and RA trials also consisted of 30 minutes of cycling at 60% VO_{2max} during the aerobic portion of the exercise. The resistance bout of each trial was comprised of the 5 exercises performed during the initial assessment. For each exercise, the participant performed 3 sets of 8 repetitions at 70% predicted 1-repetition maximum (1-RM) in the following order: overhead squat, chest press, triceps extension, shoulder press, and dead-lift. Each bout of exercise was separated by a five-minute rest period and after completion of the two bouts of exercise, participants rested on the bed for 30 minutes, while remaining in a fasted state.

For participants who were unable to maintain their predicted aerobic intensity (for example, the participant was unable to maintain 60 rpm or greater) over the 30-minute exercise

bout, the work rate was reduced by 10 Watts until the subject was able to pedal at 60 rpm, which was then maintained for the duration of the aerobic exercise bout. To ensure that participants maintained a 60% work intensity, VO_2 values during the exercise trial were compared with the predicted 60% VO_2 values from the initial assessment. This work rate adjustment was made during the first aerobic bout completed by the participant, regardless of exercise trial, and was maintained for all subsequent aerobic trials to ensure that the participant exercised at the same workload for each trial.

The exercise intensities chosen for this study were based on previous work that examined substrate utilization in combined aerobic and resistance exercise when exercise sequence was varied (Goto et al, 2007b; Kang et al, 2009). Cycling at 60% $\text{VO}_{2\text{max}}$ is regarded as moderate intensity aerobic exercise where steady state can be established within 3-4 minutes of initiation (Wasserman et al, 2005). In untrained individuals, elevated glycolytic rates and carbohydrate oxidation would most likely be maintained at this intensity for the duration of the exercise bout. The resistance exercise protocol is similar to protocols previously employed to evaluate substrate oxidation during this type of exercise (i.e. each exercise is performed at 70% 1RM for 3 sets (~30 min)) (Kang et al, 2009), which is a feasible volume of work for this group of untrained males to complete. Additionally, this intensity and volume of work will largely stimulate of glycolysis. The extent to which each parameter deviates from rest can be compared between exercise sequences for differences in substrate oxidation and energy expenditure.

Blood samples (8.5mL) were drawn at five time points during each trial: before beginning the first exercise bout, before and after the second exercise bout and at 15 and 30 minutes post-exercise. Figure 2 summarizes the timeline for blood collection during each exercise trial.

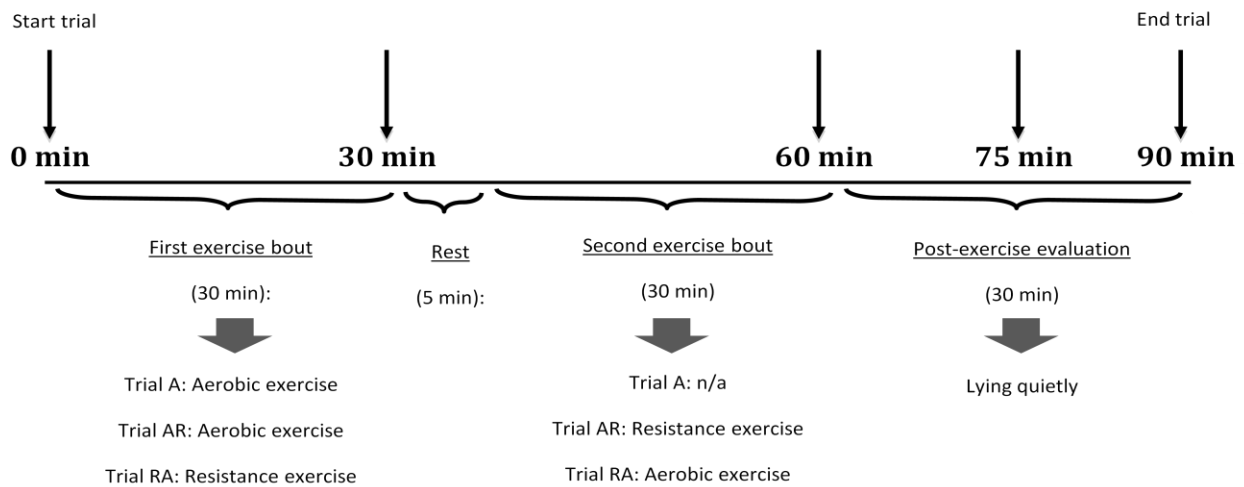


Figure 2. Exercise Trial Timeline; arrows indicate an 8.5 mL blood draw for: glucose, insulin, C-peptide, glucagon, lactate, glycerol, NEFA, triacylglycerol, epinephrine and norepinephrine

5.5 Blood Analyses

Blood was analyzed for: non-esterified fatty acids (NEFA), glycerol, glucose, insulin, C-peptide, glucagon, epinephrine, norepinephrine and lactate. In addition, a lipid profile (triacylglycerol (TAG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL)) was completed at pre-exercise time-points for each participant to establish a more detailed characterization of the study participants.

Serum was collected for glucose, insulin, C-peptide, lactate, NEFAs and glycerol. Whole blood was given 30 minutes standing time to clot and then centrifuged at 2500 rpm for 10 minutes at room temperature. Plasma samples for measuring glucagon were prepared by adding 2 mL of whole blood to test tubes containing 80 μ L of combined aprotinin and ethylenediaminetetraacetic acid (an anti-coagulant). Similarly, for catecholamines, 3mL whole

blood was added to test tubes containing 75 μ L of combined EGTA/Glutathione (also an anti-coagulant). The test tubes were then set on ice for the duration of the trial and were subsequently centrifuged at 2800 rpm for 15 minutes at 4°C.

All samples were aliquoted into a separate eppendorf for each metabolic measurement to avoid multiple freeze and re-thaw cycles. All samples were stored at -80°C. All samples, unless otherwise indicated, were run in triplicate and the average measure for the two readings with the lowest co-efficient of variance (COV) was used as the final value. Appendix 3 lists the mean COV as well as the range of COVs for each metabolite reported.

5.5.1 Serum Glucose

Serum glucose was measured using a spectrophotometer (UV160U; Shimadzu, Columbia, MD). Test tubes were filled with 10 μ L of distilled water (also termed the blank; providing a measure of background variability), glucose standard or sample. A reagent solution (Sigma-Aldrich, St. Louis, MO, USA) with active ingredients glucose oxidase, peroxidase, and o-Dianisidine dihydrochloride was prepared and 2500 μ L of this solution was added to each test tube. After vortexing, the test tubes were incubated for 30 minutes in at 37°C waterbath. When the glucose in the solution reacts with glucose oxidase, hydrogen peroxide is liberated. Peroxidase catalyzes the reaction between hydrogen peroxide and o-Diansidine dihydrochloride to form oxidized o-Dianisidine. O-Dianisidine is often used in analytical tests to produce a color change that can be detected by the spectrophotometer. The intensity of the final product, oxidized o-Dianisidine, was measured at 450 nm and compared to a standard glucose concentration.

5.5.2 Serum Insulin

Insulin concentration was measured, in duplicate, using a Coat-A-Count Insulin Radioimmunoassay kit (Siemens Healthcare Diagnostics; Deerfield, IL). 200 μL of standard, blank or sample were added to insulin-antibody coated polypropylene test tubes. 1 mL of ^{125}I -labeled insulin was added to each test tube and subsequently vortexed. ^{125}I -labeled insulin competes with insulin from the sample for sites on insulin-specific antibody that is immobilized to the test tube walls. After incubating for 24 hours at room temperature, the supernatant in each test tube was aspirated. A gamma counter (Wallac Wizard 1470 Automatic Gamma Counter; PerkinElmer Life and Analytical Sciences, Woodbridge, ON) measured the amount of ^{125}I -labeled insulin bound to the insulin antibody and using a calibration curve, computed the insulin concentration for each sample.

5.5.3 Serum C-Peptide

Serum C-peptide is a marker of insulin release since it is by-product when pro-insulin is cleaved to form insulin. Thus, C-peptide is released in a 1:1 ratio with insulin. This measurement is useful to distinguish between elevated insulin concentrations attributed to increased insulin release (where C-peptide would be proportionally elevated) or reduced insulin clearance (where C-peptide would be reduced). Using the Human C-Peptide radioimmunoassay kit (Siemens Healthcare Diagnostics; Deerfield, IL), 100 μL of serum is combined with 100 μL each of ^{125}I -Human C-Peptide and 100 μL of Human C-Peptide antibody. The sample is vortexed and incubated at 4°C for 20-24 hours before a precipitating reagent is added to the mixture. The sample is then vortexed and incubated again, for 20 min at 4°C. Subsequent centrifugation (15 minutes at 3000g) separates the sample into a precipitate and supernate, which is aspirated. The

solid precipitate is then placed in a gamma counter (Wallac Wizard 1470 Automatic Gamma Counter; PerkinElmer Life and Analytical Sciences, Woodbridge, ON) for 1 minute. The concentration of C-peptide (ng/mL) for each sample is determined by extrapolation from a concentration curve, which is plotted after performing the same procedure described above on samples of known C-Peptide concentrations.

5.5.4 Plasma Glucagon

Glucagon analysis is completed with a double-antibody radioimmunoassay test (Siemens Healthcare Diagnostics; Deerfield, IL). ¹²⁵I-labeled glucagon competes with glucagon in the sample for sites on glucagon-specific antibodies. 200 µL of calibrator or sample were combined with 100 µL of glucagon anti-serum in test tubes. All test tubes were vortexed and incubated at 4°C for 24 hours. 100 µL of ¹²⁵I-labeled glucagon is then added to all of the test tubes, which are subsequently vortexed and incubated for an additional 24 hours at 4°C. 1 mL of cold precipitating solution (ingredients: goat anti-rabbit gamma globulin and dilute polyethylene glycol in saline) was added to the test tubes which were then vortexed and centrifuged for 15 minutes at 1500g. Precipitated solid was isolated through aspiration of supernatant and each test tube was counted for 1 minute on a gamma counter (Wallac Wizard 1470 Automatic Gamma Counter; PerkinElmer Life and Analytical Sciences, Woodbridge, ON). Glucagon concentration for each sample was calculated from a calibration curve derived from known glucagon concentrations.

5.5.5 Plasma Catecholamines

Plasma catecholamine concentrations, specifically epinephrine and norepinephrine, were determined using high performance liquid chromatography. For this assay, 1 mL of plasma from each time point was added to a microcentrifuge tube that contained 20 mg acid washed alumina, 400 μ L 2.0 M Tris + 2% EDTA and 50 μ L of 3,4-dihydroxybenzylamine. The samples were then mixed by inversion for 45 minutes. The catecholamines became bound to the alumina, allowing for extraneous metabolites in the plasma sample to be removed when each microcentrifuge tube was washed with distilled water (repeated with three additional washes). 100 μ L of 0.1 M perchloric acid was then added to the catecholamine-bound alumina to release the catecholamines back into solution. The microcentrifuge tube was vortexed and centrifuged before 50 μ L of supernatant was injected into the HPLC column. The concentration of each sample was calculated based on a standard curve that was derived from known concentrations as well as an internal standard, which was 3,4-dihydroxybenzylamine.

5.5.6 PCA Extraction for Lactate and Glycerol Samples

To remove extraneous protein that interferes with the true reading of serum metabolite concentration, lactate and glycerol samples underwent a PCA extraction before being measured in their respective assays. Test tubes containing 500 μ L of 0.6M perchloric acid were set on ice and 100 μ L of serum from each sample was added to individual test tubes. Following centrifugation at 4°C for 2 min at 15,000 rpm, 250 μ L of 1.25M potassium bicarbonate was added to each test tube and allowed to set for 10 min. Centrifugation was repeated and supernatant was transferred to eppendorfs for storage at -80°C and a dilution factor of 8.5 was used for calculating final concentrations of lactate and glycerol.

5.5.7 Serum Lactate

Serum lactate was measured using a spectrofluorophotometer (RF-1501; Shimadzu, Columbia, MD). The reagent solution contained 15 mL hydrazine, 15 mL glycine and 1500 μL NAD^+ was prepared, brought to 150 mL volume using distilled water and adjusted to a pH of 10.0. Lactate dehydrogenase was diluted mixing 250 μL of lactate dehydrogenase (Sigma-Aldrich, St. Louis, MO, USA) (5200 U/mL) with 1 mL of the reagent solution. 25 μL of blank, lactate standard and sample were added to each test tube in triplicate. Subsequently, 1 mL of dilute reagent was added to each test tube and each tube was then vortexed. A baseline reading was performed on the flurometer (measurement settings: 365nm to 455nm). 25 μL of dilute lactate dehydrogenase was additionally added to all of the test tubes before being vortexed and placed in the dark to incubate for 120 minutes. Lactate in the test tubes is oxidized to pyruvate by lactate dehydrogenase. Hydrazine in the dilute reagent solution breaks down the lactate, allowing complete oxidation of lactate. Following the incubation period, a final reading was completed on each tube. The wavelengths for each sample were determined by subtracting the initial baseline reading from the final reading. NAD^+ from the dilute reagent solution was reduced to NADH which fluoresces in direct proportion to the concentration of lactate in each sample. The concentration of lactate was then calculated based on a regression equation created from the standard curve between the blank and lactate standard of known concentrations.

5.5.8 Serum Glycerol

To measure serum glycerol, a dilute 100 mL reagent solution was prepared from 5 mL hydrazine, 20 mL glycine, 160 μL NAD^+ , 40 μL ATP, 100 μL MgCl_2 , 500 μL cysteine and 50 μL of glycerol-3-phosphate dehydrogenase (Roche Diagnostics, Indianapolis, IN, USA). 50 μL

of sample was placed in a test tube and combined with 1 mL of dilute reagent. A baseline reading was performed on the spectrofluorophotometer (RF-1501; Shimadzu, Columbia, MD), with absorbance parameters between 365 to 455nm. 10 μ L of dilute glycerol kinase (glycerol kinase (450 U/mL in 1 mL of dilute reagent) (Boehringer Mannheim, Germany)) was added to each test tube, vortexed and then incubated in a dark drawer for 60 minutes. In the presence of glycerol kinase, serum glycerol reacts with ATP to form glycerol-3-P. Glycerol-3-P combines with NAD^+ from the reagent solution to yield dihydroxyacetone phosphate and more important to this assay, NADH, a fluorescing marker of glycerol concentration. The absorbance of NADH in the spectrofluorophotometer is proportional to the concentration of glycerol in each sample. A second reading on the spectrofluorophotometer was taken for each sample after the incubation period and concentrations were determined using a standard curve derived from known concentrations of five glycerol standards.

5.5.9 Serum Non-Esterified Fatty Acids

Serum non-esterified fatty acids (NEFA) concentrations were evaluated using a Wako NEFA-HR(2) kit (Wako Diagnostics; Richmond, VA) and 96 well microplate. 5 μ L of water (blank), standard or sample were added to separate wells. 200 μ L of Color Reagent A solution, containing acyl-coenzyme A synthase, coenzyme A, ATP and 4- aminoantipyrine, was added to all of the wells and the microplate was incubated in the spectrophotometer (Spectramax Plus 384; Molecular Devices, Sunnyvale, CA) for 5 minutes at 37°C. During the incubation, acyl-COA synthase reacted with the serum NEFAs in the presence of coenzyme A and ATP to form by-products, in particular, acyl-COA. Following incubation, the absorbance of each well was measured at 550 nm. 100 μ L of Color Reagent B solution (main ingredients: acyl-coenzyme A

oxidate and peroxidase) was then added to each well and the microplate underwent an additional 5 minutes of incubation at 37°C. When Color Reagent B solution was mixed with the previously incubated solution in the wells, acyl-CoA was oxidized (via acyl-CoA oxidase) to produce hydrogen peroxide. Hydrogen peroxide was then available to catalyze the final end-product formation from 4-aminoantipyrine, which fluoresces in direct proportion to the concentration of NEFA present in each sample. The absorbance of each well was obtained a second time and the final absorbance for each sample was determined by subtracting the first absorbance reading from the second absorbance reading. Sample concentrations were calculated using the following equation provided by the Wako Diagnostics kit:

Sample Concentration (mmol/L) = standard concentration X (sample absorbance/standard absorbance)

5.5.10 Serum Triacylglycerol

Fasting triacylglycerol concentrations were measured using a kit (Genzyme Diagnostics Inc., P.E.I., Canada) and samples were run in quadruplicate. 12.5 µL of blank, standard or diluted sample were added to test tubes. 125 µL of reagent (active ingredients: ATP, 4-aminoantipyrine, peroxidase, glycerol phosphate oxidase, glycerol kinase, 3,5-dichloro-2-hydroxy-benzenesulfonic acid and lipase) was added to each test tube and all samples were incubated at room temperature for 10 minutes. While incubating, lipase catalyzes the degradation of triacylglycerides into glycerol and NEFA. Glycerol combines with ATP in a reaction catalyzed by glycerol kinase to produce glycerol-1-phosphate. In the presence of glycerol phosphate oxidase, the newly formed glycerol-1-phosphate reacts with oxygen to form hydrogen peroxide. In the final reaction, hydrogen peroxide, via peroxidase, mixes with 4-aminoantipyrine

and 3,5-dichloro-2-hydroxy-benzenesulfonic acid producing a quinoneimine dye. The absorbance of the quinoneimine dye in each test tube is measured at 520 nm on a spectrophotometer (Spectramax Plus 384; Molecular Devices, Sunnyvale, CA); the absorbance of the dye is directly proportional to the concentration of triacylglycerides in the sample.

5.5.11 Serum Total Cholesterol

Fasting serum cholesterol was evaluated using a prepared kit (Diagnostic Chemicals Ltd, P.E.I., Canada). Test tubes containing 25 μ L of blank, cholesterol standard or sample were mixed with 2.5 mL of cholesterol working reagent (main ingredients: 4-aminoantipyrine, cholesterol esterase, cholesterol oxidase, phenols). After mixing, the test tubes were incubated in a water bath set at 37°C for 5 minutes. When serum cholesterol was mixed with the working reagent, cholesterol oxidase catalyzed the conversion of free cholesterol to cholesten-3-one and hydrogen peroxide. The hydrogen peroxide generated then reacted with 4-aminoantipyrine and phenol to produce quinoneimine, a chromogen. Using a spectrophotometer (Spectramax Plus 384; Molecular Devices, Sunnyvale, CA) set at 505 nm, the absorbance for each sample was determined. Total cholesterol concentration was directly proportional to the color intensity of the chromogen in each sample.

5.5.12 Serum High-density Lipoprotein

To measure high-density lipoprotein (HDL), 2 μ L of blank, HDL standard or serum was added to separate wells in a 96 well microplate. A HDL kit (Genzyme Diagnostics, Framingham, MA) provided 2 reagents that were added to the wells. 200 μ L of the first reagent, containing antihuman β -lipoprotein antibody, was added to each well, vortexed and incubated at 37°C for 10

minutes in the spectrophotometer (Spectramax Plus 384; Molecular Devices, Sunnyvale, CA). The 96 well microplate was mixed using the spectrophotometer plate mixer every 1-2 minutes for the duration of the incubation period. The absorbance of each sample was read at 550 nm before 100 μ L of a second reagent (main ingredients: cholesterol esterase, cholesterol oxidase, 4-aminoantipyrine, peroxidase) was added to all wells. The microplate was vortexed before undergoing an additional 10 minutes of incubation (with slight agitation using the plate mixer) in the spectrophotometer at 37°C. The addition of antihuman β -lipoprotein in each well liberates free HDL by binding with all lipoproteins in serum, excluding HDL. HDL is then oxidized by cholesterol oxidase to produce hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide couples with 4-aminoantipyrine to form a blue-dyed chemical complex. The absorbance of the final product in each well was measured at 550 nm and concentrations were calculated using a standard curve derived from the blank and HDL standard concentrations.

5.5.13 Serum Low-density Lipoprotein

Fasting serum low-density lipoprotein (LDL) was indirectly assessed, due to cost and kit availability limitations. The following equation (Friedewald et al, 1972) was provided by Diagnostic Chemicals Ltd (P.E.I., Canada) and estimates LDL concentration:

$$LDL (mmol/L) = total\ cholesterol - HDL - (TAG * 2.2)$$

Total cholesterol, HDL and TAG were measured directly with the procedural details described above.

5.6 Calculations

Fat oxidation that occurred during the aerobic bout of activity for each trial was estimated using the respiratory exchange ratio (RER) value recorded by the Vmax system (Vmax; SensorMedics, Yorba Linda, CA). To further evaluate energy expenditure during exercise, the following equation was used:

$$\text{Energy expenditure (kcal/min)} = 0.550 \cdot \text{VCO}_2 - 4.471 \cdot \text{VO}_2 \quad (\text{Jeukendrup and Wallis, 2005})$$

The derivation of this equation was made based on the assumption that the contribution of carbohydrate oxidation to energy expenditure is fulfilled by plasma glucose (20%) and muscle glycogen (80%) when exercise is performed at moderate intensities (50 to 75% $\text{VO}_{2 \text{ max}}$). For the evaluation of energy expenditure during recovery, the Weir equation (Weir, 1949) was used:

$$\text{Resting energy expenditure (kcal/min)} = (3.941 \cdot \text{VO}_2 \cdot 1000 + 1.106 \cdot \text{VCO}_2 \cdot 1000) \cdot 1.44$$

Respiratory measures were used to estimate potential differences in carbohydrate and lipid oxidation rates during the aerobic exercise portion of the AR trial compared to the RA trial, where the aerobic portion of the latter sequence is preceded by resistance exercise. The last 10 min of VO_2 and VCO_2 values for each aerobic exercise were averaged and incorporated into Frayn's equations (1983):

$$\text{Carbohydrate oxidation (g/min)} = 4.55 \cdot \text{VCO}_2 - 3.21 \cdot \text{VO}_2 - 2.87 \cdot n$$

$$\text{Lipid oxidation (g/min)} = 1.67 \cdot \text{VO}_2 - 1.67 \cdot \text{VCO}_2 - 1.92 \cdot n$$

Where n represents nitrogen excretion rate that was estimated to be $135 \mu\text{g/kg/min}$ (Romijn et al, 2000).

Area under the Curve (AUC) calculations

To measure AUC for the AR and RA trials, a graph of glucose and insulin concentration vs. time were separately created for each participant. Area under each curve was calculated for the following time periods: 1) 0 to 30 min to represent changes in the first bout of exercise, 2) 30 to 60 min to depict changes in the second bout of exercise, 3) 60 to 75 min to examine changes immediately post-exercise, and 4) 75 to 90 minutes to illustrate changes in early recovery. Total Exercise AUC reflects changes in plasma glucose concentration over the entire duration of exercise (0 to 60 min), while Total Recovery AUC displays changes in plasma glucose concentration from immediately post- to 30 min post-exercise (60 to 90 min).

5.7 Statistical Analyses

Data collected from this study were reported as means \pm standard deviation (SD). All power calculations and results were analyzed using SigmaPlot 11.0 (Systat Software Inc, SPSS, San Jose, CA) and values with a p-value of <0.05 were considered significant. For statistically significant data, Tukey's post-hoc analysis was employed to identify the trial and time point where the difference occurred. Trends will be identified were $p < 0.15$.

5.7.1 Statistical Power and Sample Size

Based on the study results for primary measures used to evaluate lipid and carbohydrate metabolism (i.e. glycerol, NEFA, glucose and respiratory exchange ratio (RER)), a sample size that would achieve 80% statistical power was calculated for each of the primary parameters described above. For these power calculations, the difference in value between the AR and RA exercise trials, as well as the standard deviation of the difference in means, was determined by

subtracting the value measured at 0 min from the value measured at 60 min, for each parameter. Power was re-calculated after data collection for the same primary measures (i.e. glycerol, NEFA, glucose and RER) to identify the statistical power that was achieved with the study's actual sample size (n=9). The results are presented in Appendix 4.

5.7.2 Comparison of Metabolite and Energy Parameters during Exercise in the AR and RA Trials

To evaluate the effect of exercise order on changes in metabolite concentrations and energy-related parameters, AR and RA exercise trials were evaluated using a two-way repeated measures ANOVA (time x exercise sequence). Specifically, baseline (0 min) was compared to the completion of the first exercise bout (30 min) and the completion of the second exercise bout (60 min), which were in turn compared between the AR and RA trials. Baseline (0 min) respiratory measures (i.e. VO_2 , EE and RER) were calculated from data obtained during the initial assessment, as resting values for these parameters were not collected during the exercise trials.

5.7.3 Comparison of Metabolite and Energy Parameters after the Completion of an Aerobic Exercise Bout in the AR and RA trials

To assess differences in metabolite concentrations and values for energy parameters between an aerobic bout of exercise in the AR and RA trials, a two-way repeated measures ANOVA (time x exercise sequence) was used. Statistics were generated for both pre- and post- aerobic exercise, however only post-aerobic exercise values were investigated. In both trials, there is a break that is followed by the first exercise session prior to the onset of the second

exercise session. For logistical reasons, respiratory data collection stopped with the cessation of the first exercise bout and blood samples were collected immediately following each exercise bout (prior to a 5 min rest period). While post-exercise values can be accurately compared with the data collected, pre-exercise values cannot be compared between trials because metabolite concentrations and energy parameters may have changed over the 5 min rest period, and thus may not be reflective of starting concentrations for the subsequent aerobic exercise bout in the RA trial.

5.7.4 Comparison of Metabolite and Energy Parameters Recovery in the AR and RA Trials

To investigate the effects of exercise sequence on post-exercise recovery following an AR or RA exercise trial of the same total work volume, changes in metabolite concentrations and energy-related parameters immediately post-exercise (60 min), 15 minutes into recovery (75 min) and 30 minutes into recovery (90 min) were also compared between time points and between trials. Post-exercise recovery data for VO_2 , RER and EE data at 60 min was collected for the first 5 minutes immediately post-exercise. It is therefore different from the data reported for these parameters at the 60 min time point for the exercise portion of the trial, which was calculated using the last 10 minutes of data from the exercise bout.

Area-under-the-curves (AUC) were calculated for glucose and insulin to examine the amount of circulating plasma concentrations over the duration of exercise and recovery relative to rest.

5.7.5 Evaluation of Variability in Metabolite and Energy Parameters during an Aerobic Exercise Bout Completed on Two Separate Days

Variation in performing a single bout of aerobic exercise on two separate days was evaluated (A trial vs aerobic portion of AR trial) for each measurement at baseline (0 min) and immediately post-exercise (30 min). Bland-Altman plots (Bland and Altman, 1986) were created for NEFA, glycerol, glucose, insulin, epinephrine and norepinephrine, the primary parameters used to assess carbohydrate and lipid oxidation and energy expenditure for this study. Bland-Altman plots were also created for VO_2 and RER immediately post-exercise (30 min), as baseline data for these parameters was taken from the initial assessment which was identical for both the A and AR trial. The A trial served as a control and the results for the aerobic portion of the AR trial were expressed as units of standard deviation from the results calculated for the aerobic portion of the A trial.

6.0 Results

6.1 Baseline Characteristics

Despite the tight range in age, fasting glucose as well as the number of hours reported for weekly physical activity (<3 hours / week), the group of participants that was studied was heterogeneous with respect to body composition, metabolic profile, resting energy expenditure, and maximal aerobic capacity (Tables 2 and 3). Individual predicted 1-RM strength testing also revealed heterogeneity among study participants. The average predicted 1-RM for the overhead squat, chest press, triceps extension, shoulder press and dead-lift exercises completed in this study were 64 ± 28 , 125 ± 58 , 60 ± 24 , 71 ± 37 and 168 ± 80 kg, respectively. Mean BMI was 23.9 ± 4.5 kg/m², which represents a healthy BMI classification (WHO, 1995). However, one participant was underweight with a BMI of 17 kg/m², two participants were classified as overweight (27.72 and 28.37 kg/m²), and one participant was obese (BMI: 30.6 kg/m²). This range of classifications also coincides with the range of fat and fat-free mass measurements observed in this group (Table 2). Variability was also reflected in the range of measurements for the fasting lipid profile of the participants (Table 3). With the aim to focus on untrained individuals, the heterogeneity observed in these parameters was expected from this group of male participants.

Table 2. Physical Characteristics of Participants at Initial Assessment

	Mean \pm SD	Range
Age (yrs)	25 \pm 2	21 to 27
Weight (kg)	78.8 \pm 14.5	59.3 to 102.6
Height (cm)	181.8 \pm 6.3	173.0 to 192.4
BMI (kg/m ²)	23.9 \pm 4.5	17.0 to 30.6
Body Fat (%)	22 \pm 6	14.0 to 31.4
Whole Body Fat Mass (kg)	18 \pm 7	9 to 31
Whole Body Fat Free Mass (kg)	61 \pm 8	51 to 78
REE (kcal/day)	1635 \pm 258	1379 to 2120
REE (kcal/kg/day)	21.0 \pm 2.4	17.8 to 24.5
VO _{2max} (mL/kg/min)	39.52 \pm 5.96	29.40 to 47.96

Table 3. Metabolic Profile of Participants Following an Overnight Fast

	Mean \pm SD	Range
TAG (mM)	1.15 \pm 0.89	0.44 to 3.29
LDL (mM)	2.23 \pm 0.85	0.78 to 3.72
HDL (mM)	1.33 \pm 0.33	0.92 to 1.78
Total Cholesterol (mM)	4.08 \pm 1.03	2.11 to 5.62
Total Cholesterol/HDL Ratio	3.2 \pm 1.1	1.9 to 5.2
Glucose (mM)	4.51 \pm 0.39	3.83 to 4.89

6.2 Substrate Oxidation during Exercise

RER values increased from rest to 30 min, for both the AR and RA trials (AR: 0.81 \pm 0.05 to 1.27 \pm 0.90 and RA: 0.81 \pm 0.05 to 1.07 \pm 0.07, $p < 0.05$; Table 4). At 60 min, RER was significantly elevated in the AR trial compared to rest and 30 min. In the RA trial, RER was reduced compared to 30 min ($p < 0.05$), although it was still significantly elevated compared to rest. While RER was significantly different between trials at 30 and 60 min ($p < 0.05$), RER values were similar at the completion of the aerobic exercise bout for both the AR and RA trial.

This suggests that exercise sequence does not affect substrate oxidation during an acute bout of aerobic exercise.

Carbohydrate oxidation rates were similar between the aerobic exercise bouts of both trials (AR: 2.49 ± 0.69 vs. RA: 2.47 ± 0.64 g/min). Fat oxidation rates were also similar between the aerobic bouts of the AR (0.15 ± 0.09 g/min) and RA (0.19 ± 0.09 g/min) trial.

Table 4. Metabolite Concentrations at Baseline and at Completion of Exercise

Metabolite	Trial AR			Trial RA		
	Baseline (0 min)	Completion of Aerobic Exercise (30 min)	Completion of Resistance Exercise (60 min)	Baseline (0 min)	Completion of Resistance Exercise (30 min)	Completion of Aerobic Exercise (60 min)
Energy-Related Parameters						
RER	0.81 ± 0.05 ^a	0.97 ± 0.02 ^{b*}	1.03 ± 0.05 ^{c*‡}	0.81 ± 0.05 ^a	1.07 ± 0.07 ^b	0.96 ± 0.02 ^c
VO ₂ (mL/kg/min)	3.01 ± 0.36 ^a	27.73 ± 5.17 ^{b*}	15.50 ± 1.94 ^{c*}	3.01 ± 0.36 ^a	14.81 ± 1.23 ^b	28.71 ± 4.07 ^c
EE (kcal/kg/day)	20.96 ± 2.38 ^a	157.48 ± 29.47 ^{b*}	87.65 ± 11.22 ^{c*}	20.96 ± 2.38 ^a	83.35 ± 6.77 ^b	163.29 ± 22.58 ^c
Lipid-Related Parameters						
NEFA (mM)	0.349 ± 0.150	0.356 ± 0.135	0.288 ± 0.084	0.412 ± 0.171 ^a	0.243 ± 0.096 ^b	0.362 ± 0.175 ^a
Glycerol (mM)	80.14 ± 41.78 ^a	145.77 ± 51.96 ^{b*}	140.68 ± 33.35 ^{‡b}	90.01 ± 39.77 ^a	96.81 ± 42.11 ^a	175.78 ± 70.64 ^b
Carbohydrate-Related Parameters						
Glucose (mM)	4.53 ± 0.41 ^a	4.50 ± 0.58 ^{a*‡}	5.03 ± 0.53 ^b	4.50 ± 0.64 ^a	5.18 ± 0.54 ^b	4.87 ± 0.39 ^{ab}
Insulin (μU/mL)	5.24 ± 2.65 ^a	2.54 ± 1.92 ^{b*}	3.09 ± 2.25 ^{b‡}	4.95 ± 2.64 ^a	4.71 ± 1.86 ^a	2.93 ± 2.09 ^b
Glucagon (pg/mL)	132.33 ± 28.39 ^a	149.27 ± 17.48 ^b	154.28 ± 21.44 ^c	125.51 ± 31.37 ^a	141.26 ± 28.41 ^b	164.34 ± 44.97 ^c
C-peptide (ng/mL)	2.48 ± 1.09	2.18 ± 0.91 ^{*‡}	2.54 ± 1.16 [‡]	2.50 ± 0.94	2.96 ± 1.00	2.35 ± 0.89
Lactate (mM)	2.96 ± 0.60 ^a	8.57 ± 2.67 ^{b*}	13.45 ± 3.72 ^{c*}	3.02 ± 1.04 ^a	12.42 ± 1.87 ^b	9.39 ± 2.07 ^c

Notes:

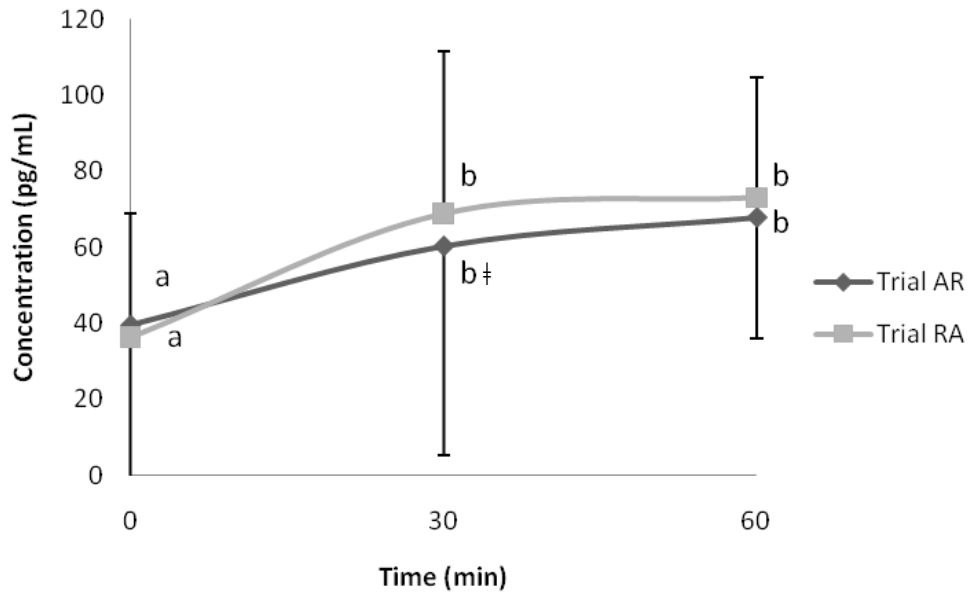
1. Data are reported in mean ± SD
2. * Denotes a significance level of p<0.05 between trials
3. Different letters denote a significance level of p<0.05 between time points (0 vs. 30 vs. 60 min) within a trial
4. ‡ Denotes a significance level of p<0.05 between the aerobic exercise bouts of the AR (30 min) and RA (60 min) trial or between resistance exercise bouts of the AR (60 min) and RA (30 min) trial
5. VO₂, EE and RER data for baseline measures were taken from the initial assessment

Plasma NEFA concentrations remained unchanged from rest throughout the entire exercise duration of the AR trial (0.349 ± 0.150 mM at 0 min, 0.356 ± 0.156 mM at 30 min, and 0.288 ± 0.084 mM at 60 min, $p > 0.05$; Table 4). In the RA trial, NEFA concentrations were reduced from 0.412 ± 0.171 to 0.243 ± 0.096 mM by the completion of the first bout of resistance exercise (30 min, $p < 0.05$), but increased during the aerobic exercise bout to return to values similar to rest by 60 min (0.36 ± 0.175 ; $p > 0.05$). Thus, both trials depicted no differences between NEFA concentrations at the end of aerobic exercise relative to rest. While there were no significant differences observed in NEFA concentrations between AR and RA trials, NEFA concentrations tended to be greater at 30 min in the AR trial compared to the RA trial ($p = 0.056$; Table 4). Plasma glycerol was significantly higher immediately after the first bout of exercise (at 30 min) for the AR trial compared to the RA trial (145.77 ± 51.96 vs. 96.81 ± 42.11 mM, respectively, $p < 0.05$; Table 4). Glycerol concentrations remained elevated during the resistance portion of the AR trial compared to rest; in contrast, glycerol concentrations increased after the completion of the aerobic exercise bout (at 60 min) in the RA trial compared to 0 and 30 min ($p < 0.05$). In addition, glycerol concentrations increased during each aerobic exercise bout and tended to be higher at 60 min in the RA trial compared to 60 min in the AR trial ($p = 0.105$; Table 4).

Epinephrine (Figure 3A) and norepinephrine (Figure 3B) concentrations were increased from rest at the completion of the first bout of exercise (from 0 to 30 min; $p < 0.05$) and remained elevated for the second exercise bout (30 versus 60 min; $p > 0.05$) for both the AR and RA trials. A comparison of the aerobic exercise bouts in both trials showed significantly higher epinephrine concentrations during the RA trial as opposed to the AR trial (AR: 60.41 ± 55.29 vs. RA: $73.01 \pm$

31.58 pg/mL; Table 5). In addition, there was a trend observed for norepinephrine concentrations to be higher at 30 min and lower at 60 min in the RA trial ($p=0.053$; Figure 3B).

(A)



(B)

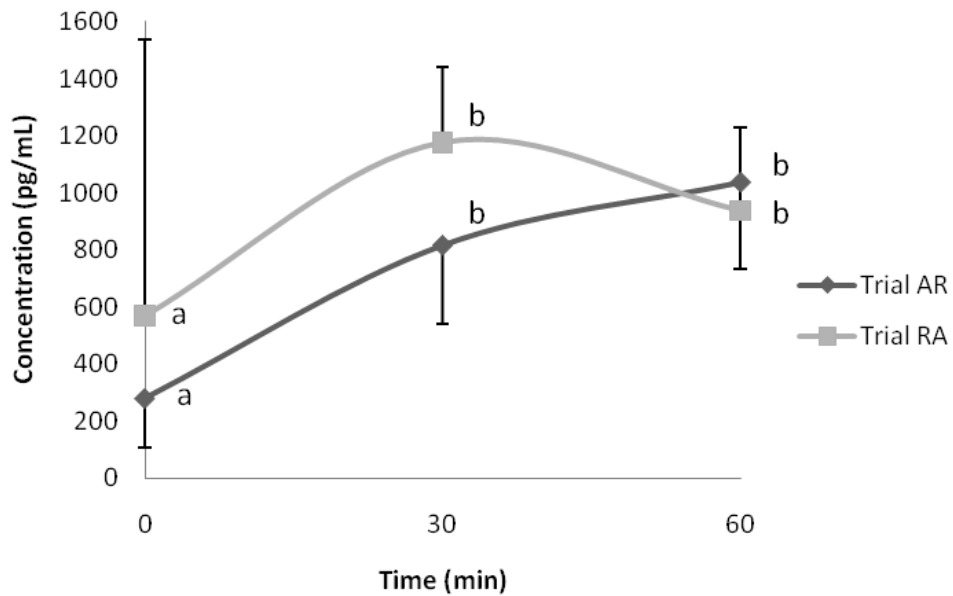


Figure 3. Changes in epinephrine (A) and norepinephrine (B) concentration from rest (0 min) to completion of exercise (60 min). * denotes a significant difference of $p < 0.05$ between trials; different letters denote a significance level of $p < 0.05$ between time points (0 vs. 30 vs. 60 min) within a trial. † denotes a significance level of $p < 0.05$ between the aerobic exercise bout of the AR (30 min) and RA (60 min) trial.

During the aerobic exercise of each trial, plasma glucose was maintained (AR: 4.53 ± 0.41 mM at 0 min versus 4.50 ± 0.58 mM at 30 min; RA: 5.18 ± 0.54 mM at 30 min versus 4.87 ± 0.39 mM at 60 min; Table 4 and Figure 4A). Plasma glucose at the end of the aerobic exercise bout from each trial was not different from rest, however it was significantly elevated after aerobic exercise in the RA trial compared to the AR trial (AR: 4.50 ± 0.58 vs. RA: 4.87 ± 0.39 mM; Table 5). Plasma glucose was increased after the completion of the resistance exercise for both trials and was elevated relative to rest for both trials ($p < 0.05$; Figure 4A). Glucose AUC during exercise did not differ between trials, however, both trials tended to be elevated for the second bout of exercise ($p = 0.115$; Figure 4B). There were no differences in total glucose AUC between either trial.

During the AR trial, plasma insulin concentrations decreased after a bout of aerobic exercise (30 min) and remained depressed following a bout of resistance exercise (60 min, $p < 0.05$; Figure 5A). In contrast, in the RA trial, insulin concentrations were maintained during the first bout of exercise (30 min) relative to rest (0 min) before declining in the aerobic exercise bout ($p < 0.05$). Insulin concentrations were significantly lower at 30 min in the AR trial compared with the RA trial (AR: 5.24 ± 2.65 $\mu\text{U/mL}$ at 0 min to 2.54 ± 1.92 $\mu\text{U/mL}$ at 30 min vs. RA: 4.95 ± 2.64 $\mu\text{U/mL}$ at 0 min to 4.71 ± 1.86 $\mu\text{U/mL}$ at 30 min). AUC for insulin during exercise was significantly elevated during the 2nd bout of exercise (30 to 60 min) compared to the 1st bout of exercise (0 to 30 min, $p < 0.05$; Figure 5B) for both trials. Similar to insulin, C-peptide concentrations, as expected, were also lower at 30 min in the AR trial (2.18 ± 0.91 ng/mL) compared to the RA trial (2.96 ± 1.00 ng/mL). C-peptide tended to be greater at 60 min vs. 30 min in the AR trial (2.54 ± 1.16 vs. 2.18 ± 0.91 ng/mL, $p = 0.089$; Table 4) and was significantly higher after completion of the aerobic exercise bout in the RA trial as opposed to the AR trial

(AR: 2.18 ± 0.91 vs. RA: 2.35 ± 0.89 ng/mL; Table 5). Glucagon concentrations were elevated after the first exercise bout and continued to rise after the second exercise bout in both trials ($p < 0.05$; Table 4), suggesting a potential increase in liver contribution to carbohydrate supply via gluconeogenesis and glycogenolysis; however there were no differences in glucagon concentrations observed between trials.

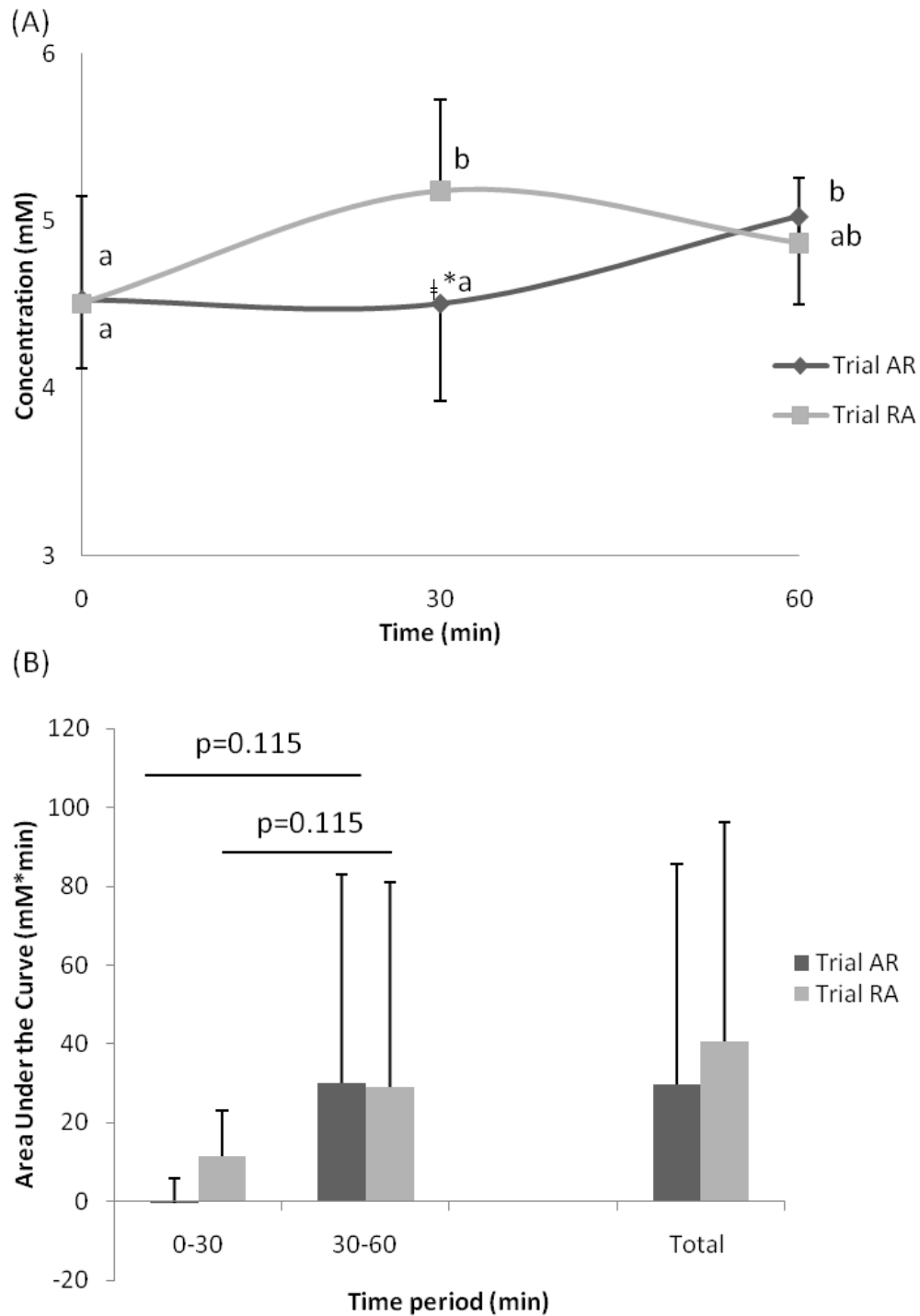


Figure 4. Glucose concentration (A) and AUC (B) from Rest (0 min) to Completion of Exercise (60 min). * denotes a significant difference of $p < 0.05$ between trials; different letters denote a significance level of $p < 0.05$ between time points (0 vs. 30 vs. 60 min) within a trial. † denotes a significance level of $p < 0.05$ between the aerobic exercise bout of the AR (30 min) and RA (60 min) trial.

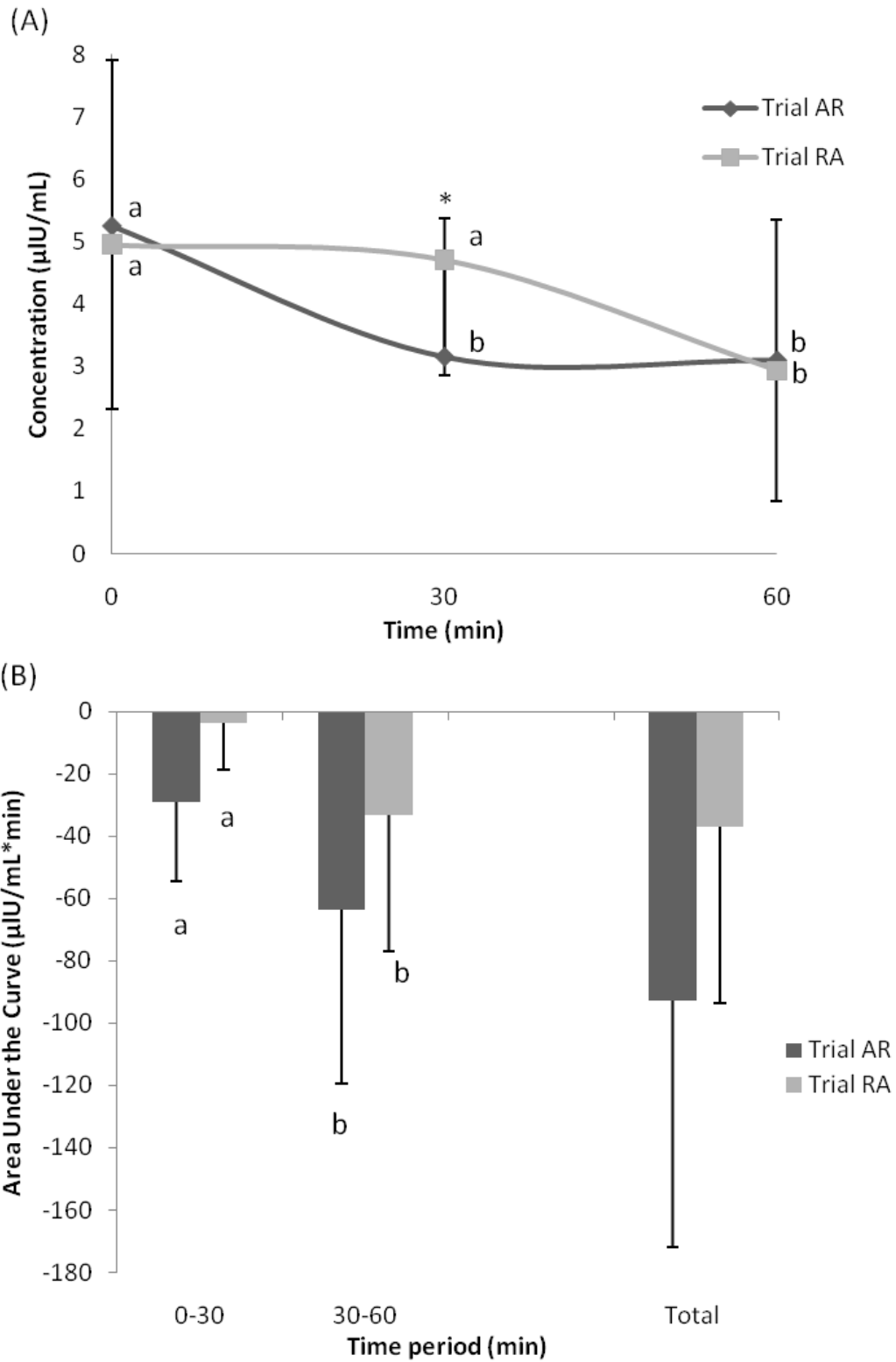


Figure 5. Insulin concentration (A) and AUC (B) from Rest (0 min) to Completion of Exercise (60 min). * denotes a significant difference of $p < 0.05$ between trials; different letters denote a significance level of $p < 0.05$ between time points (0 vs. 30 vs. 60 min) within a trial.

Lactate increased from rest during the first exercise bout and continued to increase in the second exercise bout of the AR trial. In the RA trial, lactate concentrations increased during the resistance exercise portion and then significantly declined during the aerobic exercise portion, while still remaining elevated above resting values ($p < 0.05$; Table 4). Overall, lactate concentrations were significantly higher at 30 min in the RA trial compared to the AR trial whereas at 60 min lactate was greater in the AR trial compared with the RA trial ($p < 0.05$). Interestingly, lactate concentrations reached similar values at the end of the aerobic exercise bouts for the AR and the RA trials (8.57 ± 2.67 vs. 9.39 ± 2.07 mM, respectively; Table 4). Likewise, lactate was similar at the end of the resistance exercise bouts for each trial (AR: 13.45 ± 3.72 vs. RA: 12.42 ± 1.87 mM).

6.3 *Energy Expenditure during Exercise*

Oxygen consumption increased significantly from rest for the entire duration of exercise in both the AR and RA trials ($p < 0.05$; Table 4). VO_2 was elevated following aerobic exercise and declined in the subsequent resistance exercise bout (27.73 ± 5.17 to 15.50 ± 1.94 mL/kg/min; $p < 0.05$) in the AR trial. The opposite trend was observed in the RA trial (14.81 ± 1.23 after an initial bout of resistance exercise to 28.71 ± 4.04 mL/kg/min after the second bout (aerobic) of exercise; $p < 0.05$). As expected, VO_2 was significantly different between the AR and RA trials at 30 and 60 min; this may be attributed to the higher VO_2 at the completion of the aerobic bout of exercise compared to the lower VO_2 measured at the completion of the resistance exercise bout for each trial. As well, VO_2 tended to be higher after a bout of aerobic exercise in the RA trial compared to the AR trial (AR: 27.73 ± 5.17 , RA: 28.71 ± 4.07 mL/kg/min; Table 5).

Similar to VO_2 , energy expenditure (kcal/kg/day) increased during the first bout of aerobic exercise and declined in the second bout of resistance exercise (AR trial), while still remaining elevated from rest ($p < 0.05$; Table 4). Energy expenditure was greater than at rest during the first bout of resistance exercise in the RA trial, and further increases in energy expenditure were observed following the aerobic exercise bout ($p < 0.05$). Total energy expenditure for each bout of exercise was calculated. Thirty minutes of aerobic exercise expended 256.76 ± 59.31 kcal in the AR trial, which tended to be lower than in the RA trial, where 265.82 ± 49.41 kcal was expended. Resistance exercise in the AR trial showed a 139.81 ± 54.18 kcal deficit compared with 122.69 ± 32.90 kcal in the RA trial ($p > 0.05$). Overall, the combination of both the aerobic and resistance exercise in the AR and RA trials resulted in a total net energy expenditure of 396.57 ± 80.47 kcal (AR trial) and 388.50 ± 55.79 kcal (RA trial), with no significant difference in kcal deficit between the two trials ($p > 0.05$).

6.4 Comparison of Substrate Oxidation and Energy Expenditure between Aerobic Exercise Bouts and between Resistance Exercise Bouts in the AR and RA Trial

A comparison of metabolite concentrations at the completion of aerobic exercise between trials revealed significantly greater glucose, C-peptide and epinephrine concentrations in the RA exercise trial compared to the AR (Table 5). VO_2 and energy expenditure tended to be elevated after aerobic exercise performed in the RA trial compared to the AR trial (Table 5). No significant differences were observed for RER values or for NEFA, glycerol, insulin, glucagon, lactate, and norepinephrine concentrations (Table 5).

When metabolite concentrations were compared between resistance exercise bouts of the AR and RA trials, there were significant elevations in insulin and C-peptide concentrations and

RER, with concomitant reductions in glycerol concentrations in the resistance bout of the RA trial compared to the AR trial ($p < 0.05$; Table 6).

Table 5. Metabolite Concentrations After Completion of an Aerobic Bout of Exercise

	Trial AR Post- Aerobic Exercise Bout (30 min)	Trial RA Post- Aerobic Exercise Bout (60 min)	p-value
RER	0.97 ± 0.02	0.96 ± 0.02	0.64
VO ₂ (mL/kg/min)	27.73 ± 5.17	28.71 ± 4.07	0.12
EE (kcal/kg/min)	0.109 ± 0.02	0.113 ± 0.016	0.11
NEFA (mM)	0.36 ± 0.14	0.36 ± 0.18	0.89
Glycerol (mM)	145.77 ± 51.96	175.78 ± 70.64	0.19
Glucose (mM)	4.50 ± 0.58	4.87 ± 0.39	0.01‡
Insulin (μU/mL)	3.15 ± 2.22	2.93 ± 2.09	0.50
Glucagon (pg/mL)	149.27 ± 17.48	164.34 ± 44.97	0.35
C-peptide (ng/mL)	2.18 ± 0.91	2.35 ± 0.89	0.01‡
Lactate (mM)	8.57 ± 2.67	9.39 ± 2.07	0.28
Epinephrine (pg/mL)	60.41 ± 55.29	73.01 ± 31.58	0.03‡
Norepinephrine (pg/mL)	816.60 ± 277.45	937.30 ± 291.72	0.20

Notes:

1. Data are reported in mean ± SD

2. ‡ Denotes a significance level of p<0.05 between the aerobic exercise bout of the AR and RA trials

Table 6. Metabolite Concentrations After Completion of a Resistance Bout of Exercise

	Trial AR Post- Resistance Exercise Bout (60 min)	Trial RA Post- Resistance Exercise Bout (30 min)	p-value
RER	1.03 ± 0.05	1.07 ± 0.07	0.05‡
VO ₂ (mL/kg/min)	15.50 ± 1.94	14.81 ± 1.23	0.59
EE (kcal/kg/min)	0.061 ± 0.008	0.058 ± 0.005	0.55
NEFA (mM)	0.288 ± 0.084	0.243 ± 0.096	0.91
Glycerol (mM)	140.68 ± 33.35	96.81 ± 42.11	0.01‡
Glucose (mM)	5.03 ± 0.53	5.18 ± 0.54	0.69
Insulin (μU/mL)	3.09 ± 2.25	4.71 ± 1.86	0.01‡
Glucagon (pg/mL)	154.28 ± 21.44	141.26 ± 28.41	0.14
C-peptide (ng/mL)	2.54 ± 1.16	2.96 ± 1.00	0.04‡
Lactate (mM)	13.45 ± 3.72	12.42 ± 1.87	0.34
Epinephrine (pg/mL)	67.94 ± 31.84	68.84 ± 42.62	0.06
Norepinephrine (pg/mL)	1037.91 ± 303.07	1175.75 ± 263.84	0.67

Notes:

1. Data are reported in mean ± SD
2. ‡ Denotes a significance level of p<0.05 between resistance exercise bout of the AR and RA trials

6.5 *Substrate Oxidation during Recovery*

RER, measured immediately post-exercise (60 min), was significantly higher in the AR trial compared with the RA trial (0.97 ± 0.07 vs. 0.83 ± 0.07 , respectively). There were no subsequent differences between trials for the remainder of the recovery. However, RER continued to decline significantly ($p < 0.05$) in the AR and RA trials at 75 min and 90 min relative to immediately post-exercise (60 min; Table 7), and in the AR trial, RER tended to be further reduced after 90 min compared to 75 min ($p = 0.086$). Fat oxidation rates immediately post-exercise (60 min) were significantly higher for the RA trial compared to the AR trial (0.11 ± 0.04 vs. 0.00 ± 0.10 g/min). In contrast, carbohydrate oxidation rates were significantly elevated immediately post-exercise in the AR trial compared to the RA trial (0.60 ± 0.34 vs. 0.265 ± 0.20 g/min), which is in line with the RER data. No other significant differences in fat or carbohydrate oxidation rates were observed between trials.

Plasma NEFA concentrations increased 15 min post exercise (75 min) for both trials ($p < 0.05$) and subsequently decreased modestly by 90 min whereby it was not significantly different from 60 nor 75 min in the recovery period of both trials (Table 7). In addition, the delta NEFA concentrations from 60 min to 90 min were not significantly different between trials (AR: 0.05 ± 0.11 mM vs. RA: 0.07 ± 0.12 mM) In the RA trial, glycerol concentrations were reduced 15 min post-exercise (75 min) and remained depressed at 30 min post-exercise (90 min) compared to immediately post-exercise (60 min, $p < 0.05$; Table 7). In contrast, there were no significant changes in post-exercise glycerol concentrations in the AR trial despite that glycerol concentrations tended to be reduced at 90 min compared to 60 min ($p = 0.113$) and 75 min ($p = 0.097$). Between trials, glycerol concentrations tended to be higher at 60 min in the RA trial compared to the AR trial ($p = 0.112$; Table 7).

Following the completion of exercise, catecholamine response was reduced significantly in both trials after 15 min of recovery ($p < 0.05$) and remained lower after 30 min of recovery (Figure 6A&B), similar to baseline concentrations depicted in Figures 3A&B. There were no differences in either epinephrine or norepinephrine concentrations at any time point between the AR and RA trial (Figure 6A&B).

There were also no differences in plasma glucose concentrations between trials at any time point despite the decrease in glucose concentrations that were observed for both trials after 15 min recovery (AR: 5.03 ± 0.53 at 60 min to 4.89 ± 0.58 mM at 75 min; RA: 4.87 ± 0.39 at 60 min to 4.68 ± 0.30 at 75 min, $p < 0.05$; Table 7, Figure 7A). Glucose concentrations remained significantly lower at 90 min compared to 60 min (Table 7, Figure 7A). AUC for glucose was significantly lower for the last 15 min of recovery (75 to 90 min) compared to the first 15 min immediately post-exercise (60 to 75) in both trials (AR: 38.34 mM*min for 60 to 75 min vs. -13.13 mM*min for 75 to 90 min, RA: 107.55 mM*min for 60 to 75 min vs. -1.35 mM*min for 75 to 90 min, $p < 0.05$; Figure 7B). Yet, total glucose AUC during recovery was similar between trials.

Insulin concentrations significantly increased after 15 min recovery in both the AR and RA trial and was maintained at 30 min post-exercise (Figure 8A&B, Table 6). In addition, there were no differences in insulin concentrations observed between trials. In contrast, C-peptide concentrations at 75 min were similar to immediately post-exercise (60 min) and did not significantly decline until 90 min (Table 7). Taken together, the insulin and C-peptide results suggest that there was not a continual release of insulin into circulation during recovery, but rather insulin clearance was reduced after exercise.

Contrary to changes during exercise, glucagon concentrations declined during recovery such that glucagon concentrations were significantly lower at 75 min and further reduced at 90 min in both trials (Table 7).

Table 7. Metabolite Concentrations at Completion of Exercise and During Recovery

Metabolite	Trial AR			Trial RA		
	Immediately Post-Exercise (60 min)	Post-Exercise (75 min)	Post-Exercise (90 min)	Immediately Post-Exercise (60 min)	Post-Exercise (75 min)	Post-Exercise (90 min)
Energy-Related Parameters						
RER	0.97 ± 0.07 ^{a*}	0.72 ± 0.08 ^b	0.67 ± 0.03 ^b	0.83 ± 0.07 ^a	0.71 ± 0.10 ^b	0.71 ± 0.07 ^b
VO ₂ (mL/kg/min)	5.90 ± 1.76 ^a	4.31 ± 1.41 ^b	3.75 ± 1.49 ^b	5.66 ± 0.11 ^a	4.44 ± 0.82 ^b	3.66 ± 0.57 ^b
EE (kcal/kg/day)	42.85 ± 13.17 ^a	29.41 ± 9.87 ^b	25.26 ± 9.88 ^b	39.67 ± 11.90 ^a	30.22 ± 5.68 ^b	24.77 ± 3.80 ^b
Lipid-Related Parameters						
NEFA (mM)	0.288 ± 0.084 ^a	0.372 ± 0.101 ^b	0.342 ± 0.121 ^{ab}	0.362 ± 0.175 ^a	0.495 ± 0.250 ^b	0.431 ± 0.208 ^{ab}
Glycerol (mM)	140.68 ± 33.35	147.38 ± 69.45	118.83 ± 54.02	175.78 ± 70.64 ^a	133.85 ± 41.00 ^b	104.66 ± 47.09 ^b
Carbohydrate-Related Parameters						
Glucose (mM)	5.03 ± 0.53 ^a	4.89 ± 0.58 ^b	4.61 ± 0.56 ^b	4.87 ± 0.39 ^a	4.68 ± 0.30 ^b	4.77 ± 0.37 ^b
Insulin (μU/mL)	3.09 ± 2.25 ^a	6.00 ± 5.55 ^b	5.04 ± 3.75 ^{ab}	2.93 ± 2.09 ^a	5.20 ± 3.23 ^b	4.27 ± 2.28 ^{ab}
Glucagon (pg/mL)	154.28 ± 21.44 ^a	144.40 ± 41.06 ^b	129.79 ± 48.88 ^c	164.34 ± 44.97 ^a	129.61 ± 23.66 ^b	118.80 ± 19.23 ^c
C-peptide (ng/mL)	2.54 ± 1.15 ^{ab}	2.71 ± 1.31 ^a	2.38 ± 1.19 ^b	2.35 ± 0.89 ^{ab}	2.45 ± 1.13 ^a	2.22 ± 0.99 ^b
Lactate (mM)	13.45 ± 3.72 ^{a*}	7.56 ± 2.90 ^{b*}	4.51 ± 2.83 ^c	9.39 ± 2.07 ^a	5.09 ± 1.28 ^b	3.78 ± 1.68 ^b

Notes:

1. Data are reported in mean ± SD
2. * Denotes a significance level of p<0.05 between trials
3. Different letters denote a significance level of p<0.05 between time points (60 vs. 75 vs. 90 min) within a trial

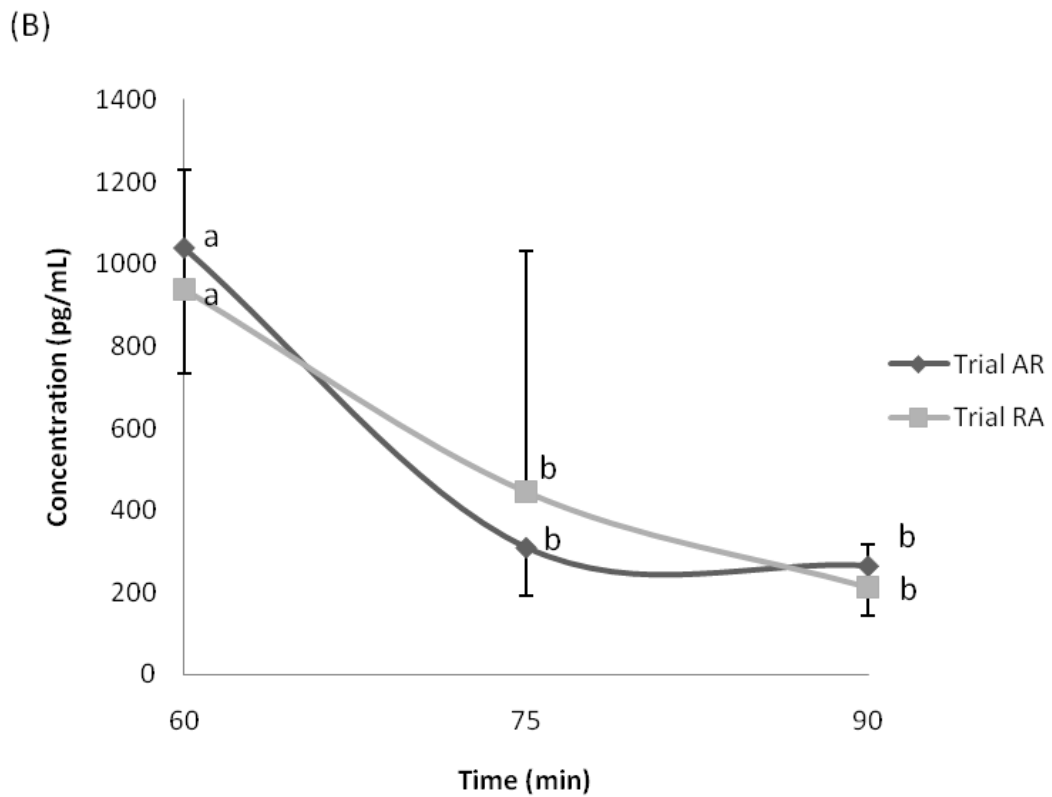
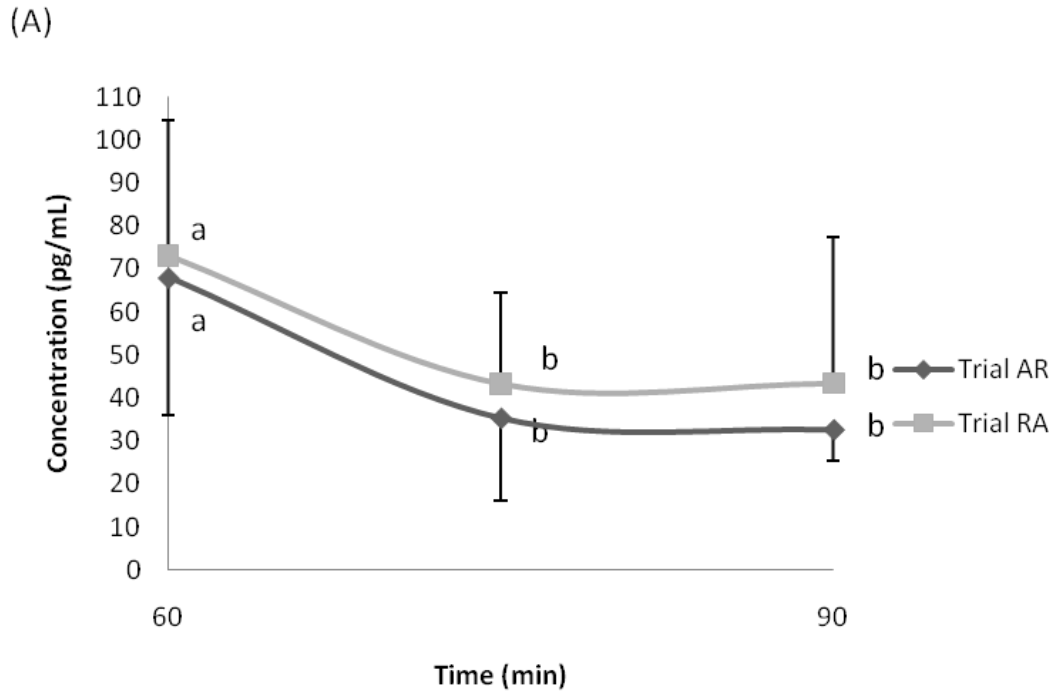


Figure 6. Epinephrine (A) and norepinephrine (B) concentration during recovery. * denotes a significant difference of $p < 0.05$ between trials; different letters denote a significance level of $p < 0.05$ between time points (60 vs. 75 vs. 90 min) within a trial.

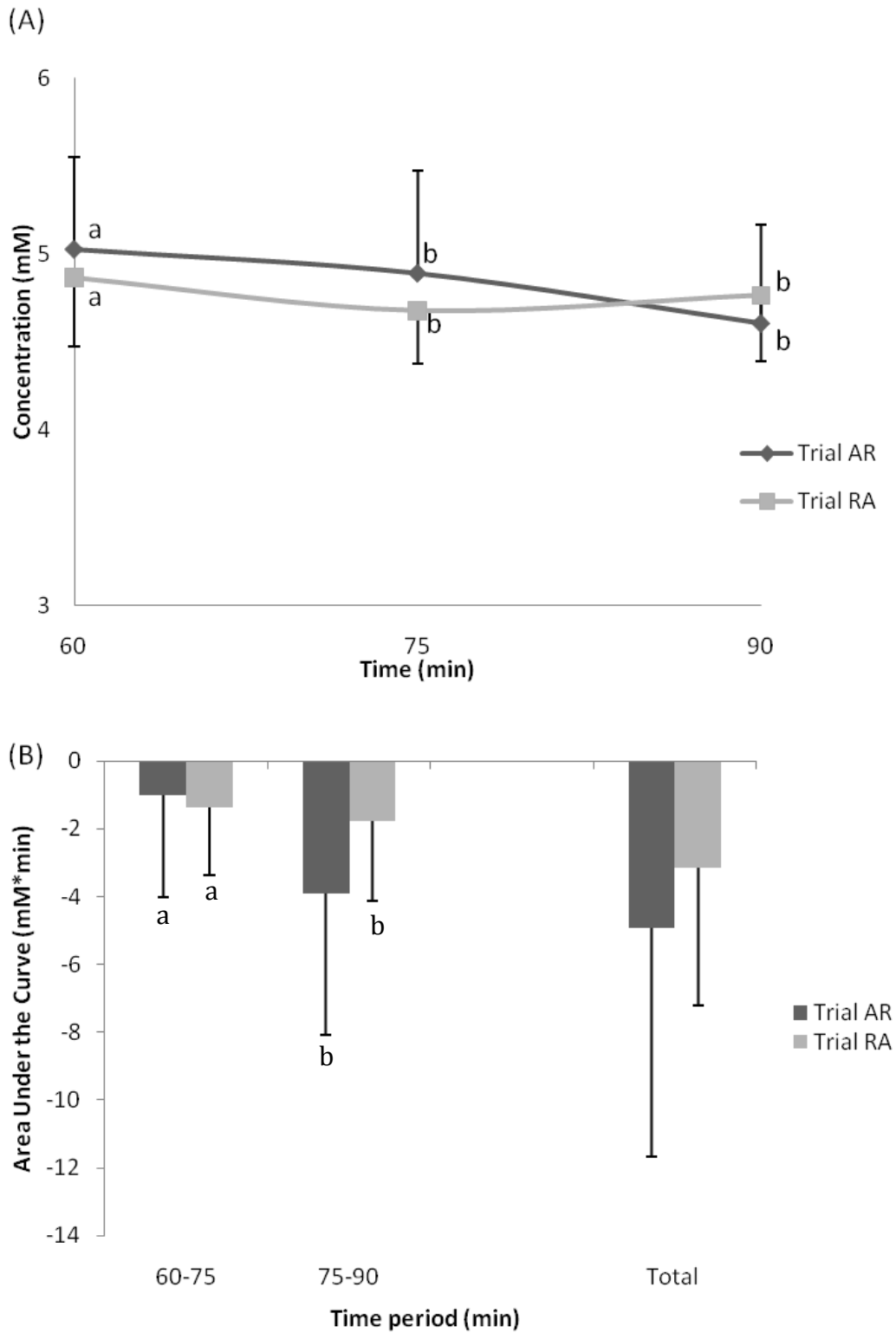


Figure 7. Glucose concentration (A) and AUC (B) during recovery. * denotes a significant difference of $p < 0.05$ between trials; different letters denote a significance level of $p < 0.05$ between time points (0 vs. 30 vs. 60 min) within a trial.

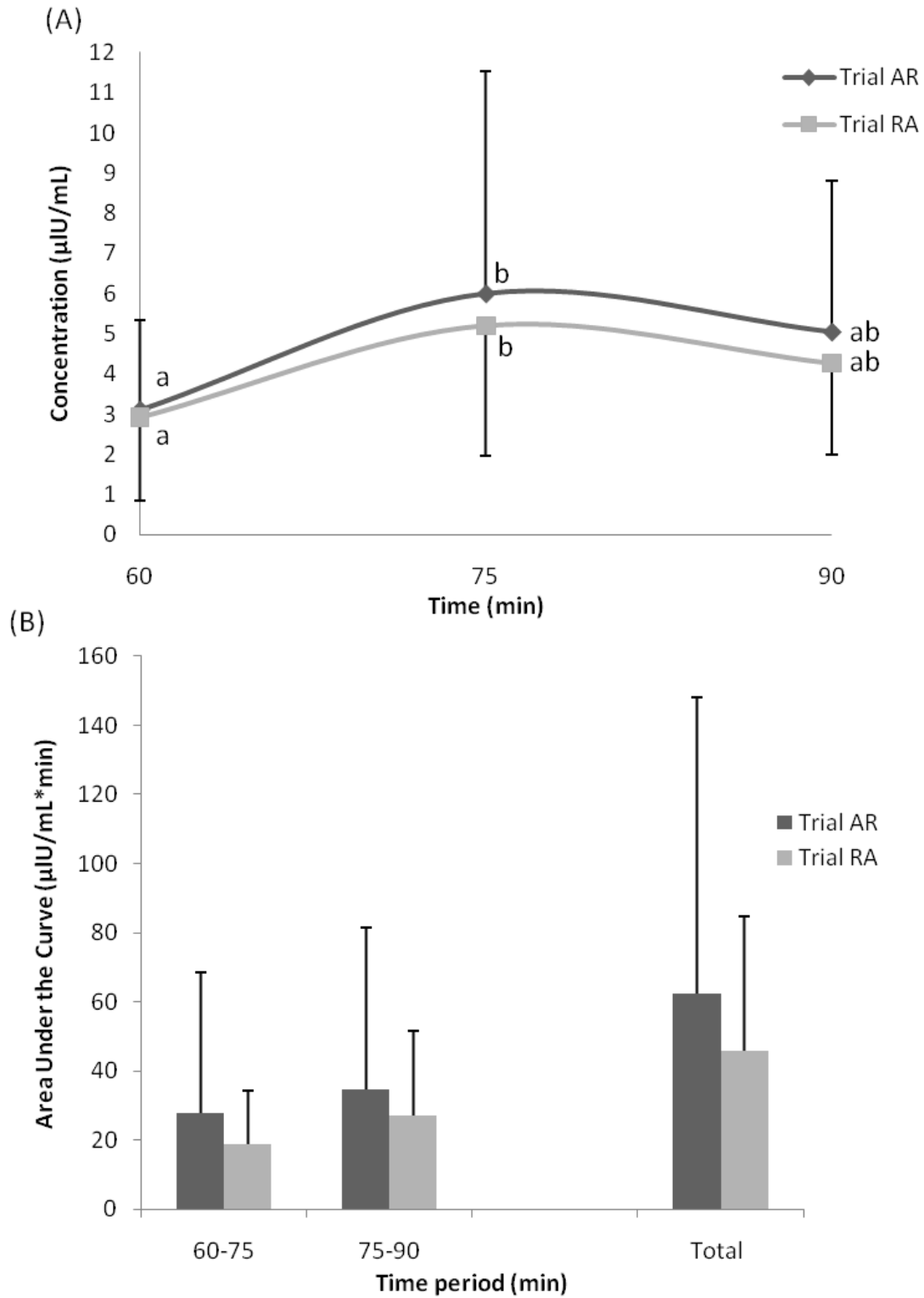


Figure 8. Insulin concentration (A) and AUC (B) during recovery. * denotes a significant difference of $p < 0.05$ between trials; different letters denote a significance level of $p < 0.05$ between time points (0 vs. 30 vs. 60 min) within a trial.

There was a significant drop in lactate concentrations from 60 to 75 min within each trial ($p < 0.05$). And, there was a further drop in the AR trial by 90 min (Table 7) compared to 75 min, while there were no significant differences from 75 to 90 min in the RA trial (Table 7). Lactate concentrations were significantly greater in the AR trial immediately post-exercise and 15 min post-exercise (60 min and 75 min, respectively) compared to the RA trial (Table 7). Moreover, the delta lactate concentrations from 60 to 90 min were significantly greater for the AR trial ($-8.94 \pm 3.91 \text{ mM}$) compared to the RA trial ($-5.61 \pm 0.83 \text{ mM}$; $p = 0.031$).

6.6 *Energy Expenditure during Recovery*

Oxygen consumption during recovery was similar between trials (Table 7). As expected, reductions in VO_2 were evident at 75 and 90 min in both trials compared to immediately post-exercise ($p < 0.05$) and although significance was not achieved, VO_2 tended to be lower at 90 min compared to 75 min in both trials ($p = 0.078$; Table 6).

The trend observed for VO_2 was paralleled by changes in energy expenditure. Energy expenditure was reduced at 75 min compared to values measured immediately post-exercise (Table 7). Energy expenditure tended to be reduced from 75 to 90 min for both trials ($p = 0.091$; Table 7). In addition, there were no significant differences between the AR and RA trials at any time point during recovery. Overall, both trials resulted in a net energy expenditure of approximately 40 kcal during the 30 min recovery period (AR: 40.34 ± 14.20 and RA: $40.59 \pm 9.75 \text{ kcal}$), which is in comparison to $34.07 \pm 5.38 \text{ kcal}$ for 30 min rest, measured during the initial assessment.

6.7 *Variation between Aerobic Exercise Bouts Performed on Separate Days*

The values displayed in the Bland-Altman plots for glucose, glycerol, and insulin at baseline (0 min) were within 2 SD of the mean difference between the A and AR trials (Figures 9 to 15 & Table 8). Similarly, Bland-Altman plots for glucose, NEFA, glycerol, epinephrine, norepinephrine, and VO_2 at completion of the aerobic exercise bout (30 min) were also within 2SD of the mean difference measured between trials A and AR (Figures 9 to 15 & Table 8). In addition, the mean deviation for all trials except VO_2 , were close to zero, demonstrating minimal day-to-day variability within each parameter (Figures 9 to 15 & Table 8). However, the Bland-Altman plot for NEFA concentration at baseline (0 min) included one participant with values above 2 SD. In addition, catecholamine concentration was elevated above 2SD at baseline (0 min) for one participant in both the epinephrine and norepinephrine Bland-Altman plots. Lastly, after completion of exercise (30 min), there was one participant who had RER values that were below 2SD of the mean. The participants that had values outside of the 2 SD were different for each parameter identified. Despite these deviations from the mean difference, parameters measured on different days using the same protocols showed no differences between trials (i.e. glucose, insulin, NEFA, glycerol, epinephrine, norepinephrine, VO_2 and RER, $p>0.05$; Table 8).

Table 8. Variability between Two Identical Bouts of Aerobic Exercise Performed on Separate Days

	0 min			30 min		
	Trial A: Aerobic Bout	Trial AR: Aerobic Bout	p-value	Trial A: Aerobic Bout	Trial AR: Aerobic Bout	p-value
Glucose (mM)	4.51 ± 0.35	4.53 ± 0.41	0.895	4.79 ± 0.64	4.50 ± 0.58	0.088
Insulin (µU/mL)	5.12 ± 2.47	5.25 ± 2.65	0.831	2.54 ± 1.92	3.15 ± 2.22	0.869
NEFA (mM)	0.391 ± 0.244	0.349 ± 0.150	0.397	0.350 ± 0.191	0.356 ± 0.135	0.397
Glycerol (mM)	77.49 ± 45.80	80.14 ± 41.78	0.715	142.20 ± 57.23	145.77 ± 51.96	0.715
Epinephrine (pg/mL)	40.76 ± 50.57	39.57 ± 40.03	0.825	58.37 ± 28.62	60.41 ± 55.49	0.825
Norepinephrine (pg/mL)	298.96 ± 328.36	278.05 ± 169.27	0.898	830.22 ± 361.04	816.60 ± 277.45	0.898
VO ₂ (mL/kg/min)				26.60 ± 4.17	27.73 ± 5.17	0.211
RER				0.97 ± 0.03	1.27 ± 0.90	0.191

Note:

1. Data are reported in mean ± SD
2. * Denotes a significance level of p<0.05 between trials

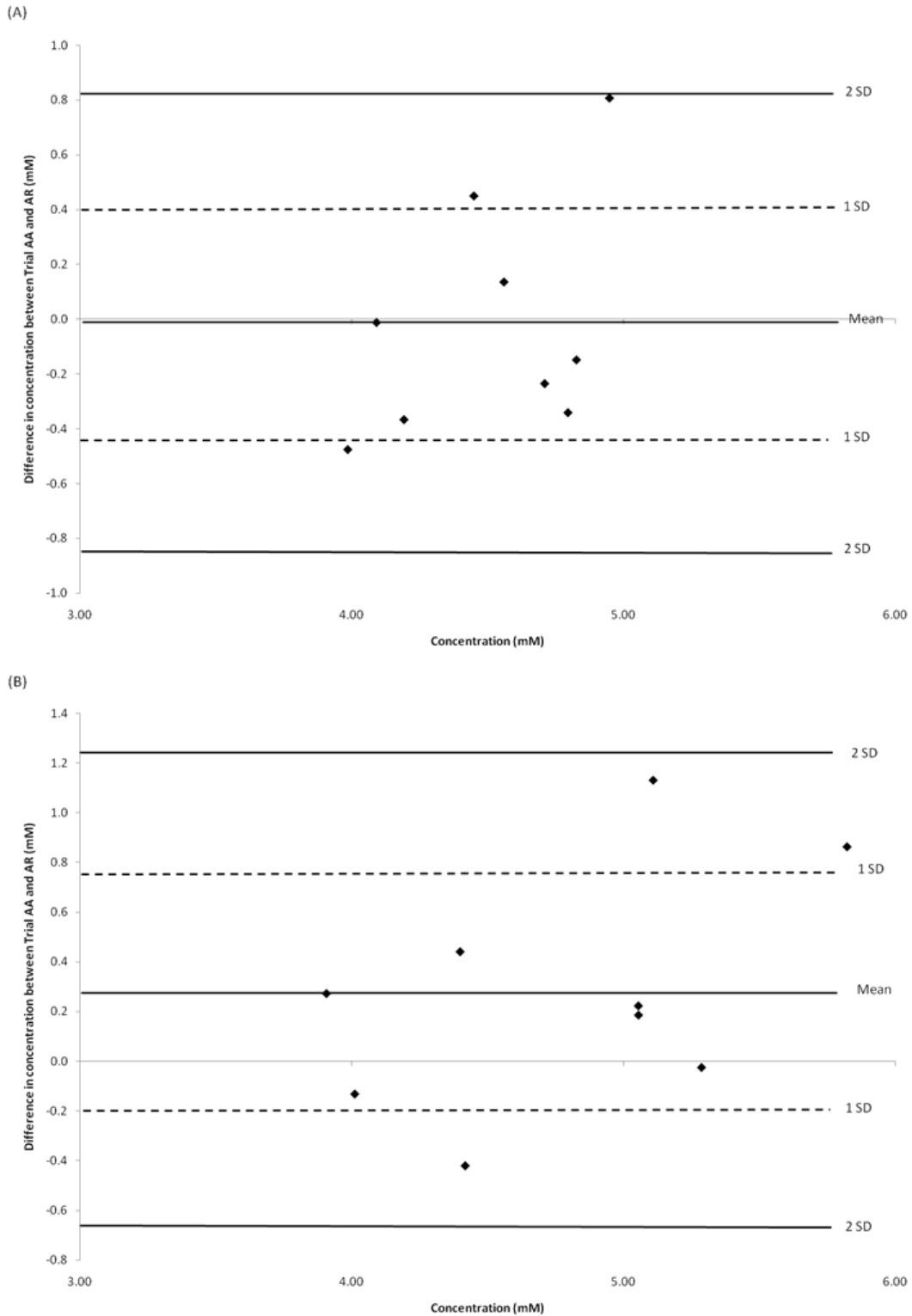


Figure 9. Bland-Altman Plot for Glucose Concentration at Baseline (A) and After Completion of Aerobic Exercise Bout (B). There were no significant differences observed between trials.

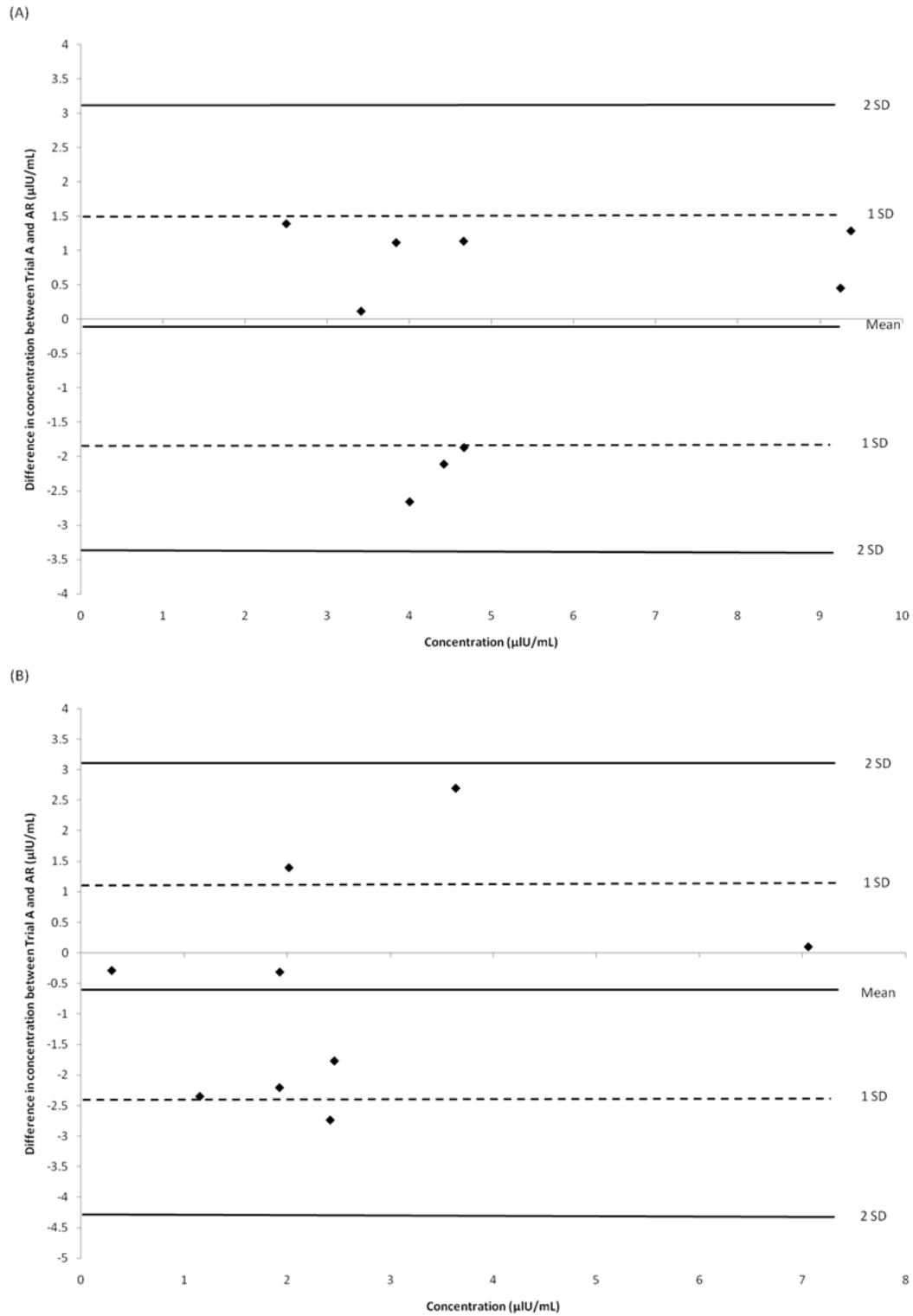


Figure 10. Bland-Altman Plot for Insulin Concentration at Baseline (A) and After Completion of Aerobic Exercise Bout (B). There were no significant differences observed between trials.

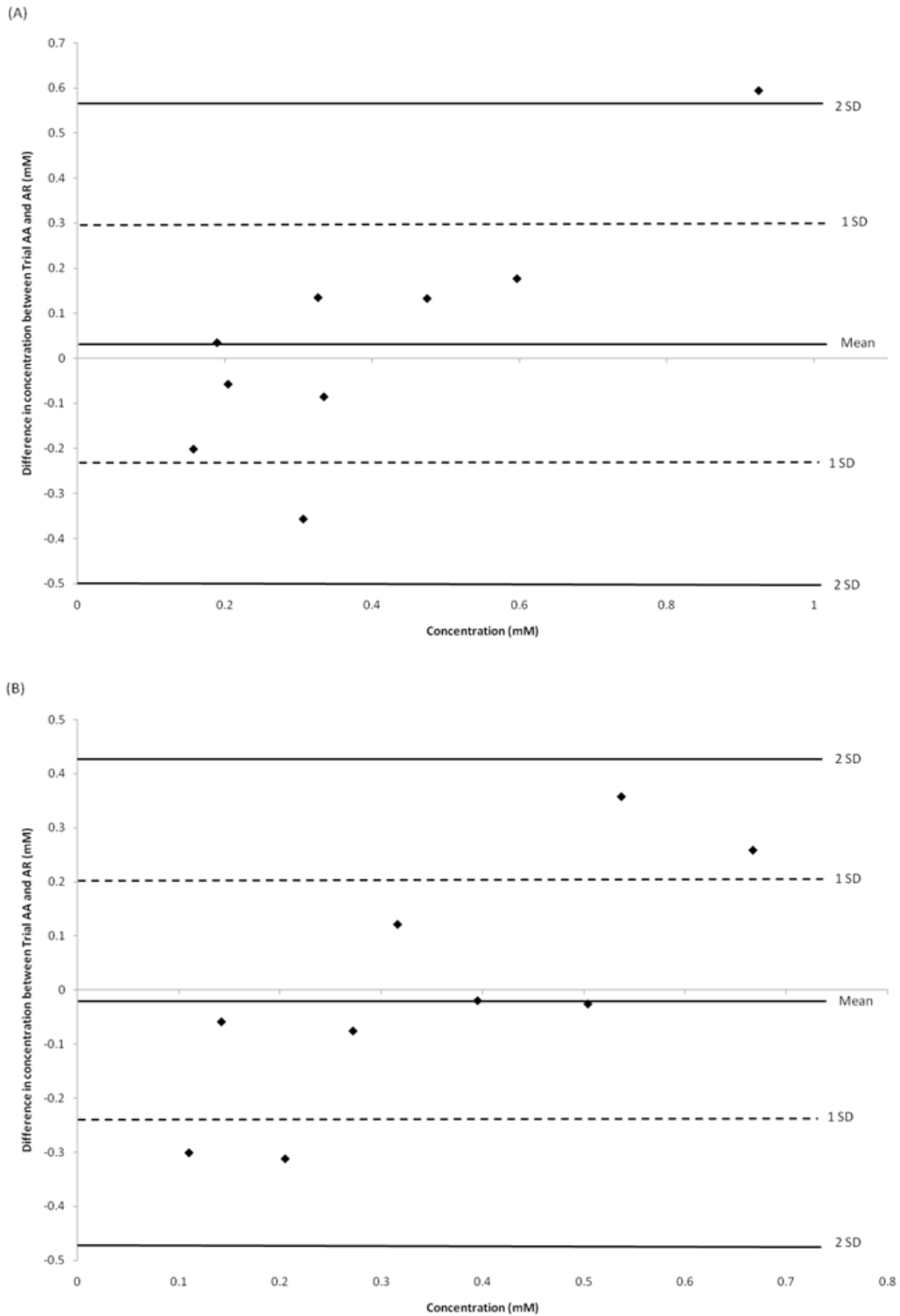


Figure 11. Bland-Altman Plot for NEFA Concentration at Baseline (A) and After Completion of Aerobic Exercise Bout (B). There were no significant differences observed between trials.

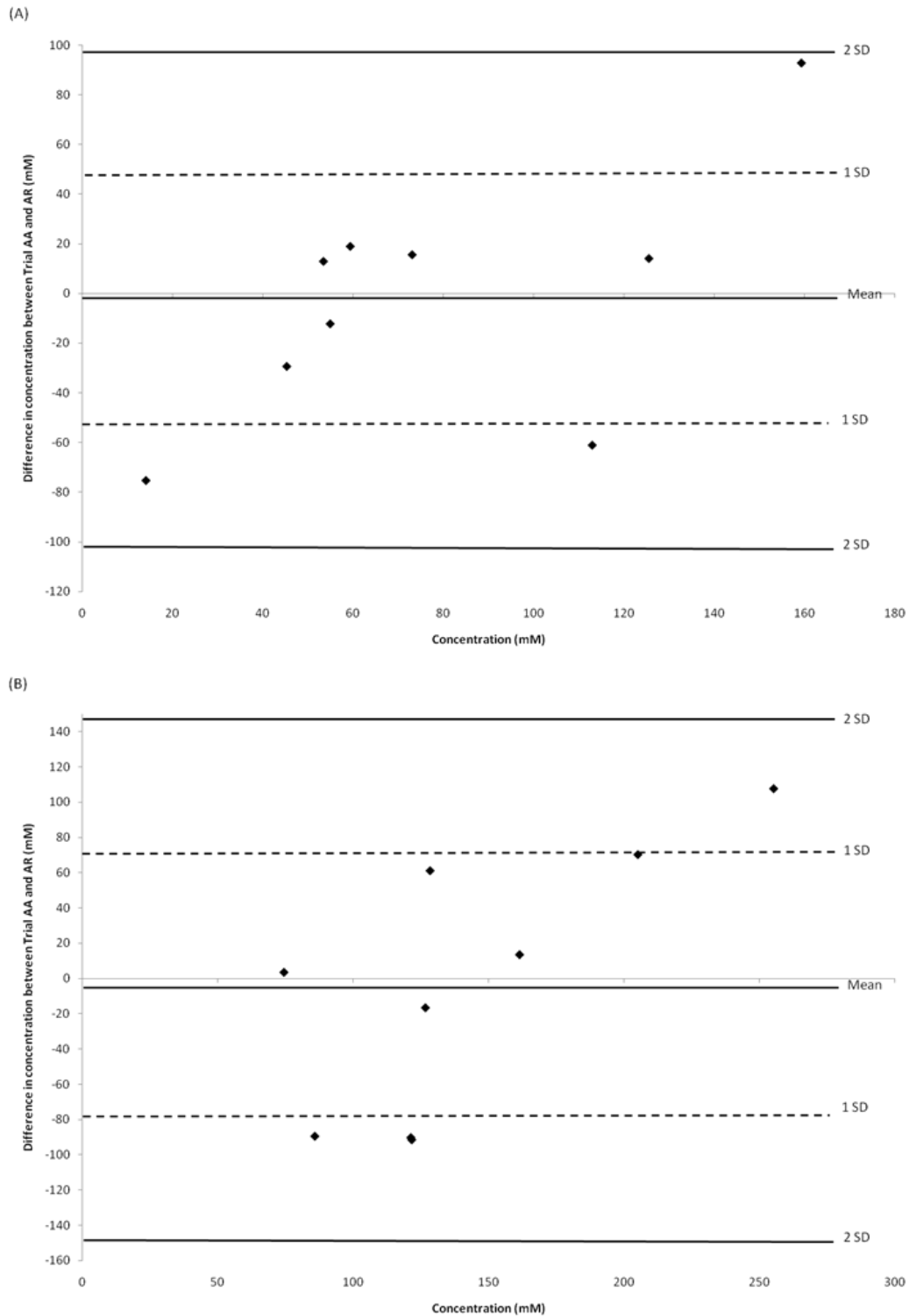


Figure 12. Bland-Altman Plot for Glycerol Concentration at Baseline (A) and After Completion of Aerobic Exercise Bout (B). There were no significant differences observed between trials.

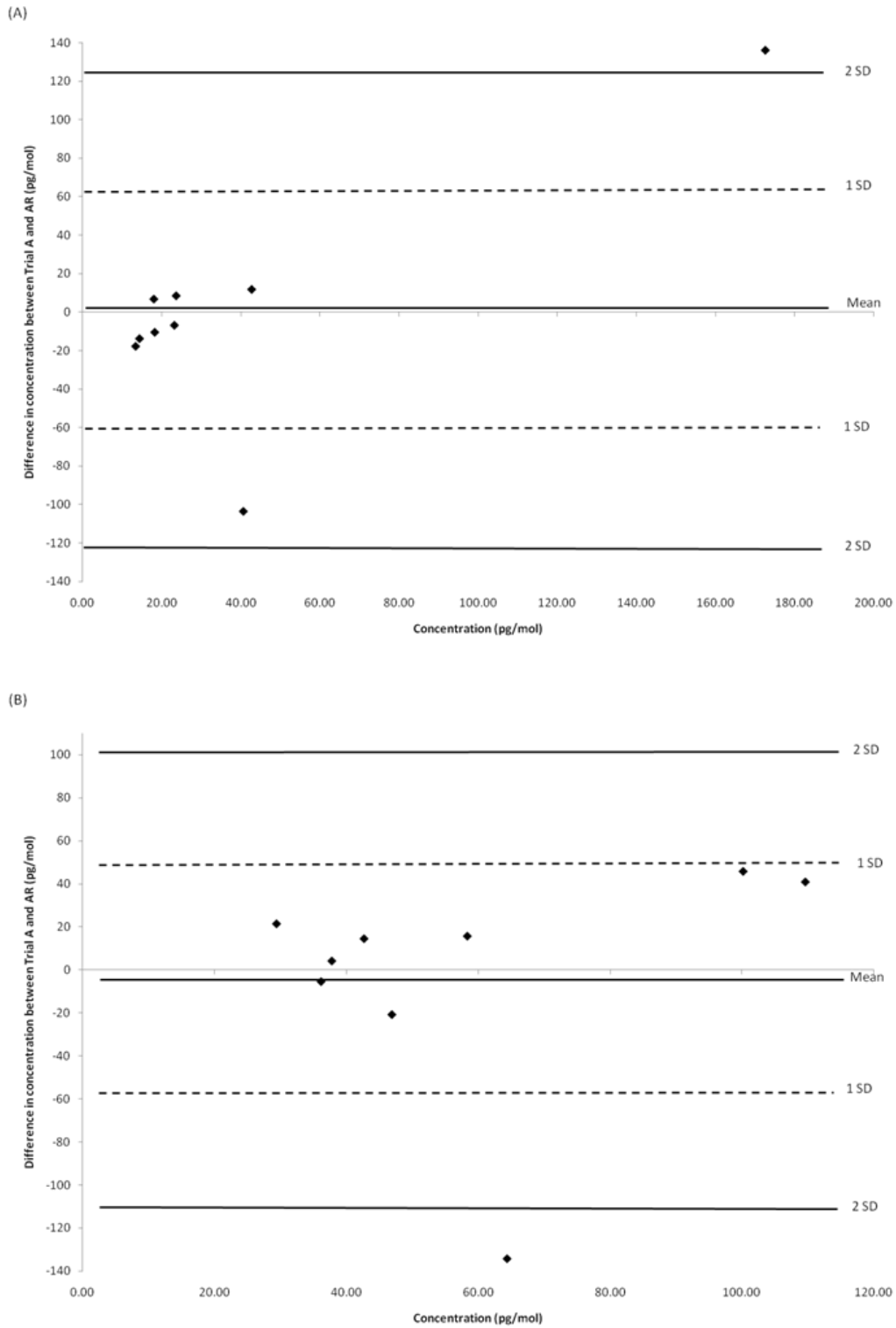


Figure 13. Bland-Altman Plot for Epinephrine Concentration at Baseline (A) and After Completion of Aerobic Exercise Bout (B). There were no significant differences observed between trials.

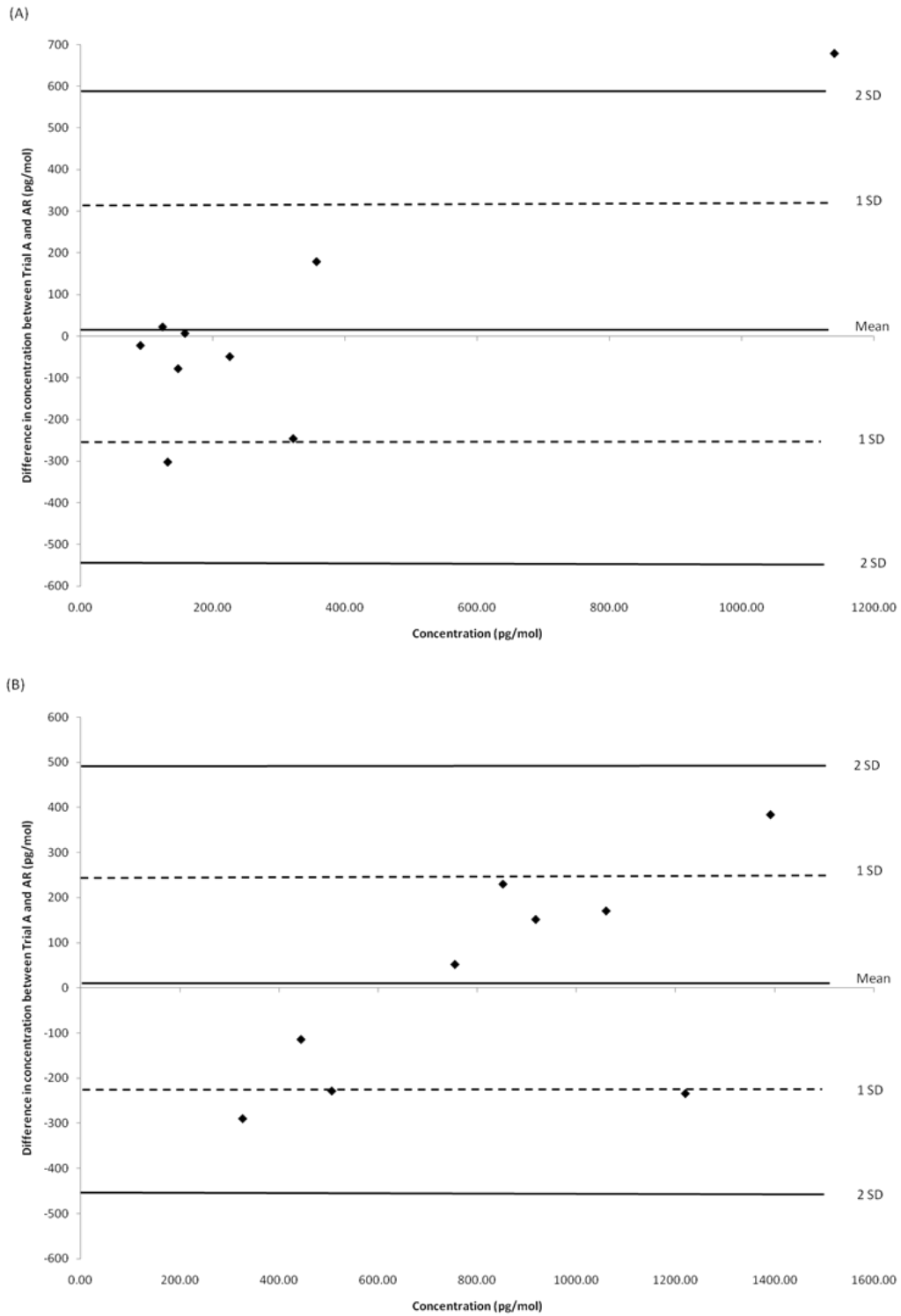


Figure 14. Bland-Altman Plot for Norepinephrine Concentration at Baseline (A) and After Completion of Aerobic Exercise Bout (B). There were no significant differences observed between trials.

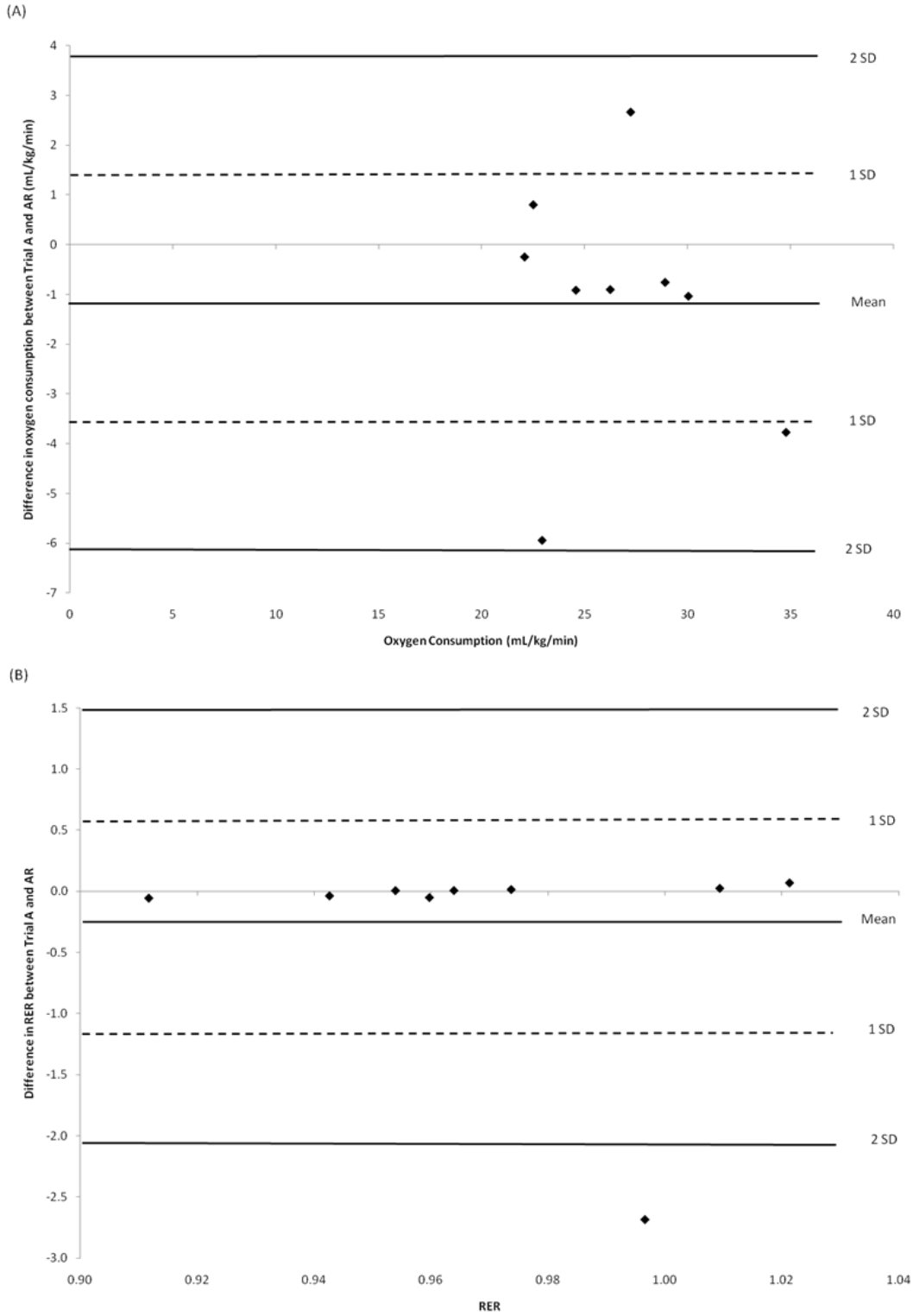


Figure 15. Bland-Altman plot For VO_2 (A) and RER (B) After the Completion of an Aerobic Exercise Bout (30 min).

7.0 Discussion

This was the first study to investigate the effects of exercise sequence in a combined aerobic and resistance exercise session on substrate utilization and energy expenditure in recreationally active males. Contrary to our hypothesis that the RA trial would result in greater NEFA availability, and thus lipid oxidation, during the aerobic exercise bout compared to the AR trial, NEFA availability and lipid oxidation were not different for the aerobic portion of each trial. While norepinephrine concentrations were elevated to the same extent during both the aerobic and resistance exercise bouts of each trial, epinephrine concentrations were significantly higher after a bout of aerobic exercise in the RA trial compared to the AR trial. This provides partial support for our hypothesis that catecholamine concentrations would be higher during the RA trial compared to the AR trial. We also hypothesized that post-exercise lipid oxidation would be augmented, and would be accompanied by an increase in energy expenditure, when resistance exercise preceded aerobic exercise in comparison to aerobic exercise preceded by a resistance exercise bout. Although we did not observe any differences in epinephrine or norepinephrine at any time during recovery, glycerol concentrations were elevated immediately post-exercise in the RA trial. An increased mobilization of lipid substrate seems likely as relative rates of lipid oxidation were significantly higher immediately post-exercise for the RA exercise sequence compared to the AR exercise sequence, but there were no changes in energy expenditure. Differences between trials were abolished 15 minutes into recovery; thus the effect of exercise sequence on post-exercise substrate oxidation and energy expenditure may be minimal but requires further investigation.

Our final hypothesis was that two identical bouts of moderate intensity aerobic exercise, performed on separate days, would elicit the same hormonal response and thus produce similar rates of carbohydrate and lipid oxidation for the duration of the aerobic exercise bout. In comparing two bouts of aerobic exercise that were performed one week apart, we found that there was minimal variability in parameters evaluated for identical bouts of moderate-intensity aerobic exercise in healthy, untrained males.

7.1 Substrate Oxidation during Exercise is Not Influenced by Exercise Sequence

The anticipated elevation in lipid oxidation in the aerobic bout of the RA trial in comparison to the aerobic bout of the AR trial was not observed. In both trials, RER values demonstrated that carbohydrate oxidation was the primary source for energy provision in the first 30 min of exercise and remained elevated and close to 1.0 regardless of the type of exercise. This was in contrast to the findings of Kang et al (2009) where RER for aerobic exercise performed subsequent to a bout of resistance exercise was approximately 0.87, substantially lower than the RER values reported in our study. In addition, RER was significantly lower when aerobic exercise was preceded by high intensity resistance exercise compared to aerobic exercise performed without any prior exercise (Kang et al, 2009). In the present study, while RER values for the aerobic exercise bout during the AR trial were elevated from rest, and in the RA trial, RER values declined slightly following resistance exercise, both trials revealed no differences in RER values between each aerobic exercise bout. In addition, substrate oxidation rates were similar between the aerobic exercise bouts of each trial suggesting that lipid oxidation was not influenced by exercise sequence. This discrepancy between Kang et al (2009)

and our study results may be due to employment of a different resistance exercise protocol: participants in Kang et al (2009) performed 3 sets of 8 repetitions for 6 exercises at 90% 8-RM, which is equivalent to completing 3 sets of 8 repetitions at 80% 1-RM for each exercise. Participants in our study performed 3 sets of 8 repetitions for 5 exercises at 70% predicted 1-RM. Although the male participants in Kang et al (2009) had slightly higher BMI values ($25.7 \pm 2.1 \text{ kg/m}^2$ compared to $23.9 \pm 4.5 \text{ kg/m}^2$ in our study), maximal oxygen consumption was similar (Kang: $35.4 \pm 5.6 \text{ mL/kg/min}$ vs. $39.52 \pm 5.96 \text{ mL/kg/min}$ in our study). Since participant characteristics were similar between studies, it is possible that a more strenuous resistance exercise protocol, similar to Kang et al (2009), would have elicited greater differences in fat oxidation rates between the aerobic bouts of the AR and RA trial in our study.

NEFA concentrations were not elevated during either AR or RA trial, and in the RA trial, NEFA concentrations were significantly reduced after an initial bout of resistance exercise. NEFA concentrations remained unchanged from rest after aerobic exercise in the AR trial while glycerol concentrations increased, suggesting that NEFA was mobilized and released from adipose tissue at the same rate as it was taken up, most likely by the exercising skeletal muscle. However, during the aerobic portion of the RA trial, there was a simultaneous increase in glycerol and NEFA concentrations, implying that while there was increased lipid mobilization, NEFA may not have been taken up by skeletal muscle. In context with RER and substrate oxidation values, NEFA were not the primary substrate oxidized for energy provision during the aerobic portion of the RA trial. According to Goto et al (2007), resistance exercise performed 20 minutes prior to aerobic exercise did not increase NEFA concentrations (Goto et al, 2007) and in

agreement with our findings, NEFA concentrations were actually depressed prior to the initiation of the subsequent bout of aerobic exercise in the RA trial. Interestingly though, in Goto et al (2007), fat oxidation was still augmented in the subsequent bout of aerobic exercise, despite lower NEFA concentrations, potentially implying that NEFA uptake exceeded NEFA mobilization from adipose tissue. This finding was not confirmed in our study and Goto et al (2007) suggest that the increased lipid oxidation in the aerobic exercise bout could be attributed to elevated catecholamine concentrations.

In our study, however, only epinephrine —and not norepinephrine— concentrations were significantly higher at the end of the aerobic exercise bout of the RA trial compared to the AR trial. Epinephrine and norepinephrine increased in the first 30 min of exercise and remained elevated in both trials above rest until 60 min. While there were no further increases in NEFA concentrations compared to rest during the aerobic bout of either trial, elevated norepinephrine concentrations —a stimulus for lipolysis in adipose tissue (Quish et al, 2005)— and concomitant increases in glycerol concentrations support increased NEFA mobilization and subsequent uptake into exercising muscle. Glycerol would likely be taken up by the liver as a precursor for gluconeogenesis; however since hepatic blood flow is reduced during exercise (Schoemaker et al, 1998), glycerol clearance from circulation would likely be slower than NEFA uptake by exercising muscles. While elevated insulin concentrations are known to promote increased NEFA uptake through CD 36 translocation (Schwenk et al, 2010), the results from our study show a drop in insulin concentrations with aerobic exercise. Thus, insulin did not likely have a role in the increased NEFA uptake observed for the aerobic portion of each trial.

However, the low plasma insulin concentrations observed during aerobic exercise in both the AR and RA trial were conducive for carbohydrate oxidation. Glucose concentrations increased after a bout of resistance exercise in the RA trial to become significantly higher during aerobic exercise (compared to the AR trial) and were coupled with a significant increase in lactate concentrations compared to rest in both trials. This was in agreement with reported RER values to suggest carbohydrate oxidation contributed most appreciably to energy provision during the subsequent bout of aerobic exercise. Yet, increased plasma glucose concentrations in the RA trial did not result in increased glycolysis compared to the AR trial, as Frayn's estimations (1983) of carbohydrate oxidation rates as well as RER values indicate no differences between trials.

In addition, energy provision was similar between trials. It was expected that muscle fatigue from prior resistance exercise would decrease mechanical efficiency during the subsequent bout of aerobic exercise in the RA trial compared to the aerobic exercise in the AR trial. However, VO_2 and energy expenditure were similar for both trials suggesting that prior exercise did not influence the mechanical efficiency and factors that promote fatigue in the exercising muscle.

Mechanistically, a rise in AMP: ATP ratio during the resistance exercise bout (RA trial), although not measured in this study, would be expected to activate the AMPK pathway (Dreyer et al, 2006). The resultant phosphorylation of PFK would increase glycogenolysis and glycolysis within the exercising muscle. In addition, elevated Ca^{2+} and epinephrine concentrations would also activate glycolytic enzymes, promoting pyruvate production (Mourtzakis et al, 2006). While increased epinephrine and glucose concentrations were observed for the aerobic bout of the RA trial compared to the AR

trail, it remains unclear why these increases in hormone and substrate did not stimulate increased carbohydrate oxidation. Higher pyruvate concentrations, from increased glycolysis, stimulate PDH activity, producing acetyl-CoA as substrate for the TCA cycle.

Although AMPK activation also removes inhibition of ACC on CPT1 to stimulate increased transport of NEFAs into mitochondria, lipid oxidation as estimated by RER values, still contributed less to energy provision than carbohydrate oxidation. Perhaps the initial depression in plasma NEFA concentrations after resistance exercise in the RA trial limited the amount of substrate taken up by skeletal muscle, and thus less NEFA was available for CPT1 transport into mitochondria for beta-oxidation. As well, Watt et al (2004) found carbohydrate oxidation to be greatest during the first 30 minutes of exercise. Since the baseline for initiation of aerobic exercise was altered by prior resistance exercise in the RA trial, the increased catecholamine concentrations, reduced NEFA concentrations and increased plasma glucose availability should potentially favor a greater reliance on carbohydrate oxidation during the aerobic exercise bout. This would be in comparison to the aerobic bout of the AR trial, where exercise commenced from rest. Thus, skeletal muscle was not primed with energy substrate from a previous exercise bout to favor selection of carbohydrates as opposed to lipids for oxidation during the aerobic portion of the AR trial.

In contrast, when resistance exercise was compared between trials, elevated insulin and C-peptide concentrations along with higher RER values were observed in the RA trial, suggesting a greater reliance on carbohydrate oxidation, and potentially a greater activation of the AMPK pathway. In addition, glycerol concentrations were maintained similar to rest after a bout of resistance exercise in the RA trial compared to

the AR trial where glycerol concentrations were significantly elevated compared to rest (as well as compared to a bout of resistance exercise in the RA trial). Since there was a drop in NEFA concentrations after resistance exercise in the RA trial (with no changes in NEFA concentration in the AR trial), this suggests that insulin could have potentially mediated an increase in NEFA uptake during resistance exercise in the RA trial. This would likely increase lipid availability for energy provision in the subsequent bout of aerobic exercise in the RA trial. However RER was 0.96 which indicates a primary reliance on carbohydrate oxidation and thus it remains unclear if resistance exercise performed in a RA exercise sequence, has the potential to significantly influence substrate oxidation.

The increased lactate production observed throughout the duration of both trials, with greatest production occurring after resistance exercise, was likely the result of an increase in pyruvate availability. This could have implications for estimation of carbohydrate and lipid oxidation as Kang et al (2009) note that increased acidosis (i.e. lactate production) can interfere with the assessment of substrate oxidation from indirect calorimetry, such that carbohydrate oxidation may be overestimated while lipid oxidation is underestimated. Lactate concentrations measured in our study were higher than concentrations measured after resistance exercise in Goto et al (2007), which may confound our RER values. Therefore, caution should be used to assess the effect of exercise sequence on substrate oxidation at the intensities used in our study.

7.2 Substrate oxidation during Recovery is Minimally Influenced by Exercise Sequence

This is the first study to our knowledge that evaluates post-exercise substrate oxidation when exercise sequence is altered in a combined aerobic and resistance exercise session. The findings from this study supported our hypothesis that fat oxidation would be increased immediately post-exercise in a resistance-aerobic exercise sequence as opposed to an aerobic-resistance exercise sequence. Although RER declined over the 30 min recovery for both trials, RER was significantly lower in the RA trial immediately post-exercise, indicating an increased contribution of lipid oxidation to energy provision. According to Frayn's 1983 estimations of substrate utilization, lipid oxidation was greater immediately post-exercise in the RA trial, compared to the AR trial (RA: 0.11 ± 0.04 vs. AR: 0.00 ± 0.10 g/min). However, this occurred in absence of changes in energy expenditure. NEFA concentrations were elevated immediately after cessation of exercise in both trials, while glycerol concentrations were only significantly elevated in the RA trial in comparison to the remainder of recovery. It is likely that elevated catecholamine concentrations at the cessation of exercise promoted increased TAG breakdown early in recovery. Insulin levels, which were depressed immediately after exercise, could have potentially had a role in the initial increase in plasma NEFA availability. However, insulin concentrations were elevated by 15 min post-exercise and therefore, were not likely responsible for the elevated circulating NEFA concentrations during the remainder of the recovery period in both trials. The mechanism for continued elevation of NEFA concentrations post-exercise, in accord with other studies (Bahr et al, 1991; Kiens and Richter, 1998; Goto et al, 2007b; Henderson et al, 2007; Magkos et al, 2007) is still unclear. Goto et al (2007) have alluded to growth hormone, which was not measured in

this study, as a potential stimulus for lipolysis later in recovery. Glycerol concentrations, in contrast, decreased during recovery in the RA trial and were most likely recycled in the liver as a precursor for gluconeogenesis. Interestingly, despite the initial differences in substrate selection between trials in early recovery (60 min), oxygen consumption and energy expenditure during recovery (at 75 and 90 min) were similar for both trials. This is in contrast to Drummond et al (2005) who reported increased oxygen consumption, and thus energy expenditure for the first 10 minutes immediately following an AR exercise sequence in comparison to a RA exercise sequence. However, differences in respiratory measures had disappeared by 20 min post exercise (Drummond et al, 2005) and the strength of their suggestion that an AR sequence promotes increased energy expenditure post-exercise requires further assessment. The differences in post-exercise oxygen consumption and energy expenditure observed between our study and Drummond et al (2005) could be the result of different exercise programs. In our study, participants cycled at 60% $VO_{2\text{ max}}$ for 30 min, compared to 70% $VO_{2\text{ max}}$ for 25 min in the Drummond et al (2005) study. In addition, both studies prescribed 3 sets of 5 exercises at 70% 1RM for the resistance exercise bout, however the repetitions and thus the total volume of work in our study was lower compared to Drummond et al (2005). Since higher intensity exercise elicits greater post-exercise oxygen consumption and energy expenditure (Thorton and Potteiger, 2002), our study could potentially have found greater differences in recovery between the AR and RA trial if prescribed work intensity had been greater.

Catecholamine concentration declined over the 30 min recovery, and it is assumed that Ca^{2+} concentration was also reduced with the cessation of exercise. The drop in concentration for both of these parameters would reduce the activation of the AMPK

pathway, which would have important implications on substrate selection: glycogenolytic and glycolytic enzyme activity would be reduced. Thus, acetyl-CoA would be derived from beta-oxidation. The excess lactate produced during exercise could be taken up and oxidized by skeletal muscle, in addition to serving as a precursor for liver gluconeogenesis; thus, the decrease in lactate concentrations observed during post-exercise could support increased glycogen re-synthesis during early recovery. Surprisingly, the drop in lactate concentrations during the 30 min recovery, were greater for the AR trial compared to the RA trial. Since lactate concentrations were lower in the RA trial, the higher delta lactate concentrations for the AR trial versus the RA trial could be: 1) a consequence of prior lactate utilization during the aerobic exercise bout of the RA trial or 2) increased lactate clearance after resistance exercise in the AR trial.

Plasma glucose concentrations were reduced, in concert with depressed RER values, during the first 15 min of recovery for both the AR and RA trial. This could be explained by greater glucose uptake by skeletal muscle; other studies have suggested that glucose uptake remains elevated after exercise for up to 2 hours (Dreyer et al, 2008), while carbohydrate oxidation remains depressed to promote glycogen re-synthesis (Richter et al, 1989; van Loon et al, 2003). AUC for glucose during recovery was similar between trials, but was lower during the last 15 min of the 30 min recovery, indicating plasma glucose concentrations were reduced 15 minutes after cessation of exercise. Enhanced glucose uptake (presumably by the exercising skeletal muscle) would be facilitated by the elevated insulin concentrations during recovery; insulin facilitates GLUT 4 translocation as well as CD 36 translocation (Schwenk et al, 2010) and would likely provide substrate for glycogen re-synthesis and lipid oxidation respectively. Insulin

also activates glycogen synthase (Shepherd et al, 1995); if this enzyme had been measured in this study, it would most likely have been elevated post-exercise, confirming the switch from carbohydrate oxidation to synthesis after cessation of exercise.

7.3 Minimal Variability Between Identical Aerobic Exercise Bouts Performed on Separate Days

One of the secondary aims of this study was to determine the day-to-day variability in metabolic and energy-related parameters for moderate-intensity aerobic exercise. Bland-Altman plots for primary parameters (i.e. glucose, insulin, NEFA, glycerol, epinephrine, norepinephrine, VO_2 and RER) showed no significant differences between the A and AR trials for fasting (0 min) and after completion of exercise (30 min). This indicated that the likelihood of day-to-day variability confounding results from identical exercise performed on separate days was minimal. However, inter-subject variability was large based on the large standard deviation values. Thus variability between the A and AR trials was higher relative to the mean difference measured between trials for each of the primary parameters. This large variation between trials was expected with our heterogeneous group of study participants; had our study sample been homogenous (i.e. similar body composition, fasting metabolite concentrations and exercise capacities), we would have expected reduced inter-subject variability and in turn less day-to-day variability between trials. With respect to the data points that were plotted above 2 SD (i.e. Bland-Altman plots for glucose, NEFA, epinephrine and norepinephrine), these deviations occurred at baseline and were not observed by the completion of exercise.

In addition, the trend observed for elevated glucose concentrations at the completion of the aerobic exercise bout in the AR trial compared to the A trial ($p=0.088$) should be investigated further with a larger, more homogenous sample size. Since both

exercise bouts were performed at the same intensity and same time of day, the same hormonal responses should elicit similar changes in glucose metabolism.

Overall, the comparison of primary parameters evaluated in trial A and trial AR suggested that the repeatability for aerobic exercise of identical volume, intensity and duration performed on different days is adequate for attaining results with minimal variation.

7.4 Limitations

Diet prior to each exercise trial was not controlled for in this study. Thus, the influence of food prior to the exercise trial is unknown for its effect on substrate selection, subsequent oxidation as well as resting energy expenditure. However, participants completed an overnight fast prior to each trial, to ensure that fasting metabolite values (e.g. glucose, NEFA, epinephrine) were consistent between trials for each participant as well as between participants.

Participants were not instructed to void prior to completing the BIA procedure in the initial assessment. While a full bladder has the potential to give a false body fat percentage reading, a pilot study was completed to estimate the amount of variance between BIA measurements taken pre- and post-voiding. The difference between the two body fat percentage values calculated was not significant (Appendix 2), indicating that the results obtained in this study were likely an accurate evaluation of body composition for each participant.

Baseline (0 min) measures of VO_2 and VCO_2 were not collected during the exercise trials. Instead of providing a rest period to collect baseline data, participants

began cycling shortly after the catheter was inserted. Therefore, resting oxygen consumption, energy expenditure and RER values were derived from initial assessment data and do not allow for normalization of exercise values to their respective baseline values for each trial.

Oxygen consumption and carbon dioxide output were measured continuously during the aerobic and resistance bouts of each exercise trial. The equipment used measured respiratory data breath-by-breath. Since each exercise performed in the resistance exercise bout lasted a short duration (approximately 1 to 2 min per set) with intermittent rest periods (30 to 60 sec in between each set), it was not practical to stop data collection during these breaks in exercise and would likely lead to greater errors associated with the measurements. Therefore, VO_2 and EE calculated for the resistance exercise bout of each trial included both rest and exercise data and we would expect measurements of these parameters to likely be higher than the values reported in this study, had only VO_2 and VCO_2 during each exercise been used to calculate EE. However, in a real-world setting, resistance exercise is performed with rest periods in between sets and thus the values reported in this study, from a practical perspective, are an accurate depiction of the changes in VO_2 and in turn, EE that occur over the duration of a resistance exercise session.

Energy expenditure was calculated from indirect calorimetry. There were no significant differences between aerobic bouts of exercise for the AR and RA trial as well during recovery. This suggested that exercise sequence did not affect energy expenditure during exercise or post-exercise, however high lactate concentrations could have confounded the estimations based on VO_2 and VCO_2 values in this study (Kang et al,

2009). Therefore, further investigation is required to determine if indirect calorimetry was either: 1) not sensitive enough to detect changes in energy expenditure between trials or 2) there in fact was not a true difference in substrate oxidation patterns when exercise sequence was altered.

Finally, this study was conducted on a small sample size (n=9). There were trends observed during exercise and recovery that would likely have reached significance with the inclusion of more participants. For example, NEFA concentration after 30 minutes of exercise tended to be higher (p=0.056) for the AR trial compared to the RA trial. More insight into substrate selection and subsequent oxidation would be gained if trends were strengthened to achieve significance.

7.5 Practical Implications and Future Directions

It was anticipated that the results from this study could be applied to the prescription of exercise programs designed to promote weight loss. The results from this study suggest that energy expenditure both during exercise and during early recovery (up to 30 minutes post-exercise) were not influenced by exercise sequence in a combined aerobic and resistance exercise session. However, lipid and carbohydrate oxidation were different between the AR and RA trial immediately post-exercise. Future studies should prescribe higher intensity exercise and increase the volume of work to determine if differences in substrate selection immediately post-exercise would be of a greater magnitude and/or sustained for a longer duration in recovery. The influence of a more strenuous exercise protocol on energy expenditure could be evaluated for possible differences between exercise sequences that we did not observe with the exercise

protocol used in this study. Therefore, substrate oxidation and energy expenditure should be evaluated for a longer duration (e.g. 24 hours) and at frequent intervals (e.g. every hour). Regarding the differences and trends seen in the RA trial compared to the AR trial, this would provide greater insight into: 1) how long the increase in plasma NEFA concentrations persist post-exercise and 2) whether the increased plasma NEFA concentrations observed in the recovery period of the RA trial were taken up by skeletal muscle for lipid oxidation

This study measured blood metabolites and used respiratory data to estimate substrate oxidation rates. Future research should measure glycolytic and oxidative enzyme activity as well as take muscle biopsies to investigate changes in intramuscular glycogen and triacylglyceride content during exercise and recovery.

8.0 Conclusions

It can be concluded that contrary to the first hypothesis, a RA exercise sequence did not differ with respect to substrate selection and oxidation from an AR exercise sequence. Although NEFA concentration was reduced prior to the start of aerobic exercise in the RA exercise sequence, it did not appear to influence RER; in addition, lipid and carbohydrate oxidation rates remained similar for both the AR and RA trials for the duration of exercise. With respect to recovery, lipid oxidation rates were greater immediately post-exercise in the RA trial as compared to the AR trial. However, differences in substrate oxidation for the remainder of the 30 min recovery were similar between trials. Although, there were no significant differences in NEFA or glycerol concentration between trials post-exercise, elevations in plasma NEFA and glycerol concentration persisted for the duration of recovery in the RA trial, and thus it would be

worth investigating if lipid oxidation rates remain similar between trials, or if differences between exercise sequences would emerge later in recovery.

Our hypothesis that catecholamine response to exercise would be heightened with a RA exercise sequence was partially confirmed. Epinephrine concentrations were significantly higher after aerobic exercise in the RA trial compared to the AR trial, while norepinephrine concentrations remained similar between trials. There was, however, a tendency for norepinephrine to be elevated after completion of a resistance exercise bout. This provides support for the main finding from this study that with only a difference in epinephrine, and not norepinephrine, concentrations between the aerobic bouts of each trial, catecholamine stimulation of skeletal muscle does not likely affect substrate oxidation or utilization when exercise sequence is altered.

As expected, when two identical bouts of moderate-intensity aerobic exercise performed on separate days, metabolite or energy-related parameters were similar between trials. This strengthens our study's ability to compare the crude differences that result from alteration of exercise sequence, with minimal day-to-day variation that could confound results.

Overall, exercise sequence did not affect substrate oxidation during exercise and very minimally, if at all, during early recovery in healthy, young males. This study highlights the importance of exercise, while suggesting that exercise sequence will not affect the results achieved through a combined aerobic and resistance training program.

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Appendices

Appendix One. Incremental Exercise Test Protocol

UNIVERSITY OF WATERLOO OFFICE OF RESEARCH ETHICS STANDARD
OPERATING PROCEDURES UWSOP212- Protocol for Conducting an Exercise Test
to Measure Oxygen Consumption (VO₂) on a Cycle Ergometer

Purpose: This SOP describes the procedures for the setup, calibration and execution of an exercise test to measure oxygen consumption (VO₂) on a cycle ergometer.

Responsibility: Faculty researchers or supervisors, undergraduate and graduate students

1.0 Preparation of participants

1.1 Each potential participant will be asked if he/she has any conditions that would affect his/her ability to participate in exercise, and will be asked to complete the Health Status Form. Persons with known cardiovascular disease or any other condition that would compromise his/her ability to safely participate in exercise are advised that they cannot participate in the exercise test. According to the American College of Sports Medicine (ACSM) guidelines, any participant will not be permitted to start the exercise test if blood pressure is measured as equal to or above 144/90 mmHg. Likewise, the exercise test will be concluded if blood pressure rises above 250/110 mmHg at any point during the test. A medical health questionnaire, appropriate for the particular participant population, should also be administered at the start of the testing session to identify any co-morbidities the participant may have as well as determine the participant's physical activity level.

1.2 Potential participants will be asked to wear a loose fitting t-shirt, comfortable pants/shorts and running shoes on the day of the test. Participants will also be asked to refrain from consumption of alcohol or engaging in heavy exercise 24 hours prior to the test. It is recommended that no food be consumed 2 hours prior to exercise, as this may affect the participant's ability to optimally perform the test and the participant may develop nausea during the test if consuming food close to the time of testing.

1.3 Potential participants are also advised to avoid participation in the exercise test if he/she is allergic to rubbing alcohol, as it is used to prepare the skin for electrode placement to measure heart rate.

2.0 Calibration of equipment

2.1 The Vmax system (Sensormedics Corp.) is turned on and both the flow and gas exchange equipment are calibrated according to the Vmax software system requirements. Calibration values must be saved in order for the Vmax system to allow the

commencement of an exercise test.

3.0 Exercise test procedures

3.1 Participant information (height, weight and birth date) is collected and entered into the Vmax system; this information is required and used by the Vmax system to calculate accurate respiratory values. An exercise test is then selected from the Vmax system software (selection depends on the respiratory variables desired for collection).

3.2 Three electrodes are placed on the participant's chest, using the CM5 placement (center of the manubrium and on the 5th ribs) and the participant is connected to the electrocardiogram (ECG) machine as per UWSOP-209 to provide a continuous measurement of heart rate at rest and during exercise. Blood pressure will also be monitored at rest and every two minutes during exercise using a manual sphygmomanometer and stethoscope.

3.3 The participant will be fitted with a Hans-Rudolph facemask or mouthpiece and nose clip, depending on apparatus preference, availability or study protocol used within the laboratory. The facemask is fitted snugly onto the participant's nose first and then pulled down and cupped under the chin. It is secured to the face with a mesh-netting that clips onto each side of the facemask. As the facemask needs to be air-tight, the participant will be asked to cover the tube that allows for air intake with his/her hand (~2 seconds) and will be asked to forcefully exhale to assure that there are no air leaks. If air leaks exist, the mask will be adjusted or tightened and the air-leak test will be repeated. The facemask and flow sensor are cleaned and sterilized between participants; see section 4.0 below.

The mouthpiece has a bite block component which fits inside the participant's mouth, like a snorkel mouthpiece. The nose clips are then placed on the participant's nose to occlude airflow into the nostrils. The mouthpiece and nose clip are cleaned and sterilized between participants; see section 4.0 below.

3.4 The cycle ergometer seat height is adjusted to be level with the participant's hip height (i.e. when the participant sits on the cycle ergometer, there will be a slight bend in the knees) and handlebars are adjusted to ensure maximal comfort.

3.5 Exercise measures

Incremental exercise

The incremental exercise test takes 8 to 12 minutes to complete and will include a 2-3 minute resting period, in which the participant will sit on the cycle ergometer while cardiovascular, respiratory and rating of perceived exertion values are collected. Immediately following the rest stage, the participant will begin his/her first stage, which will serve as a warm up. The work rate will be between ~10 to 50 Watts, depending on the participant's physical activity level (which is determined in the medical health questionnaire, completed at the same time as the consent form).

Pedaling resistance is increased at the beginning of each new stage according to the participant's rating of perceived exertion level from the previous stage, and is highly specific to each participant. Typically, work rates are increased by ~10 to 25 Watts in untrained individuals and by ~50 Watts in trained participants or those that participate regularly in physical activity.

Each stage is 1-3 minutes long and the participant will complete 2-3 stages for a submaximal exercise test or between 4 to 6 stages for a maximal test. For a sub-maximal test, the target for each stage is longer (i.e. ~3 minutes) to ensure the participant has reached steady state before increasing the pedaling intensity; the accuracy and consistency of the values collected at each of these stages is critical, as this data will be used to predict maximal oxygen consumption (VO₂ max). If a maximal test is being completed, a two-minute stage duration should provide sufficient time for attaining steady state values before increasing the work rate (this data will not be involved in any prediction equations); VO₂ max will be determined from the last stage.

Heart rate

Heart rate is measured continuously at rest and during the exercise test. Values are automatically transferred and recorded from the ECG machine onto the Vmax system.

Blood pressure

Blood pressure values are collected at rest and every two minutes during the exercise test.

Respiratory values

Gas exchange values are measured using the facemask or the mouthpiece and nose clips and are automatically recorded on the Vmax system.

Rating of perceived exertion

Participants are asked to subjectively assess the relative effort levels imposed on him/her by the exercise, using the Borg scale (6-20). These values are collected at rest and every two minutes during the exercise test and will be used to determine the increase in pedaling resistance for the following exercise stage.

Revolutions per minute

The revolutions per minute (RPM) as digitally displayed on the cycle ergometer should be between 50 to 100 rpm for the entire test.

3.6 The exercise test is terminated for the following reasons:

- Pre-determined endpoint (ex. 85% of age-predicted maximum heart rate); as occurs with a sub-maximal test
- Participant is fatigued and requests to stop the test
- RPM cannot be maintained above 50
- ECG abnormalities
- Chest pain, shortness of breath, dizziness, light-headedness

- Blood pressure fails to rise or drops > 10 mmHg below baseline with increased work rate, SBP is > 250 mmHg or DBP > 115 mmHg

3.7 Immediately after the exercise test is stopped, the pedaling intensity is reduced to a light intensity (ex. 25 Watts), for a five minute cool-down period. The mouthpiece or facemask is removed from the participant's mouth or face, respectively and heart rate and blood pressure will continue to be monitored according to the same protocols used during the test.

3.8 Heart rate and blood pressure should decrease, although they will most likely be higher than pre-exercise levels as the body continues to recover from the exercise. If the participant demonstrates any signs of discomfort (ex. Light-headedness), the participant will be assisted off of the cycle ergometer and onto the floor, with feet elevated, to alleviate the abnormal feelings induced by the exercise. In the event of a medical emergency, call 911 immediately.

3.9 The exercise test pulmonary results are averaged over 10, 20, 30 or 60 seconds and tabulated on the computer screen. The results can be saved to the Vmax system or printed off for analysis.

4.0 Equipment cleaning and sterilization

4.1 The mouthpiece and noseclips or facemask and flow sensor are washed in warm water that has a small amount (approximately 1 teaspoon) of Terga-Zyme added to the mixture. Equipment is then rinsed and placed in Cidex, a disinfectant, for 10 minutes. The mouthpiece and noseclips or facemask are submerged in Cidex. The flow sensor should be placed in a separate, shallower container of Cidex, such that the adaptor piece which connects the flow sensor to the Vmax system is not exposed to the disinfectant (as it can cause damage to this part of the apparatus).

After 10 minutes in Cidex, the equipment is rinsed in warm water and allowed to air dry for future use. Prior to the next user, the facemask is wiped with 0.5% hydrogen peroxide to rid of any Cidex residue.

4.2 The two samples lines are disconnected from the Vmax system and hung to air dry before the next use.

4.3 The cycle ergometer seat and handles as well as the blood pressure cuff are wiped down with 3% hydrogen peroxide solution.

5.0 Discussion of risks

5.1 The risks of doing incremental exercise to a participant's functional limit are very similar to the risks of doing heavy voluntary exercise. There is a very slight chance that an apparently healthy individual will have a cardiovascular complication that has not

been previously detected during normal medical examinations. There is no way to predict this potential complication.

5.2 In a very small group of individuals, a skin rash may occur due to the adhesive on the electrodes used to measure heart rate. The rash is expected to disappear within a few days. Participants are advised to avoid scratching the rash and to keep the area clean.

On occasion a slightly pink to reddish mark may occur at the site of the electrodes when removed. While this generally dissipates within minutes, in some people, it may take up to two days.

Rubbing alcohol is used to clean the skin before electrode placement to measure heart rate. Therefore, participants are advised not to participate in the exercise test if he/she is allergic to rubbing alcohol.

5.3 The mouthpiece and noseclips or facemask and flow sensor are sterilized after each participant's use to eliminate any risk of spread of infection.

5.4 Details about all risks are communicated to participants in the Information Consent Letter.

Appendix Two. Pilot Bioelectrical Impedance Analysis (BIA) Data

In this thesis study, participants did not void prior to BIA assessment. This pilot study was to evaluate the differences in body fat percentage measurements pre- and post-voiding to estimate error associated with not including this procedure in the original testing protocol.

Protocol

Participants were asked to consume 500 mL of water 30 to 60 min and this was followed by an initial BIA assessment (pre-voiding). Following the pre-voiding measurement, participants were asked to void and a second BIA measurement was collected (post-voiding).

Participant	Body Fat Percentage (%)		Difference in Pre – Post Measurement (%)
	Pre-voiding	Post-voiding	
01	26.98	26.83	0.15
02	25.38	25.51	-0.13
03	30.27	30.10	0.17
04	17.44	17.52	-0.08
05	24.46	24.54	-0.08
<i>Average</i>	24.90	24.90	0.01
<i>SD</i>	4.72	4.63	0.12
<i>p-value</i>			0.918

Appendix Three. Mean Co-efficient of Variance (COV) for Metabolite Measures

Metabolite	COV \pm SE (%)	Range of COVs
Glucose	1.77 \pm 3.51	0.00 to 36.47
NEFA	4.98 \pm 2.73	0.90 to 15.70
Glycerol	1.03 \pm 1.04	0.00 to 6.97
Lactate	1.25 \pm 1.11	0.00 to 5.09
Insulin	20.64 \pm 22.55	0.00 to 124.10
Glucagon	10.25 \pm 12.60	0.08 to 67.61
C-peptide	9.49 \pm 7.45	0.34 to 40.12
Cholesterol	1.47 \pm 1.19	0.00 to 3.19
HDL	4.66 \pm 2.72	2.00 to 9.90
Triacylglycerol	2.21 \pm 1.09	0.58 to 3.66

SE, standard error of the mean

Appendix Four. Sample Size and Power Calculation Results

Variable	Exercise		Post-Exercise Recovery	
	<i>Sample size to achieve 80% statistical power</i>	<i>Statistical power (%) for study sample size (n=9)</i>	<i>Sample size to achieve 80% statistical power</i>	<i>Statistical power (%) for study sample size (n=9)</i>
Glucose	113	7.68	92	5.00
NEFA	63	5.00	24	17.80
Glycerol	38	5.00	132	5.00
RER	8	86.00	20	65.6

Notes:

1. Values reported in the table represent power achieved when differences are compared between the AR and RA trial.
2. Values at 60 min and at 90 min were compared between trials to determine sample size to reach 80% statistical power during exercise and post-exercise recovery, respectively