THE EFFECT OF CARBOHYDRATE LOADING ON PERFORMANCE

IN THE FOLLICULAR AND LUTEAL MENSTRUAL CYCLE PHASES

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ABSTRACT

The purpose of this study was to examine the effect of carbohydrate loading (CHO) on physiologic and performance variables in seven moderately trained females (aged 30.9 ± 9.6 years; VO_2max 51.8 ± 2.3 ml/kg/minute) during the follicular and luteal menstrual cycle phases. Participants completed a 60 minute treadmill run at 70% VO_2max followed by a timed run to exhaustion at 80% VO_2max under four conditions: (1) follicular phase, CHO loading (F/CHO); (2) follicular phase, normal mixed diet (F/NMD); (3) luteal phase, CHO loading (L/CHO); (4) luteal phase, normal mixed diet (L/NMD). CHO loading was accomplished using a modified regime and a tapered running program. Performance time to volitional exhaustion at 80% VO_2max was significantly increased in L/CHO over L/NMD ($p<0.05$). There was a significant menstrual phase difference in RER and oxidation values, irrespective of diet intervention. RER and estimated CHO oxidation values were significantly greater in the luteal phase than the follicular phase. Fat oxidation was significantly greater in the follicular phase than the luteal phase. The pattern of blood substrate response showed an general increase in utilization of blood glucose, lactate, free fatty acids, and glycerol during the 60 minute treadmill run at 70%VO_2max. Due to inadmissible lab values, the sample was not large enough to run a valid ANOVA. It is suggested from these results that a CHO loading regime in the luteal menstrual cycle phase may offer a performance advantage to the moderately trained female endurance athlete compared to a normal mixed diet in the same phase.

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DEDICATION

This thesis is dedicated to all those people in my life who have left me with more questions than answers, thereby inadvertently spurring on my journey into the realm of research and critical evaluation:

To my loving husband Patrick who has challenged my way of thinking and then provided the constant love and support to see me through tough changes both personally and professionally.

To my wonderful son Kevin, through your resilience you gave me the courage to just 'try harder' when I felt like quitting.

To my Dad and Mom, Dale and Joanne, for never doubting I could finish.

To my Sisters and friends, for your constant support and timely reminders about what's really important in life.

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CHAPTER 1

SCIENTIFIC FRAMEWORK

1.1 Introduction

Athletes today are continually pushing the limits of their physical performances. Endurance competitions range upwards in length from one hour, although there is a small margin between winning and losing. Success of endurance athletes, at any level, is mainly dependent on their level of training, which works to maximize the individual's genetic potential for sport specific tasks. However, athletes often search for other aspects, such as ergogenic aids, that will give them a competitive edge (Williams, 1998).

The term ergogenic aid in the realm of sport refers to something that enhances or improves work capacity, or sport performance, beyond what is possible through natural ability or training (Williams, 1998). Nutritional ergogenic aids, which either serve to promote muscle tissue growth, increase stored energy, or aid the rate of energy generated to support muscular work, are targeted to endurance athletes (Williams, 1998). These ergogenic aids include the manipulation of micronutrients and macronutrients. Micronutrients include the use of vitamins (involved in generating energy), minerals (involved in the delivery of energy or energy pathways), or even antioxidants, which might counteract accumulation of substances that interfere with the delivery of energy to the working muscles (Williams, 1998). The manipulation of dietary macronutrients, such

as carbohydrate, fat and protein, are also used in order to maximize initial body stores of energy prior to exercise, to prolong stores during an endurance event, or to provide enough substrate to promote muscular growth (Williams, 1998).

Carbohydrate loading is one technique that is often recommended to endurance athletes to enhance performance (Robergs & Roberts, 1997; Williams, 1998). This ergogenic technique involves manipulating dietary carbohydrate intake in an effort to maximize body energy stores, particularly muscle glycogen stores (Bergstrom, Hermansen, Hultman, & Saltin, 1967; Sherman, Costill, Fink, & Miller, 1981). The traditional seven day carbohydrate loading method involved completing an exhaustive bout of exercise followed by three days of restricting dietary carbohydrate intake (15% energy intake; E_{in}) in an effort to empty muscle glycogen stores (Bergstrom et al., 1967). This was followed by a high dietary carbohydrate intake (75% E_{in} or greater) while tapering exercise intensity and volume. Since then, a modified carbohydrate loading technique has been recommended, eliminating the low carbohydrate diet and exhaustive exercise portions of the original technique. In this modified technique, a high dietary carbohydrate intake was combined with an exercise depletion taper acting in a similar fashion as the traditional method (Sherman et al., 1981). Both techniques have been positively linked to super-compensated muscle glycogen stores (Bergstrom et al., 1967; Kochan, Lamb, Lutz, Perrill, Reimann, & Schlender, 1979; Sherman et al., 1981; Madsen, Pedersen, Rose, & Richter, 1990; Tarnopolsky, Atkinson, Phillips, & MacDougall, 1995), which are associated with an increased time to fatigue during prolonged, intense exercise (Bergstrom et al., 1967; Lamb, Snyder and Baur, 1991;

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Tarnopolsky et al., 1995). Thus, the higher muscle glycogen levels are thought to be a key component in enhancing exercise performance.

Although the reported use of manipulating carbohydrate intake to improve performance dates back to the 1960's (Bergstrom et al., 1967), its ability to enhance endurance performance in both male and female endurance athletes is now an issue of debate in the literature. Work by Tarnopolsky and associates (1995) first suggested that the nutrition recommendation for carbohydrate loading founded in male endurance athletes may not be valid for equally trained female endurance athletes. This gender difference suggested that the unique influence of the menstrual cycle needed to be taken into consideration in these popular nutrition recommendations.

The influence of the menstrual cycle, or more specifically estrogen and progesterone, on substrate utilization during exercise and athletic performance is not clearly understood. Studies examining the effect of the menstrual cycle phase on variables such as maximal oxygen consumption, ventilatory drive, or specific endocrine hormones have shown very inconsistent results (Jurkowski, Jones, Toews, & Sutton, 1981; Schoene, Roberston, Peirson, & Peterson, 1981; Pivarnik, Marichal, Spillman, & Morrow, 1992; Lebrun, McKenzie, Prior, & Taunton, 1995). Likewise, the unique interplay of the sex steroid hormones, estrogen and progesterone, and their overall effect on substrate metabolism during exercise, is not well understood in the female athlete (Nicklas, Hackney, & Sharp, 1989; Hackney, 1990; Lebrun et al., 1995; Tarnopolsky et al., 1995). Thus, the purpose of this study was to examine how exercise performance is affected by carbohydrate loading and the menstrual cycle phase in moderately trained athletes.

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1.2 Review of Literature

1.2.1 Carbohydrate Metabolism During Steady State Exercise

Energy for muscular contraction is supplied through a variety of pathways, all of which end in the formation of adenosine triphosphate (ATP), the body's common energy currency. Under steady state conditions during prolonged submaximal exercise intensities of 65 – 85% VO_2max, carbohydrate is a primary energy substrate (Saltin & Karlsson, 1971). The extensive degradation of muscle glycogen stores at these intensities is thought to be the result of the recruitment of both slow oxidative and fast glycolytic muscle fibers in which the processes of glycogenolysis and glycolysis produce the ATP for muscular work. (Gollnick, Piehl, Saubert, Armstrong, & Saltin, 1972; Vollestad & Blom, 1985; Zachwieja, Costill, Pascoe, Robergs, & Fink, 1991).

Energy for the initial muscular contraction at any exercise intensity is generated through the phosphagen system where ATP is created through the dephosphorlyation of creatine phosphate (Robergs & Roberts, 1997). This reaction does not require oxygen to proceed; however, it is a short - lived energy source limited by muscle stores (Robergs & Roberts, 1997). To maintain the rate of muscle contraction, there is an increased rate of glycolysis with or without oxygen (Robergs & Roberts, 1997).

Glycolysis involves a series of reactions that catabolizes glucose into usable energy (ATP) in the presence or absence of oxygen (Robergs & Roberts, 1997). Glucose is present via entry from the blood or generated through the process of glycogenolysis (Robergs & Roberts, 1997). Blood glucose then enters the muscle cell

with the assistance of a glucose transport protein (GLUT4 is the major transporter) (Robergs & Roberts, 1997). Within the cell, the glucose molecule undergoes phosphorylation on the sixth carbon to form glucose-6-phosphate at the expense of one ATP molecule (Robergs & Roberts, 1997). This process is regulated in the muscle by hexokinase, an enyzme located on the outer mitochondrial membrane (Robergs & Roberts, 1997). This conversion is irreversible and ensures the muscle fiber has glucose for either glycolysis or glycogenesis (Robergs & Roberts, 1997). In the process of glycolysis, nine reactions occur where glucose-6-phosphate is further metabolized to pyruvate, ATP and nicotinamide adenine dinucleotide (*reduced form*; NADH)(Robergs & Roberts, 1997). The intensity of the exercise determines the fate of pyruvate in generating adequate energy in order to sustain continued muscle contractions (Robergs & Roberts, 1997).

During steady state exercise at moderate intensities pyruvate, in the presence of pyruvate dehydrogenase, in the mitochondria produces acetyl CoA, CO_2 and NADH. Acetyl CoA enters the tricarboxylic acid cycle (TCA) and in conjunction with the electron transport chain produces large quantities of ATP for sustained muscular contraction (Robergs & Roberts, 1997). This final process requires adequate oxygen delivery to the muscle and thus is dependent on cardiorespiratory factors (i.e., adequate blood perfusion, proper hydration) (Robergs & Roberts, 1997). The inability of the cardiorespiratory system to deliver oxygen for this purpose has been associated with the onset of fatigue (inability to continue muscle contraction at a given rate) (Robergs & Roberts, 1997). When oxygen supply to the muscle is insufficient, or the rate of pyruvate exceeds its entry into the mitochondria (e.g. high rates of muscular contraction),

pyruvate, in the presence of lactate dehydrogenase, can be converted to lactate (Roberg and Roberts, 1997). The resultant lactate can be removed to other tissues (i.e. skeletal muscle, liver) for metabolism and ultimately ATP generation (Robergs & Roberts, 1997).

In addition to blood glucose, a ready and rapid supply of glucose-6-phosphate is available within the muscle through the process of glycogenolysis (Robergs & Roberts, 1997). The initial enzyme, phosphorylase, acts on alpha 1-4 glycosidic bonds to remove single glucose residues from the glycogen molecule until only four glucose units remain (Robergs & Roberts, 1997). A second enzyme, transferase, removes groups of three glucose molecules and then debranching enzyme cleaves the last alpha 1-6 bond to release the final glucose unit (Robergs & Roberts, 1997). These enzymes are activated by an increase in epinephrine (a neurotransmitter for sympathetic nerve stimulation). Ephinephrine, in turn, increases the concentration of cyclic adenosine monophosphate (*cAMP*) (Robergs & Roberts, 1997). *cAMP* is involved with the phosphorylation of the phosphorylase enzyme, which converts phosporylase to the active form, along with the inactivation of glycogen synthase (the enzyme responsible for glycogen synthesis), thus providing a negative feedback mechanism (Robergs & Roberts, 1997). In addition, the increase in calcium ion concentration, released from the sacroplasmic reticulum, and inorganic phosphate (from CrP breakdown) during the initiation of muscle contraction, assists with maintaining the direction of the reaction sequence because both calcium and inorganic phosphate stimulate the conversion of phosphorylase to the active form (Robergs & Roberts, 1997). The process of glycolysis requires one ATP molecule for the phosphorylation of glucose to glucose-6-phosphate (Robergs & Roberts, 1997). This

process provides rapid production of glucose-6-phosphate to be used in glycolysis and, ultimately, the TCA cycle and electron transport system generating large quantities of ATP for muscular contraction (Robergs & Roberts, 1997).

The Cori cycle also provides another, albeit limited, source of glucose for muscle metabolism during submaximal exercise. In the event that the rate of pyruvate production exceeds the rate of pyruvate entry into the mitochondria it may be reduced to lactate (Robergs & Roberts, 1997). This process requires the enzyme lactate dehydrogenase along with the electrons and protons provided by NADH (Robergs & Roberts, 1997). Under steady state conditions, NADH is oxidized generating NAD^+ for use in the production of more pyruvate from glyceraldehyde-3-phosphate, thus continuing the process of glycolysis and supporting a high rate of ATP generation (Robergs & Roberts, 1997). As the level of lactate rises, it is removed for further metabolism in other tissues (Robergs & Roberts, 1997). For example, the liver may take up the excess lactate and convert it to glucose-6 phosphate through gluconeogenesis, which is released back into the blood stream and transported to working muscles for metabolism (Robergs & Roberts, 1997).

Finally, the break down of muscle fibers with muscular contraction during exercise may provide another small amount of glucose for energy during submaximal work (Robergs & Roberts, 1997). Alanine, a nonessential amino acid, is released during the catabolism of muscle proteins and then cycled to the liver (Robergs & Roberts, 1997). The liver further catabolizes the nitrogen portion of the amino acid to urea and recycles the carbon backbone converting it to glucose (Robergs & Roberts, 1997). This glucose then enters the blood stream and can be taken up by muscle. It may be

processed through the glycolytic pathway and TCA cycle depending on the availability of oxygen (Robergs & Roberts, 1997). Thus, the muscular contraction in itself provides a source of carbohydrate for metabolism though this would not be appreciable in trained individuals who experience less muscle damage with the eccentric contraction of running and/or would not occur until the exercise had been excessive compared to the individuals' level of training (Robergs & Roberts, 1997). Though there are many other pathways in which to generate energy, those involving carbohydrate metabolism through the oxidative pathways generate sufficient amounts of energy at a rate that is suitable for exercising at intensities of 65% - 85% VO_2max typical of endurance activities (Saltin & Karlsson, 1971). Goedecke, Gibson, Grobler, Collins, Noakes, and Lambert (2000) reported that the relationship between muscle metabolism of carbohydrate, dietary intake, and muslce glycogen stores were sufficiently reflected in RER (indirect measurement) duirng exercise at 70%VO_2max. Also, dietary intake and muscle glycogen content were responsible for up to 60% of the inter-individual variance in RER measurements (Goedecke et al., 2000).

Thus utilization of carbohydrate at these exercise intensities can be seen with either indirect measurements, such as respiratory exchange ratio, or direct measurement such as muscle or liver biopsy during steady state exhaustive exercise (Saltin & Karlsson, 1971). Theoretically then, by maximizing the body's capacity to store carbohydrate (liver and muscle glycogen stores) through diet and training, the ability to perform exercise at these intensities would be enhanced (Sherman & Wimer, 1991).

1.2.2 Dietary Carbohydrate and Body Glycogen Stores

Carbohydrates or "hydrates of carbon" (Groff, Gropper, & Hunt, 1995) are chemical compounds that are composed of carbon, oxygen and hydrogen atoms typically arranged in a ratio of one carbon molecule (C) to one water molecule (H_2O) (Whitney & Rolfes, 1999). Dietary carbohydrates are chemically known as monosaccharides (e.g., glucose, fructose and galactose) and disaccharides (e.g., sucrose, composed of one glucose unit plus one fructose unit); both are commonly termed simple sugars (Whitney & Rolfes, 1999). In addition, polysaccharides are nutritionally important in providing energy for the body (Whitney & Rolfes, 1999). These carbohydrates are referred to broadly as starch, fiber, and glycogen (Whitney & Rolfes, 1999). Starch and fiber are commonly termed 'complex carbohydrates' though only starch (amylose and/or amylopectin) produces sufficient amounts of glucose once digested, making it nutritionally important when dealing with carbohydrate loading (Groff et al., 1995; Whitney & Rolfes, 1999).

In general, the final products of carbohydrate digestion, with the exception of fibre (non-starch polysaccharides), are glucose, fructose and galactose. Further processing by the liver transforms most fructose and galactose to glucose derivatives (Groff et al., 1995; Whitney & Rolfes, 1999). Thus, the dietary carbohydrate eaten provides the body with glucose to be used as energy (Whitney & Rolfes, 1999). Glucose is then transformed into adenosine triphosphate (ATP), the common energy currency for muscular work through a variety of metabolic pathways (Whitney & Rolfes, 1999). Ultimately, dietary carbohydrates (simple sugars and starch) serve to provide four kilocalories of energy (heat) per gram (Whitney & Rolfes, 1999).

Glucose and its storage form in the body, glycogen, provide approximately 40% of the body's energy needs at rest and even more during exercise depending on the type, intensity and duration of the activity (Williams, 1998). As the stores of glycogen in the body are limited, stored fat and protein will also provide some energy for exercise though this is influenced by intensity, type and duration of the activity (William, 1998). Also, the composition of macronutrients digested before, during and after exercise may influence the mix of fuel used for the body to perform exercise (Robergs & Roberts, 1997). Finally, the fuel utilized for exercise, will also be affected by the level of training the athlete has experienced (Greiwe, Hickner, Hansen, Racette, Chen, & Holloszy, 1999).

For the undertaking of endurance events (marathons, cross-country skiing, distance swimming or cycling), where exercise intensities are between $65 - 85\%$ VO$_2$max , glucose has been shown to be a preferred source of fuel (Saltin & Karlsson, 1971). The amount of stored glycogen (liver and glycogen stores) in the body will provide the majority of fuel for endurance activity (Bergstrom et al., 1967; Hultman & Nilsson, 1971). Liver stores are estimated to be approximately 70 grams of glycogen, while total muscle reserves are greater at approximately 400 grams for the average male endurance athlete (Sherman & Wimer, 1991). Theoretically, these limited stores would provide enough fuel for running approximately 28 km at 70% VO$_2$max without any dietary intervention before or during exercise (Sherman & Wimer, 1991).

An early study by Levine, Gordon and Derick (1924) demonstrated that feeding a high carbohydrate diet before a marathon, plus additional carbohydrate supplement during the race (at the 24 km point), prevented hypoglycemia commonly observed in the

runners at that time near the marathon's end, and thus improved performance. Many more investigations have examined the effect of the timing, type and amount of carbohydrate intake on endurance exercise performance (Costill, Coyle, & Dalsky et al., 1977; Roberts, Noble, Hayden and Taylor, 1988; Lamb et al., 1991; Tarnopolsky et al., 1995; Hawley, Palmer, & Noakes, 1997a; Whitley, Humphreys, Campbell, Keegan Jayanetti et al., 1998). Early carbohydrate loading studies have considered these aspects of dietary carbohydrate intake, along with the effect of training on determining muscle glycogen stores and, ultimately, performance for the endurance athlete (Shepley, MacDougall, Cipriano, Sutton, Tarnopolsky & Coates, 1992; Greiwe et al., 1999).

1.2.3 Determinants of Muscle Glycogen Stores

Aerobic power and aerobic endurance require the supplying of power or fuel to the working muscles. These terms encompass both chemically breaking down fuels while using oxygen and transporting the oxygen to the working muscle via pulmonary, cardiac, blood, vascular and cellular mechanisms (Thoden, 1991). Carbohydrate loading comprises depleting muscle glycogen stores and then manipulating dietary intake to replenish these muscle tissue energy supplies (Bergstrom et al., 1967). Whether these processes improve aerobic power or aerobic endurance or athletic performance depends on a number of factors. Factors traditionally discussed as determinants of muscle glycogen stores are nutrition status, timing and type of carbohydrate consumed, level of endurance training, and intensity of exercise.

The potential mechanism for the ergogenic effect of carbohydrates, relative to carbohydrate loading, depends on the interactions of glucose uptake in the muscle cells

(Greiwe et al., 1999) and the ability of glycogen synthase to create glycogen stores (Kochan et al., 1979). The relationship between glucose uptake and glycogen synthase activity is inversely affected by the amount of glycogen present in the muscle after exercise, and also affected by the diet given (Hultman, Bergstrom, & Roch-Norlund, 1971; Wahren, Alborg, Felig, & Jorfeldt, 1971; Bergstrom, Hultman, & Roch-Norlund, 1972; Kochan et al., 1979; Zachwieja et al., 1991) and the level of endurance training (Shepley, et al., 1992). Diet and exercise manipulations independently influence muscle glycogen stores by affecting the relationship between available glucose for uptake and the stimulation of glycogen synthase enzyme necessary for glucose storage (Hultman et al., 1971; Zachwieja et al, 1991). However, the specific combined actions of diet and exercise are reported to be the most potent stimulators for increasing muscle glycogen (Bergstrom et al., 1967; Hultman et al., 1971; Kochan et al., 1979; Costill, Fink, Maresh, Witten and Miller 1981).

1.2.3.1 Nutrition Status

Dietary intake can be solely responsible for both the partial depletion and replenishment of muscle glycogen stores (Hultman et al., 1971). Past investigations have shown that several days of low carbohydrate intake or starvation (3-10 days) lead to a slow degradation (30 – 40%) of muscle glycogen stores (Hultman et al., 1971) and certain depletion of liver glycogen stores after 24 hours (Hultman & Nilsson, 1971). By feeding a diet high in carbohydrate (60% or greater of energy intake as CHO), muscle and liver glycogen returns to normal levels within 24 hours (Hultman et al., 1971; Robergs & Roberts, 1997). In contrast, if initial carbohydrate intake was adequate (45-60% intake of energy as CHO), then the influence of additional carbohydrate on muscle

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glycogen stores was negligible in most of the studies reviewed (Hultman et al., 1971; Kochan et al., 1979), unless exercise had first impacted muscle glycogen stores. The exception was an investigation by Hawley, Palmer and Noakes (1997a) where, with normal mixed diet (426 g CHO or 5.9 g/kg BM), muscle glycogen was 459 mmol/kg dry tissue weight. This was significantly increased to 565 mmol/kg dry tissue weight when supplemented with CHO to 661g/day or 9.3 g/kg BM. The rate of muscle glycogen utilization during a one hour cycle trial (80% VO_2max) was similar between the two diets and the higher muscle glycogen level did not improve performance (Hawley et al., 1997a). The lack of performance improvement may be attributed to the testing protocol, which did not exceed 60 minutes, nor did it include a high intensity time trial to exhaustion as is suggested in the literature .

1.2.3.2 Type of Carbohydrate and Timing of Intake

The type of carbohydrate, complex compared to simple sugars, was investigated by Costill et al. (1981). They found that in the first 24 hours both types of carbohydrate produced similar increases in muscle glycogen (Costill et al., 1981). However, complex carbohydrates were better at replenishing muscle glycogen stores after 48 hours (Costill et al., 1981). Roberts and associates (1988) also investigated the effect of simple and complex dietary carbohydrates in male marathon runners and concluded that, in conjunction with glycogen - depletion exercise, a diet high in either simple or complex carbohydrates would produce significant increases in skeletal muscle glycogen stores.

The frequency of feeding (two meals versus seven meals) made no difference in muscle glycogen restoration with isocaloric high CHO diets (Costill et al., 1981).

Finally, Lamb et al.(1991) looked at the efficacy of a liquid (supplement) versus a solid (pasta) high carbohydrate diet in male runners (n=14). They found that total muscle glycogen concentrations were not significantly different between a high carbohydrate diet in liquid (supplement) or solid form (pasta), nor was there a difference in performance during a run to exhaustion at 75% VO_2max (Lamb et al., 1991).

1.2.3.3 Intensity and Duration of Exercise and level of Endurance Training

Muscle glycogen stores are independently influenced by intensity and duration of exercise and by endurance training. Carbohydrate is the primary energy metabolite for endurance exercise at 65% VO_2max - 85% VO_2max (Saltin & Karlsson, 1971). The relationship between these intensities is thought to be curvilinear with most muscle glycogen used at intensities of 70 – 80% VO_2max (Saltin & Karlsson, 1971). Exhaustion after greater than 60 minutes of cycling at these intensities coincided with depleted or nearly depleted muscle glycogen (Bergstrom et al., 1967; Bergstrom & Hultman, 1967; Saltin & Karlsson, 1971). The larger the degradation of muscle glycogen the greater the metabolic stimulus, via the activation of glycogen synthase, for muscle glycogen resynthesis (Hultman, 1967; Kochan et al., 1979; Zachwieja et al., 1991). The depletion exercise, then, independently influences the rate and extent of muscle glycogen resynthesis.

Endurance training also was shown to independently impact muscle glycogen content. Greiwe et al. (1999) conducted a before and after study of carbohydrate loading in a group of sedentary women (n=4) and men (n=2). They found that carbohydrate loading after completing a 10 week endurance training program for cycling significantly

improved their muscle glycogen accumulation and muscle glycogen content compared to carbohydrate loading before the endurance training program. Muscle GLUT – 4, a cellular glucose transport protein, was found to increase two-fold in the trained state and was correlated with the increased muscle glycogen content (Greiwe et al., 1999). No change was found in muscle glycogen synthase activity (Greiwe et al., 1999). Other changes noted with training included increased citrate synthase concentrations (enzyme involved in mitochondrial oxidative processes) and higher hexokinase concentrations (rate limiting enzyme involved glucose transport) (Greiwe et al., 1999). Shepley and associates (1992) also found that a high intensity, low volume taper (low volume interval training plus running ~ 70% VO_2max) or rest only (seven days) running tapers produced increases in muscle glycogen content. These findings again suggest that exercise intensity and training are both independent stimuli for increasing muscle glycogen content.

Thus, the manipulation of either dietary carbohydrate intake or the amount and intensity of the exercise bout were thought to be key components in enhancing exercise performance (Bergstrom et al., 1967; Lamb et al., 1991; Tarnopolsky et al., 1995). This has led to the promotion of regular carbohydrate consumption and/ or advocacy of carbohydrate manipulation as an ergogenic aid for endurance athletes.

1.2.4 Common Nutrition Practices of Endurance Athletes

Generally, a diet high in carbohydrate (55-70% of calories) is a popular recommendation given to endurance athletes in an effort to maximize their glycogen stores on a daily basis (Clark, 1997; Williams, 1998). This recommendation is often

translated into the regular consumption of a minimum 5 – 7 g CHO/kg for regular training, and up to 10 g CHO/kg before competitive events, so the athlete ensures that there is an optimal level of preferential fuel available in the working muscles (Burke, Cox, Cummings, & Desbrow, 2001). Many top endurance athletes, both male and female, are very interested in nutrition and its effect on performance, yet the dietary requirements of endurance training and competition programs (e.g., carbohydrate loading) can be challenging to implement (Burke et al., 2001). The actual dietary practices of male and female athletes within similar endurance sports are different from each other and from the popular high carbohydrate recommendations (Burke et al., 2001).

Highly trained male endurance athletes have reported high energy intakes along with a carbohydrate intake of 8 – 11g/kg/day (Burke, 2001). Both macro and micronutrient content of their diets would easily meet recommendations for athletes and general population health due to their high caloric intake. Common competition practices for male endurance athletes include carbohydrate loading, attention to pre - stage and post - stage recovery meals and ensuring they are well hydrated (Burke, Gollan, & Read, 1991). However, female endurance athletes are less likely to achieve these nutrition goals due to either periodic or chronic restriction of energy intake in effort to maintain low body fat levels (Burke et al., 2001).

This theory is supported by Pate, Sargent, and Baldwin (1990), who compared non-competitive female runners (2.75 hr/wk running; n = 103) to inactive females (aerobic exercise participation of no more than once per week; n = 74). They found the female runners adopted more preventative healthy eating behaviors than the inactive

controls (Pate et al., 1990). These healthy eating behaviors included eating a higher percent of their total energy intake from carbohydrates (consumed more whole grains, fruits and vegetables), along with eating less fat and less protein, both of which are often recommended to athletes (and for general preventative health)(Pate et al., 1990). However, the runners reported total energy intake was only 4% greater (1560 kcal/day) than their inactive counterparts (1500 kcal/day) and was less than necessary for their estimated energy expenditure (Pate et al., 1990). Total caloric intake of both groups would be considered less than acceptable at 1500 – 1600 kcal/day according to healthy eating guidelines for Canadians (Health Canada, 1992). Low energy intake is often associated with restrictive dietary behaviors and low nutrient density (e.g. low calcium intake due to elimination of milk and milk products). However, under-reporting of dietary intake may also have been a source of error. The phenomenon of under-reporting has been documented to occur in 25% of women who report low fat and alcohol intakes (Black, Goldberg, Jebb, Livingstone, Cole, & Prentice, 1991).

Also, the limitation of the food recording method (prospective journals) and voluntary exercise estimate was acknowledged by the authors as a source of error, although they concluded this finding to be similar to other previous studies (Pate et al., 1990). The recommendation of 60% energy intake from carbohydrates implies that the diet is sufficient in total grams of carbohydrate for repeated bouts of muscular work. However, the low overall energy intake of female athletes would indicate that the female runners' overall carbohydrate intake would be less than the recommended 5 - 7 g CHO/kg necessary for repeated bouts of endurance exercise training. It would also make the recommendation for carbohydrate loading (i.e., 70 – 80% energy from

carbohydrate) difficult to achieve, and certainly they would be consuming less than the amount associated with successful improvement of performance in male athletes. More recently, this has been shown to be the case for females with energy intakes of less than 2000 kcal/day (Tarnopolsky, Zawada, Richmond, Carter, Shearer, Graham, Philips, 2001). Tarnopolsky and associates (2001), reported that by increasing the energy intake of well trained female athletes by 30%, that carbohydrate loading (70 – 80% CHO) increased muscle glycogen stores in the follicular menstrual cycle phase to a similar magnitude as that seen in men (Tarnopolsky et al., 2001). Furthermore, other studies have also re-examined the issue of females and carbohydrate loading and found that, when the level of carbohydrate intake is approximately 8g/kg body weight and energy level is sufficient, then carbohydrate loading is possible in both menstrual cycle phases (Walker Heigenhauser, Hultman, & Spriet, 2000; James, Lorraine, Cullen, Goodman, Dawson, Palmer & Fourneir, 2001; Paul, Mulroy, Horner, Jacobs & Lamb, 2001). Previous conclusions of gender differences in response to carbohydrate loading may have been an issue of diet controls. However, the issue of carbohydrate loading enhancing exercise performance has not yet been resolved.

1.2.5 Carbohydrate Loading and Performance

The combined depletion taper and high carbohydrate diet characteristic of the carbohydrate loading regimen provides, theoretically, both the metabolic stimulus for glycogenesis (with the exercise depletion taper) and adequate dietary carbohydrate for muscle glycogen synthesis. Sherman et al. (1981) found that a modified carbohydrate loading regimen, consisting of 3 days of normal mixed diet (50% CHO) followed by 3

days of a high carbohydrate diet (70% CHO) combined with 5 days of exercise tapered in volume and intensity (depletion taper), successfully elevated muscle glycogen levels and eliminated the unpleasant side effects of the traditional carbohydrate loading practices. This has been seen repeatedly in other studies using running exercise (Madsen et al., 1990; Tarnopolsky et al., 1995).

Higher muscle glycogen content has been correlated to longer time to exhaustion (timed exercise trial) or improved exercise performance (e.g. increased distance during steady state exercise) at intensities of 65 – 80% VO_2max (Bergstrom et al., 1967; Shepley et al., 1992; Tarnopolsky et al., 1995). However, although it is often an undisputed fact in popular literature that the carbohydrate loading protocol increases muscle glycogen stores and exercise performance, many research studies have not found improvements in exercise performance. The variety of testing protocols used to measure performance in carbohydrate loading studies has contributed to the equivocal nature of this body of literature. For example, studies using a steady state exercise protocol for less than 90 minutes generally do not see significant improvements in performance (Sherman et al., 1981; Madsen et al., 1990; Hawley et al., 1997a). In a review of this topic, Hawley, Dennis, and Noakes (1994) suggested that a timed distance trial, or an exercise protocol where a submaximal bout of exercise is followed by a timed bout of high intensity exercise to exhaustion would better reflect the capacity for exercise after carbohydrate loading. A recent study conducted by Tarnopolsky et al. (1995) used the combined protocol to determine performance where a moderate intensity run was followed closely by a high intensity run to fatigue. The carbohydrate loading technique when tested in this fashion significantly improved exercise performance for the male

subjects (Tarnopolsky et al., 1995). However, despite these positive improvements in well-trained male runners the same investigation by Tarnopolsky et al. (1995) also found that equally trained women in their follicular menstrual cycle did not significantly improve performance under the same protocol and testing conditions.

1.2.6 Sex Differences in Carbohydrate Loading

Recent comparison studies have revealed that common generalizations stemming from research in male endurance athletes may not be valid for females (Tarnopolsky et al., 1995; Tarnopolsky, MacDougall, Atkinson, Tarnopolsky, & Sutton, 1990; Horton, Pagliassotti, Hobbs, & Hill, 1998). Though carbohydrate loading is well accepted and recommended to both male and female athletes, there is some suggestion that women may not respond as well as their male counterparts to this technique.

Initial research examining sex differences in metabolism during exercise found that compared to training - matched males, females metabolized more lipid, and thus, inherently less carbohydrate than the men for the same relative exercise intensity (Tarnopolsky et al., 1990; Horton et al., 1998). This sex difference was thought to impair the ability of the female athlete to augment muscle glycogen stores and to enhance performance (Tarnopolsky et al., 1990; Horton et al., 1998). In addition, Tarnopolsky et al. (1995) reported that a modified carbohydrate loading regimen did not produce increases in muscle glycogen compared to training matched males, nor was there a benefit to exercise performance for female athletes during their follicular menstrual phase. Furthermore, it had been hypothesized that for the athletic female the

changing levels of estrogen and progesterone throughout the menstrual cycle may impact substrate utilization, and subsequent muscle glycogen storage associated with carbohydrate loading, which in turn may effect exercise performance (Bonen, Haynes, Watson – Wright, Sopper, Pierce, & Graham, 1983; Nicklas et al., 1989; Tarnopolsky et al., 1995).

Previous research suggests that the different sex-steroid milieu in each menstrual cycle phase may govern substrate utilization in females (Matute & Kalkoff, 1973; Ahmed- Sorour & Bailey, 1981; Bonen et al., 1983; Kendrick, Steffen, Rumsey, & Goldberg, 1987; Lebrun et al., 1995). An investigation by Nicklas et al. (1989), with moderately trained eumenorrheic females under the conditions of normal mixed diet and depletion exercise, found a greater rate of glycogen repletion in skeletal muscle, and a trend toward both increased glycogen utilization and increased performance times in the luteal phase (i.e., estrogen and progesterone combined). Despite this finding, no differences were found in absolute glycogen levels between the phases (Nicklas et al., 1989). However, in contrast Hackney (1990; 1999) noted a slightly greater glycogen deposition at rest in the luteal menstrual cycle phase compared to the follicular phase with only normal mixed diet (no depletion exercise). Furthermore, Walker and associates (2000), found that a 7-day carbohydrate loading regimen significantly impacted muscle glycogen stores and moderately improved endurance cycling performance during the luteal menstrual cycle phase. These findings supported the idea that the unique female hormonal milieu affects fuel utilization and storage throughout the menstrual cycle, and thus, potentially affects athletic performance in each phase.

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Recently, well - controlled studies have emerged that reveal, when given

sufficient total energy intake, and an absolute level of CHO >8.0g/kg lean body mass,

women have the capacity to increase muscle glycogen stores to supranormal levels in

both the follicular and luteal menstrual cycle phases (Walker et al., 2000; Tarnopolsky et

al., 2001; James et al., 2001; Paul et al., 2001). Tarnopolsky et al. (2001) demonstrated

that in the follicular phase, seven moderately trained females (three eumenorrheic

females, plus four on oral contraceptives) ingesting 8.8 g CHO/kg body mass (11.3 g/kg

LBM) plus increased energy intake (34% above NMD) for four days, alongside an

exercise depletion taper, increased muscle glycogen levels by 17%. No performaonce

measures were undertaken. Further to this, James et al. (2001) found by ensuring that

six well - trained female endurance athletes (all on oral contraceptives) ingested 12g

CHO/kg lean body mass (LBM) three days and completed a deletion exercise taper,

produced significant increased muscle glycogen levels in both the follicular phase (78%)

and the luteal phase (82%). No performance measure was undertaken. To date, the

influence of carbohydrate loading on endurance performance has not been clearly

established for either the luteal or follicular menstrual cycle phase.

In regard to the luteal phase, carbohydrate loading was reported by Walker et al.

(2000) to make a modest improvement in cycling endurance performance (90 minutes of

steady state cycling at 80% VO_2max) during the luteal phase, while Paul et al. (2001)

reported no improvement in performance (cycling intervals at 72% VO_2max) with

carbohydrate loading in the follicular phase. These studies differ in testing protocols,

dietary controls, and menstrual cycle phase controls. All of these factors may have

influenced their conclusions. It is not known if any current studies have used a single

subject repeated measure design to look at the effect of carbohydrate loading in both the luteal and follicular menstrual cycle phases.

1.2.7 Female Menstrual Cycle

The menstrual cycle events depend upon sex-steroid hormones produced in the hypothalamus-pituitary axis, and in the ovaries (Guyton & Hall, 1996). The sex-steroid hormones elicit changes in the endometrial lining of the uterus in preparation for conception and gestation (Hatcher, Stewart, Trussell, Kowal, Guest, Stewart & Cates, 1990; Guyton & Hall, 1996). In the absence of conception a short paucity in sex-steroid hormone secretion occurs and the endometrial lining is shed (Hatcher et al., 1990; Guyton & Hall, 1996). These mechanisms signal the body to begin the cycle again. The sex - steroid hormones secreted by the hypothalamic – pituitary axis begin to influence the ovaries at puberty and then in a cyclical fashion they rise and fall throughout each menstrual cycle (Guyton & Hall, 1996). In response to the initial stimuli from the hypothalamic – pituitary axis the ovaries secrete other sex – steroid hormones (Guyton & Hall, 1996).

The unique cyclical rise and fall in the female sex-steroid hormones in the menstrual cycle are depicted in Figure 1.1. The average length of the menstrual cycle is 28 days, however, only 10 –15 percent of women's cycles are actually this length (Berkow, 1992). The normal variation in menstrual cycle length can range from 18 to 45 days (Berkow, 1992). The cyclical pattern of events characteristic of the menstrual cycle can be divided into three distinct phases based on known endocrinologic patterns (Berkow, 1992).

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Figure 1.1 Cyclic hormone changes during a 28 - day menstrual cycle.
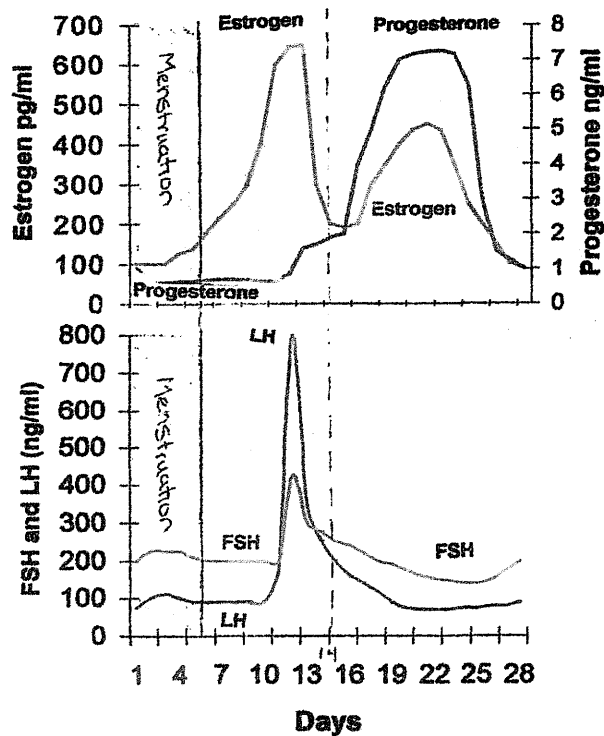
Note. Figure 1.1 represents the cyclic hormonal changes during the menstrual cycle. Adapted from Guyton and Hall (1996). For a 28-day menstrual cycle, days 0 – 11 represent the follicular phase, days 12-14 represent the period of ovulation, and days 15 – 28 represent the luteal phase.

The development of the follicles in the ovary up to the time of the release of a mature ovum (oocyte) is commonly termed the follicular menstrual cycle phase (Robergs & Roberts, 1997). The onset of endometrial shedding marked by blood flow begins the follicular phase and is referred to as day 1 of the menstrual cycle (Berkow, 1992). The length of the follicular phase depends on the time it takes the ovum to mature (Berkow, 1992). This phase usually lasts between 9 – 23 days (Robergs & Roberts, 1997). The follicular phase is marked by a slight increase in the amount of

follicle stimulating hormone (FSH) secreted from the anterior pituitary gland, which functions to stimulate the growth of follicles in an ovary (Berkow, 1992). The anterior pituitary gland also begins to gradually increase the secretion of luteinizing hormone (LH) (Berkow, 1992). The amount of LH secreted dramatically increases approximately 36 – 42 hours before ovulation followed by a dramatic drop to back baseline levels (Berkow, 1992). The follicles within the ovary, once stimulated by FSH and LH, begin to secrete the sex-steroid hormones, estrogen and progesterone, in small quantities (Berkow, 1992). Estrogen secretion accelerates near ovulation, peaking just prior to the massive surge in LH (Berkow, 1992). As LH levels peak, estrogen levels drop off, though they remain above the baseline levels in the early follicular stage (Berkow, 1992). The surge in LH marks the beginning of the ovulatory phase (Berkow, 1992).

The second phase is commonly referred to as the ovulatory phase and is characterized by the release of a mature ovum (Guyton & Hall, 1996). Ovulation occurs after massive release of LH from the anterior pituitary (Berkow, 1992). The LH surge is short lived and is followed by a sharp decline in its production along with a concurrent fall in estrogen production by the ovaries (Berkow, 1992). After the ovum is released, the remaining follicle cells are transformed into granulosa cells (lutenization) and begin producing progesterone (Guyton & Hall, 1996). Progesterone levels increase significantly with ovulation (Berkow, 1992) and continue to increase in the third phase of the menstrual cycle.

Following ovulation, the remaining corpus luteum develops and begins to secrete large amount of progestins and a smaller amount of estrogen (Guyton & Hall,

1996). This change in dominant sex-steroid hormone marks the beginning of the luteal menstrual cycle phase.   The luteal phase has the most uniform duration being approximately 14 days in length (Berkow, 1992), but can be shorter in athletic women (Robergs & Roberts, 1997).   The amount of progesterone, and to a lesser degree estrogen, peaks within seven to eight days (Berkow, 1992; Guyton & Hall, 1996). The estrogen secreted produces a negative feedback effect on the anterior pituitary gland that decreases remaining secretions of FSH and LH (Guyton & Hall, 1996). This causes degeneration of the corpus luteum and eventual reduction of estrogen and progesterone produced (Berkow, 1992; Guyton & Hall, 1996). The paucity in progesterone and estrogen secretion leads to menstruation by the uterus (Guyton & Hall, 1996) and the hormonal cycle begins again.

1.2.8 Endurance Exercise Performance and the Effect of Menstrual Cycle
Phase or Oral Contraceptives.

For women, the complex and changing hormonal milieu presented throughout the menstrual cycle has stimulated research that has attempted to gain an understanding of female exercise performance. Early investigations lacked sufficient methods for detecting menstrual cycle phase and thus its effect on exercise performance (Lebrun, 1993). The more recent information available with regards to female exercise performance, even with the precise measurement of hormone levels in each menstrual cycle phase, remains relatively equivocal (Bunt, 1990; Lebrun, 1993)

A review by Bunt (1990) discusses the difficulty in determining hormonal status (estrogen or progesterone) and its metabolic effect within one month or even over a few

months. Blood samples only provide information on the concentration of a given hormone for a moment in time and provide little information on women's hormonal status (Bunt, 1990). Hormonal status is affected not only by a single hormone's absolute concentration, but by the ratio of other sex steroid hormones, the state of the cellular receptor in the target tissue and the effect of other metabolic hormones (Bunt, 1990). These factors, along with a women's individualized response to sex-steroid hormones, have made discerning the effect of any one single sex steroid hormone difficult from a single blood assay, and may partially account for the equivocal nature of this body of literature.

Aerobic capacity was found to vary throughout the menstrual cycle suggesting a slight deleterious effect on aerobic capacity (maximal oxygen consumption) in the luteal menstrual cycle phase (Schoene et al., 1981; Lebrun et al., 1995). In addition to this finding, Schoene et al. (1981) noted that this phenomenon was significant only for non-trained women and disappeared in highly trained female athletes. Both of these studies utilized cycling as the mode of exercise employing progressive work tests to determine maximal oxygen consumption. Schoene et al. (1981) also found that time to exhaustion for the non – athletes in the luteal phase was decreased compared to the follicular menstrual cycle phase, although no significant change in performance was detected for the athletes.

In contrast, an investigation by Pivarnik et al. (1992) did not find any significant differences in aerobic capacity between the menstrual cycle phases in moderately trained athletes (42.5 ml/kg VO_2max) with 60 minutes of cycling at 65% VO_2max. However, the participants reported a higher perceived exertion rating during the luteal phase along

with greater cardiovascular strain (Pivarnik et al., 1992). Unfortunately, this study did not measure exercise performance.

Jurkowski and assossicates (1981) also found that cardiorespiratory adaptations and aerobic capacity were not significantly affected by menstrual cycle phase at low and moderate exercise intensities (33% and 66% VO_2max, respectively) in moderately trained athletes (n=9). However, in contrast to the findings of Schoene et al. (1981), they found that lactate levels were lower and exercise performance nearly doubled at high intensities (90% VO_2max) during the luteal phase (Jurkowski et al., 1981). Furthermore, Nicklas et al. (1989) tested six moderately trained eumenorrheic females in midfollicular and midluteal phases and also found that performance may be affected by menstrual cycle phase. This investigation found greater muscle glycogen repletion (after 90 minutes cycling at 60% VO_2max and exhaustive intervals followed by three days of mixed diet and rest), along with tendency for a greater work time to exhaustion (cycling at 70% VO_2max) in the luteal menstrual cycle phase compared to the follicular phase (Nicklas et al., 1989).

To further complicate the discussion regarding exercise performance, female athletes' menstrual cycle hormones may be exogenously controlled by oral contraceptive preparations. There is a great amount of diversity in oral contraceptive agents in regards to estrogen and progestin type and content, which has confounded the interpretation of the studies reviewed.

De Bruyn-Prevost, Masset, and Sturbois (1984) conducted an investigation with 14 females comparing the effect of follicular, ovulatory and luteal phases in eumenorhheic females (n=7) and oral contraceptive users (n=7) on aerobic endurance

performance measured by a maximal oxygen consumption cycling test. No differences were found between menstrual cycle phase or the oral contraceptive group in regards to maximal oxygen consumption capacity, heart rate, and blood lactate levels (De Bruyn-Prevost et al., 1984). A major limitation of this investigation is that they did not mention the type of oral contraceptive used in the study.

However, in support of this finding Grucza, Pekkarinen, Titov, Kononoff, and Hanninen (1993) reported that maximal oxygen consumption did not differ between menstrual cycle phase or oral contraceptive groups for moderately trained women exercising at 50% VO_2max for 90 minutes. Interestingly, this investigation did find a slight change in thermoregulatory response between menstrual cycle phase showing an increase in temperature and sweating in the luteal phase. Also, the triphasic low dose oral contraceptives (50 –150 ug levennorgestrel and 30 – 40 ug ethynylestradiol) reduced differences in sweating and made the thermoregulatory response to exercise more uniform (Grucza et al., 1993).

In contrast, Lebrun (1993) found that there was a 5% decrease in aerobic capacity (VO_2max) of highly trained women who were put on a low dose triphasic oral contraceptive for two months. However, this change did not alter their aerobic endurance performance at 90% VO_2max. It was suggested that the impairment may be caused by a change in cellular metabolism as maximal heart rate, respiratory exchange ratio, and ventilation remained similar (Lebrun, 1993).

Bemben, Boileau, Bahr, Nelson and Misner (1992) reported that oral contraceptive users displayed a significantly lower blood glucose level and reduced carbohydrate utilization during moderate intensity exercise (50%VO_2max) for 90

minutes compared to eumenorrheic controls. The carbohydrate sparing response during exercise was thought to reflect contra-insulin effects of increased growth hormone levels found during exercise in the oral contraceptive users. This is supported somewhat in an earlier study conducted by Bonen, Haynes and Graham (1991). These investigators reported lower insulin concentrations for both women using low dose oral contraceptives and in the luteal menstrual cycle phase. These changes were only significant at lower exercise intensities (Bonen et al., 1991) and may not apply to more athletic populations. The evidence presented in this review is again confounded by level of training, dose of exogenous steroids, and perhaps the intensity of the exercise testing protocols, and thus no clear conclusions about the effect of oral contraceptives on exercise performance or substrate metabolism can be determined.

1.2.9 Potential Metabolic Effects of Female Sex - Steroid Hormones on Substrate Metabolism during Exercise.

The full biochemical and physiologic pathways of the sex-steroid hormones, estrogen and progesterone, and their effect on carbohydrate and lipid metabolism are complicated and, as such, are quite beyond the scope of this review and investigation. However, it is necessary to derive a basic understanding of their role in the regulation of female substrate metabolism at rest and during exercise. Much of this work is generalized from testing in animals due to its truly invasive nature.

The limitations in the study of sex-steroid hormones are common to all metabolic investigations, where the effect of a particular hormone is dependent on its absolute concentration, the number and state of receptor binding sites in target tissues, cellular

transport, tissue specific responses, and finally the interplay, or antagonistic effect of other metabolic hormones (Bunt, 1990).

The information presented in this review is largely borrowed from investigations in animals, and thus, is rather theoretical as estrogen and progesterone receptor sites have not been established in non - traditional target tissues (e.g., skeletal muscle) in humans. However, there is some support for the extrapolation of the animal literature to humans in this area of interest. For example, estrogen receptors have now been established in human vascular smooth muscle cells and endothelium, myocardium and cardiac fibroblasts (Pelzer, Shamim, Wolfges, Shumann, 1997). Also, estrogen, glucocorticoid and androgen receptors are known to occur in mature human adipocytes (Pedersen, Fuglsig, Sjorgen, Richelsen, 1996). To date there is no known receptor sites specifically for progesterone in the above stated tissues. Finally, sex steroid receptors have not been established in skeletal muscle tissue.

Beyond the regulation of the female menstrual cycle, the ovarian hormones estrogen and progesterone have been shown to modify energy substrate utilization (Kendrick et al., 1987). Both physiological (low) and pharmological (high) doses of estrogen are shown to reduce the rate of glycogen utilization (spare glycogen) in liver, myocardial and skeletal (vastus lateralis) muscle tissue in rats during exercise compared to ovariectomized controls (Kendrick et al., 1987). The authors of this investigation suggested that the glycogen sparing effect of estrogen likely was associated with increased activity of lipoprotein lipase in adipose tissue or other changes in lipid metabolism (Kendrick et al., 1987). Also, Kendrick and Ellis (1991) reported an increase in plasma fatty acids during prolonged submaximal exercise in the male rats

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receiving exogenous estrogen compared to controls (males with no estrogen). The increased fat oxidation with the administration of estrogen, in turn, was found to spare glycogen use in both red (slow oxidative) and white (glycolytic) muscle fibers and increase work capacity during exercise (Kendrick & Ellis, 1991). These findings agree with an earlier investigation by Ahmed-Sorour and Bailey (1981) who found that the absence of ovarian hormones lead to poor blood glucose regulation and low liver, uterus, skeletal and cardiac glycogen stores in rats. The exogenous administration of estrogen (alone) to ovariectomized rats was associated with the greatest increases in body stores of glycogen and was accompanied by a improved insulinemic response from the pancreas (Ahmed-Sorour & Bailey, 1981). This suggests that estrogen alone increased circulating insulin levels, which promoted glycogenesis, and thus it has a very important role in the basic regulation of glucose (Ahmed-Sorour & Bailey, 1981).

However, elevated estrogen by itself (without a corresponding elevation of progesterone) is characteristic of only the follicular phase of a menstrual cycle. The effect of progesterone alone, or in combination with estrogen, must also be considered as this hormone is present in varying doses throughout the luteal menstrual cycle phase. Matute and Kalkhoff (1973) looked at the singular effects of estrogen and progesterone along with the combination of estrogen and progesterone on liver carbohydrate metabolism. They reported that progesterone, by itself, or in combination with estrogen, created larger hepatic stores of glycogen than the effects of estrogen alone (Matute & Kalkhoff, 1973). These authors also found that the administration of estrogen and progesterone combined, had a suppressive effect on the incorporation of gluconeogenic amino acids into glucose. Both of these actions promoted better glycemic control

compared to the ovariectomized controls, thus provided an appropriate balance of carbohydrate storage and utilization throughout the menstrual cycle (Matute & Kalkhoff, 1973).

The purported action of progesterone is supported by a later investigation by Ahmed-Sorour and Bailey (1981), who also looked at the interaction of progesterone and estrogen, as would occur in the luteal menstrual cycle phase. They reported that progesterone, which is known to promote a catabolic state similar to glucocorticoids, antagonized the glycogenic effects of estrogen in some tissues (cardiac and skeletal muscle) with the exception of liver tissue, where it increased the liver glycogen stores beyond the estrogen alone treatment (Ahmed-Sorour & Bailey, 1981). The mechanisms behind the change in action of the combined hormones in the liver was hypothesized to be either a result of inducing greater glycogen synthetase, or progesterone's catabolic effect on protein, which may have increased the amount of available gluconeogenic amino acids providing greater substrate for gluconeogenesis, or possibly suppressed glycogenolysis in the liver (Ahmed-Sorour & Bailey, 1981).

In general, the physiological action of progesterone is thought to exert a catabolic effect on the body, particularly protein breakdown (which generates gluconeogenic amino acids), similar to the influence of glucocorticoids (Guyton & Hall, 1996). Matute and Kalkoff (1973), have concluded that the presence of both progesterone (catabolic) and estrogen (anabolic) hormones play an important role in regulating a suitable balance between fuel storage and release in the female both at rest and during exercise. The idea of antagonistic action or state between the major sex-steroid hormones, estrogen and progesterone, was hypothesized also to be a result of

increased pancreatic insulin secretions (though this alone could not explain the magnitude of change in storage), or decreased glycogenolysis (suppressed hepatic glucose release), or increased peripheral utilization of glucose (Matute & Kalkoff, 1973). This would serve the maternal host well under conditions of pregnancy (fed and fasted), where the female must become somewhat catabolic giving up enough fuel for fetal growth, yet maintain her own homeostatis through anabolic processes (Matute & Kalkoff, 1973).

A dose-response relationship, between the sex-steroid hormones, and their ability to exert a particular effect in the body at rest, and during exercise was examined by Kendrick et al. (1987). They found that the specific effect (positive or negative) of the ovarian hormones was related to dose but reached a measurable upper and lower plateau. Thus, the usefulness of these studies are also limited by the different doses (both physiologic and pharmacologic) of estrogen and progesterone employed and may be responsible for some equivocal findings by other investigators. Unfortunately, the effect of the ovarian hormones on metabolism of fuel in non-traditional target tissues (e.g., skeletal muscle, liver and myocardium) has not been extensively studied in a human model.

The available information of the effect of ovarian hormones (or simply menstrual cycle phase) in humans is limited to a broader whole body metabolic effect. The measurement parameters have traditionally been respiratory quotient, and plasma metabolites, both at rest and during exercise. Also, a few studies have employed measures of muscle glycogen stores, utilization and oxidation values. However, most studies have primarily looked at estrogen and/or the follicular phase and little is know

about the effects of progesterone (alone, or in combination with estrogen) as found in the luteal phase.

Reinke, Ansah and Voigt (1972) explored carbohydrate and lipid metabolism at rest in 10 eumenorrheic women (fasted state) using a same subject design. Each of the women were tested during the follicular phase (moderate – high estrogen levels), ovulation (decreased estrogen levels) and the luteal phase (elevated estrogen and progesterone levels combined) (Reinke, Ansah & Voigt, 1972). No significant differences in plasma glucose, pyruvate, lactate, acetoacetate, beta-hydroxybutyrate, glycerid-glycerol or total glycerol were found between the three phases at rest, with the exception of increased plasma free fatty acids in the luteal phase (Reinke, Ansah, & Voigt, 1972). This was hypothesized by the authors to be the result of increased lipolysis (adipocytes) under the influence of elevated estrogen concentrations found in the luteal menstrual cycle phase (Reinke, Ansah, & Voigt, 1972).

Similarly, Hackney, McCracken-Compton, and Ainsworth (1994) reported that during 30 minutes of exercise at low to moderate intensities (35% - 60%VO_2max) in the luteal phase, substrate responses favored lipolysis compared to a greater degree of carbohydrate oxidation in the follicular phase. However, this effect was intensity dependent and no significant differences were found between the menstrual cycle phases at an intensity corresponding to 75% VO_2max (Hackney et al., 1994). The ability of estrogen to enhance lipolytic activity was thought to contribute to the low carbohydrate oxidation values at lower – mid exercise intensity in the midluteal phase when absolute hormone concentrations of estrogen are elevated (Hackney et al., 1994), which is

supported by the singular effects of estrogen during exercise derived from animal data (Kendrick et al., 1987; Kendrick & Ellis, 1991).

Also, in support of this finding, Nicklas et al., (1989) found no difference in glycogen utilization during prolonged exercise (90 minutes cycling at 70% VO_2max) between the follicular and luteal menstrual cycle phases. Nor did blood glucose, lactate, free fatty acids or RER measuresments differ between the menstrual cycle phases. In addition, Kanaley, Boileau, Bahr, Misner, and Nelson (1992) found no differences in growth hormone or substrate metabolism between the menstrual cycle phases with prolonged exercise (90 minutes) at a lower intensity of 60%VO_2max and concluded that menstrual status had little effect on substrate metabolism.

On the contrary, a more recent investigation by Hackney (1999) showed that exercise at 70% VO_2max in the follicular phase induced greater muscle glycogen utilization (measured via muscle biopsy), whereas the same exercise protocol in the luteal phase induced greater lipid oxidation (calculated from end value RER). Again, the author hypothesized that the high estrogen levels in the luteal phase were likely responsible for the enhanced lipid metabolism, as estrogen suppresses insulin activity and increases growth hormone levels promoting the lipolysis (Hackney, 1999).

Overall, in this body of literature, the investigative focus has been directed towards the effects of estrogen on exercise performance. There is little acknowledgement of the potential effects of elevated progesterone levels (found in the luteal menstrual cycle phase) on substrate metabolism during exercise. Finally, in general, some of the limiting issues that have made comparisons difficult include small

sample sizes, testing was completed at different points within a particular menstrual cycle phase, and a great variety of exercise testing protocols.

1.3 Statement of the Problem and Hypotheses

1.3.1 Statement of the Problem

The influence of the menstrual cycle, or more specifically estrogen and progesterone, on substrate utilization during exercise is not clearly understood. To date, five studies are known to have investigated the effects of carbohydrate loading on exercise substrate metabolism, or performance, throughout the menstrual cycle phases (Tarnopolsky et al., 1995; Walker et al., 2000; James et al., 2001; Paul et al., 2001; Tarnopolsky et al., 2001). Currently, in athletic females it has been shown that carbohydrate loading does not improve performance in the follicular phase, which is characterized by primarily estrogen (Tarnopolsky et al., 1995; Paul et al., 2001). One study has shown that carbohydrate loading marginally improved endurance exercise performance in the luteal phase (Walker et al., 2000). These studies have used different modes of endurance exercise, different dietary levels of carbohydrate, and different exercise testing protocols, which may have contributed to the equivocal performance results with carbohydrate loading in females. No study to date, however, has examined carbohydrate loading in the same subjects over both, the follicular and luteal menstrual cycle phases, or by using same testing protocol in both menstrual cycle phases.

The purpose of this study, therefore, was to examine and compare the effects of carbohydrate loading on running performance and selected physiologic variables in moderately trained females during both the follicular and luteal menstrual cycle phases. The effect of carbohydrate loading on running time to exhaustion was investigated during each menstrual cycle phase. In addition, the effect of carbohydrate loading on RER, oxidation values, blood glucose, lactate, glycerol and free fatty acids during exercise was explored during each menstrual cycle phase.

1.3.2 Hypotheses

The major hypotheses were that:

1. Carbohydrate loading in the luteal menstrual cycle phase compared to normal mixed diet in the luteal phase would increase time to exhaustion during a high intensity treadmill run (preceded by a moderate intensity endurance run).

2. Carbohydrate loading in the follicular menstrual cycle phase compared to normal mixed diet in the follicular phase would not increase time to exhaustion during a high intensity treadmill run (preceded by a moderate intensity endurance run).

3. RER and carbohydrate oxidation values would be greater in the luteal menstrual cycle phase over the follicular phase and would also be greater with carbohydrate loading compared to the normal mixed diet, during the 60 minute run at 70% VO_2max.

In addition, four secondary hypotheses tested the effect of carbohydrate loading on blood substrate metabolism during exercise. Estrogen has been shown to promote

increased lipolysis and reduce the rate of glycogen utilization at rest and during exercise (Kendrick et al., 1987; Kendrick & Ellis 1991;Ahmed-Sorour & Bailey, 1981). Progesterone is thought to antagonize the effects of estrogen, thereby interfering with the lipolysis and promoting a greater reliance on carbohydrate in the luteal phase (Matute & Kalkhoff, 1973). Besides these two hormonal influences, a high dietary carbohydrate intake is associated with greater RER and carbohydrate oxidation values during exercise (Bergstrom et al., 1967; Whitley et al., 1998). Thus, it was predicted that:

1. Blood glucose level would increase to a greater extent in the luteal phase compared to the follicular phase, and in the carbohydrate loading condition compared to the NMD trial, during the 60 minute run at 70% VO_2max.

2. Bood lactate levels would increase to a greater extent in the luteal phase compared to the follicular phase, and in the carbohydrate loading condition compared to the NMD condition, during the 60 minute run at 70% VO_2max.

3. Glycerol level in the blood would be greater in the follicular phase than in the luteal phase, and in the NMD condition compared to the carbohydrate loading condition, during the 60 minute run at 70% VO_2max.

4. Free fatty acid levels in the blood would be greater in the follicular phase than in the luteal phase, and in the NMD condition compared to the carbohydrate loading condition, during the 60 minute run at 70% VO_2max.

1.3.3 Limitations

1. Participants were volunteers from Saskatoon and surrounding area, and therefore were not randomly selected.

2. The lifestyle, activity and dietary habits of the free-living participants could not be completely controlled. However, as these participants acted as their own controls, it is assumed that all variables would be relatively similar across tests for each individual participant.

3. All information about menstrual cycle length and past history, habitual dietary patterns and habitual physical activity was voluntary. The accuracy of this information is dependent on the ability of the participants to unerringly report portion sizes, food items, training regimen, and menstrual cycle.

4. The small number of participants in the study leads to poor statistical power.

5. Subgroups within our sample with obvious menstrual cycle differences could not be accurately represented at this time due to the small number of participants.

6. The number of participants in this study was small (n=9) and may not accurately represent different populations of female athletes. Inferences should cautiously be made to general athletic populations at this time.

1.3.4 Delimitations

1. Direct inferences about the study findings should only be applied to female athletes aged 18 – 43 years of age who are moderately trained.

2. The exercise protocol included a 60-minute run at a moderate intensity and a high intensity timed run to fatigue. Other endurance exercise protocols (e.g., cycling) may produce different physiological variations in the response to carbohydrate loading.

3. A modified carbohydrate loading protocol (4 loading days of 70- 80% energy from carbohydrate and tapered running program, plus a 5th day for testing) was used for this study. Other carbohydrate loading protocols may produce variations in the results reported in this study.

4. The dietary aspect of the carbohydrate loading regimen relied mainly on polysaccharides in the form of starch (amylose and amylopectin) along with smaller amounts of monosaccharides (glucose polymers) and disaccharides as the main fuel substrate. Diets were low in total fibre (both insoluble and soluble fibre) content. Other carbohydrate and fibre combinations may produce variable results.

CHAPTER 2

METHODS

2.1 Research Design

This study used a randomized within-within subject repeated measures design in which every participant was involved in completing a carbohydrate loading and a normal mixed diet control intervention in both the luteal and follicular menstrual cycle phases. All participants were required to complete testing in each of the following conditions:

Experimental Conditions:

1) Follicular – Carbohydrate Loading

2) Luteal – Carbohydrate Loading

Control Conditions:

1) Follicular – Normal Mixed Diet,

2) Luteal –Normal Mixed diet.

The initial order of testing for each participant was randomized in a counterbalanced fashion in the four conditions in effort to eliminate a learning or carry-over effect. This design assisted in controlling for natural individual variations in menstrual cycle length and hormone levels, sleep patterns, stress level, normal mixed diet patterns, exercise habits and other lifestyle factors that might be considered confounding variables in a matched control design. It was assumed for the purpose of the analysis that these extraneous variables remained relatively constant for each subject throughout her participation in the study.

The treatment was either a four day modified carbohydrate loading regimen (high carbohydrate diet and depletion exercise taper) or the participant's normal mixed diet and regular training routine as the control. The dependent variables of interest were (1) Performance defined as time to exhaustion during a treadmill run at 80% VO_2max, (2) Substrate utilization during endurance exercise at 70% VO_2max measured as (a) respiratory exchange ratio, (b) calculated average oxidation values for carbohydrate and fat, and (c) blood substrate measures during exercise for glucose, lactate, free fatty acids, and glycerol.

2.2 Participants

Fifteen females volunteered for this study. The participants were recruited from the Saskatoon area by advertisement in the Star Phoenix, by poster advertisement placed in local health club facilities, through personal recruitment at a local running club, and by word of mouth (Appendix A).

As suggested in previous literature (Lebrun et al., 1995; Tarnopolsky et al., 1995) participants' training history (Appendix C) and menstrual cycle status (Appendix D) were determined by voluntary self-report. Eligibility for inclusion in this study required that:

1) participants were accustomed to running,

2) participants were moderately trained and scored approximately 50 ml/kg/minute on pre-test of maximal oxygen consumption (VO_2max) (Thoden, 1991),

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3) menstrual cycles occurred at a regular monthly interval of between 24 –

   35 days in length, and

4) participants reported no history of abnormal gynecological

   complications.

Further to this, the use of oral contraceptive is popular among females today, and thus represents another situation where the changing hormonal milieu has the potential to affect various aspects of endurance exercise performance, though there has been little agreement on this topic in the literature (Lebrun, 1993). The participants were grouped by oral contraceptive use in the following manner:

1) no oral contraceptives use within the last six months with the return of

   regular menses (no oral contraceptive group) or,

2) currently using oral contraceptives for no less than two years and

   menstrual cycle was stable on the stated dose (oral contraceptive group).

Participants were informed about the intent of the study and commitment required of them and written consent was obtained (Appendix B). Nine participants between the ages of 18 and 43 years of age were accepted.

2.3 General Procedures

2.3.1 Determining Eligibility, Familiarization to Lab and Testing Order

The University of Saskatchewan Human Ethics Committee approved this study. All participants were informed of testing procedures and provided written informed consent (Appendix B). No financial incentives of any kind were given to

participants of this study. This study entailed a minimum of seven visitations to the Exercise Physiology Laboratory at the College of Kinesiology, University of Saskatchewan.

The initial visit was used to establish the baseline fitness level (maximal oxygen test) and eligibility of the participants for inclusion in the study. At this time the participants were familiarized with the lab setting and staff, along with the procedure for an initial VO_2 max test. Anthropometric measurements of height and weight were recorded (Appendix E) and a maximal oxygen consumption (VO_2 max) treadmill test was completed. Participants then completed a self - reported record of their training history (Appendix C), menstrual history and current menstrual cycle day (Appendix D). Participants meeting all eligibility inclusion criteria also received instruction on the use of an estimated three - day food record (Appendix F) by a registered dietitian. A number of the subjects chose to return to the lab at their convenience to meet with the dietitian regarding the food record, dietary instructions, or for their skin fold measurements. The remaining visits were used for carbohydrate loading exercise regimens and testing days.

Once subjects confirmed their current menstrual phase, a suitable time to commence the study interventions was estimated. Individual calendars (Appendix D) were used to track their menstrual cycles and plot plausible testing days. Subjects were randomly assigned to start any intervention or testing so that any learning effect to testing would be reduced or evenly distributed among menstrual cycle phases.

2.4 Pre-experimental Testing Protocol

2.4.1 Maximal Oxygen Consumption (VO$_2$max, ml/kg · min)

Two to three weeks prior to any intervention, each subject performed a maximal oxygen consumption test. This test was used to describe the participant's initial fitness level and was used to estimate the exercise intensity of future submaximal running protocols for each subject. A modified running protocol (Thoden, 1991) was chosen for the maximal oxygen consumption test (VO$_2$max). The VO$_2$max test began with a short warm-up (3 – 5 minutes) at an average speed of 8 km/hr. Each subject then ran at a constant speed of 9.6 – 12.8 km per hour throughout the test while the grade intermittently increased two percent every two minutes. The test continued until oxygen consumption ceased to increase linearly with the rising workload and approached a plateau or a slight drop in VO$_2$max value. This change was accompanied by a respiratory exchange ratio of 1.00 – 1.10, and a heart rate close to the age predicted maximum (220 - age) (Thoden, 1991). Following the test, all participants reported that they were unable to continue any further activity at the given intensity.

A metabolic cart (Sensor Medics Vmax 29 series, Anaheim, CA) was used to measure breath by breath ventilations during the VO$_2$max test. The respiratory exchange ratio (RER) and oxygen uptake was calculated on-line and recorded every 20 seconds. Energy, fat, and carbohydrate utilization was estimated from the RER and VO$_2$ data. For the purpose of this study, it was assumed that the RER reflects

the non-protein respiratory quotient and VO_2 measurement (Tarnopolsky et al., 1990; Robergs & Roberts, 1997).

2.4.2 Anthropometric Measurements

Anthropometric measurements were used to describe the physical characteristics of the participants. Body size was determined by standard procedures for height, weight, body mass index and five different sites for skinfold measurements (biceps, triceps, subscapular, iliac crest, and medial calf) (Canadian Physical Activity, Fitness and Lifestyle Appraisal, 1996).

Height was measured, without shoes, using a stadiometer and recorded to the nearest 0.1 centimeter. Participants, dressed in light athletic clothing without shoes, were weighed to the nearest 0.5 kilograms on a Toledo Digital Scale (model no. 1939, Toledo Canada Inc.).

The Canadian Physical Activity, Fitness and Lifestyle Appraisal (1996) uses the sum of five skinfold measurements as a descriptive measurement of adiposity, and fat distribution. These sums are used to determine whether participants are within a normal range for age and gender and free from morbidity and mortality risks (Canadian Physical Activity, Fitness and Lifestyle Appraisal, 1996). The body mass index and sum of the five skin fold measurements (biceps, triceps, subscapular, iliac crest, and medial calf) were used as additional descriptors of body composition (Canadian Physical Activity, Fitness and Lifestyle Appraisal, 1996).

Skinfold measurements were completed by the same anthropometrist using a Harpenden caliper (Harpenden, England). All skinfold measurements were taken

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from the right side of the body to the nearest 0.2mm thickness and were repeated

two to three times per site according to the Canadian Physical Activity, Fitness and

Lifestyle Appraisal (1996) protocol. The primary researcher completed all

calculations. Anthropometric measurements took approximately 20 minutes to

complete and were obtained during the course of the study at the convenience of the

participant. See Appendix E for form used in skinfold measurements.

2.4.3 Determination of Menstrual Phase

Previous menstrual history (cycle length and date of last menses) was obtained

using a self-report form (Appendix D). This information was used along with a

general ovulatory guideline (Hygeia, Sciences, Inc., New York, 1999) to estimate

suitable test days for each menstrual cycle phase. For example, with a 32 day

menstrual cycle, ovulation is expected around day 15 plus or minus one to two days,

whereas for a 24 - day cycle ovulation is estimated on or around day 9. The average

menstrual cycle length, last date of menses, and length of menses, plotted on

individual calendars (Appendix D) were used to track any deviance from the self-

reports so adjustments or rescheduling could be made throughout the course of the

study.

In addition, a FIRST RESPONSE ovulation predictor test kit (Hygeia Sciences,

Inc., New York, 1999) was used by participants in the non oral contraceptive group

to verify they were not in the ovulation stage of the menstrual cycle during any

testing. Finally, confirmation of mid to late luteal menstrual cycle phase was

verified with resting blood progesterone measurements. Blood progesterone levels of

>7.5 nmol/L were considered indicative of luteal phase in the non-oral contraceptive users and a value of 0.6 - 2.2 nmol/L was deemed sufficient for the oral contraceptive users (Saskatchewan Provincial Medical Laboratory, Regina, SK, 1998). Testing of serum progesterone levels was restricted to five of the nine participants (both non-oral contraceptive and contraceptive users) due to financial restraints.

2.4.4 Training History

Training history and energy expenditure estimates were used primarily to ensure that the participants had sufficient training volume and intensity to be eligible for the study. Recording the participants' usual training routines helped to ensure that the group was homogeneous in respect to their level of training as VO_2max may be confounded by genetic aspects (Tarnopolsky et al., 1995; Robergs & Roberts, 1997). Criteria for inclusion required the participants to have regularly engaged in endurance-type activities at a minimum frequency of 4 days/week for 35 minutes or more, coupled with a VO_2max score of approximately 49 ml/kg/min. The training history was also used to describe the group and to determine the individual energy expenditure of participants in order to provide them with appropriate caloric intake for the carbohydrate menus. The training history form is provided in Appendix C.

This self-report measure estimated the number of training weeks per year, frequency per week, duration in hours per week, and a voluntary estimate of intensity of each session based on light, medium and hard categories. It also included the average number of hours of sleep per day that was used in a computer

analysis of daily activity energy expenditure. The voluntary information gathered was entered into the FUEL Nutrition Software (Logiform, Sillery (Quebec), Canada, 1991). The FUEL program estimates daily energy expenditure derived from the number of hours spent sleeping and daily activities of living, light to moderate activities (walking, housework, etc.) and in planned training activities. The energy expenditure equation used a standard number of hours for daily activities of living as set out by the FUEL program, with the exception of the participants' individual sleep patterns. This standard energy expenditure took the individual's height, weight and gender into account along with specific speeds and intensities of exercise reported. The program used a standard energy prediction equation in determining its energy requirement. This information assisted with matching the participants' energy expenditure and requirements with their individual energy intake for the carbohydrate loading trials.

2.4.5 Diet Preparation

A registered dietitian instructed and assisted participants in recording food and beverage intake for three days (two weekdays and one weekend day)(Appendix F). A list of likes and dislikes was also compiled for each participant. Each participant acted as her own control, thus it was assumed that the average macronutrient composition and caloric value of each diet would remain relatively stable during the course of the testing through the spring to late summer season.

All dietary records were analyzed using FUEL nutrition analysis software (Logiform, Sillery (Quebec), Canada, 1991). Manual adjustments were made when

specific foods were not available in the analysis program. The averages for daily

caloric intake and the amount of carbohydrate, fat and protein typically consumed

per day were calculated and used to develop a four day high carbohydrate (78% of

total energy) take home menu for the carbohydrate loading periods. The menus

consisted of 6.54 – 7.76g CHO/kg body weight and incorporated both complex and

simple sugars including a glucose polymer powder (Polycose, Ross Laboratories,

Quebec, Canada) and sport nutrition supplement bar (Sport Boost Bar, Mead

Johnson, Ontario, Canada). The meal pattern for the carbohydrate loading menus

consisted of three meals and three snacks to be consumed at regular intervals during

the day. This menu pattern was chosen so to ensure the participants would receive

sufficient carbohydrate in the first 24 hours after the sequential depletion exercise

bouts (Burke, Collier, Beasley, Davis, Fricker, Heeley, et al., 1995). The dietitian

reviewed the tailored menus with each participant prior to the implementation phase

so that each would have time to prepare and be familiar with her menu (See

Appendix G for example of CHO loading menu). The participants were instructed to

consume their normal mixed diet for the control trials. The participants were also

requested to be well hydrated, rested, and to refrain from the use of alcohol and

caffeine 24 hours prior to each test. The participants were asked to return all menus

to the primary investigator in an effort to promote dietary compliance.

Six of the participants were thought to inadvertently under-report overall

caloric intake compared to estimated caloric output from reported exercise regimens

and lifestyle. This has been documented to occur in 25% of women with dietary

intakes low in fat and alcohol (Black, Goldberg, Jebb, Livingstone, Cole, & Prentice,

1991). Thus, after careful personal interviews, caloric counts, and assessment of exercise in both typical and upcoming depletion exercise regimens, the calorie level of the four day menu was increased by an average of 23% (ranging from 100 – 800 kcal/day), a level more reflective of their energy output. The caloric cost of the exercise taper was also accounted for in the carbohydrate loading menu so that the individual would remain eucaloric. Participants were required to consume all supplements and a majority of menu items in order to maintain a constant carbohydrate, fat, and protein intake during the experimental protocol. The dietitian was on call and available to all participants in the event that the menus needed to be adjusted. Few requests for adjustments were made.

2.4.6 Diet and Exercise Tapering Regimen for Carbohydrate Loading Trials

On day one of the four-day program, each participant began following her tailored menu in a free-living situation and recorded any substitutions in the foods eaten if different in type or portion from the given menu. Frequent communication between the subject and registered dietitian during the four-day diet regimen was encouraged to assist with menu compliance. At the same time, the participants also began the depletion exercise taper. This consisted of treadmill running at 65% VO_2max, conducted on a motorized treadmill in the exercise physiology lab for 90 minutes on day one, 60 minutes on day two, and 30 minutes on day three, followed by a day of rest from all strenuous or moderate exercise on day four (Figure 2.1).

2.5 Test Day Protocol

Participants reported to the exercise physiology laboratory, at the same time of day, on four separate occasions for testing. Each test day was scheduled in accordance with the appropriate menstrual cycle day for the individual. Participant test days reflected the mid-late follicular phase and the mid-late luteal phase. Participants were instructed to arrive at the same time on day five for the carbohydrate loading trials and on the scheduled day for control trials. Participants not on oral contraceptives voided and performed the urine analysis with the First Response test kit for luteinizing hormone. The researcher confirmed that the urine analysis was negative before further intervention was allowed. In the advent that a subject produced a positive result for luteinizing hormone, testing was rescheduled for a more appropriate time the following month. Resting blood variables for serum progesterone, glucose, lactate, glycerol and free fatty acids were drawn from the anticubetal vein in the non-dominant arm via either saline lock or simple venipuncture technique (described below). Weight was measured in light athletic clothing prior to testing.

The participants briefly warmed up before they began a 60 minute run on the treadmill (no gradient) at 70% VO$_2$max. During the endurance run, blood was drawn at the 30 and 60 minute time points. There was a short 15 to 20 minute rest period after the last blood draw. The participants briefly warmed up again and then completed a timed performance run to volitional fatigue at 80% VO$_2$max. Verbal encouragement was consistent throughout all four trials. The treadmill protocol in all conditions remained identical to each participant's initial trial. Participants were not

made aware of time that elapsed in their performance trial until all conditions were completed.

Figure 2.1 Schematic diagram of diet treatment and exercise design

DAY | 1 | 2 | 3 | 4 | 5

F/NMD
L/NMD   ◄──────── 63%CHO; 24%FAT; 15%PRO ────────►
Exercise   normal training routine, variable intensity   Test

F/CHO
L/CHO   ◄──────── 78%CHO; 13%FAT; 13%PRO ────────►
Exercise @
65% VO2max 90min   60min   30min   Rest   Test
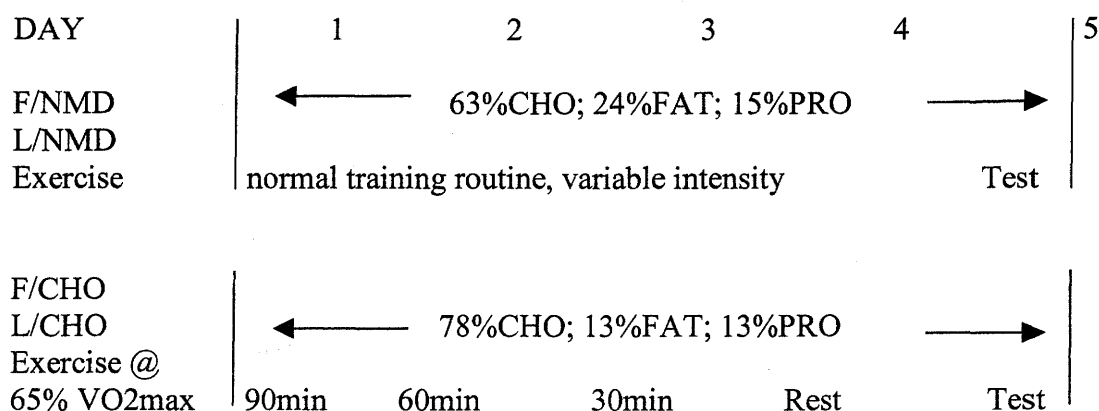
Note. Schematic diagram of 4 day modified carbohydrate loading diet and exercise tapering protocol. Normal mixed diet (NMD); Carbohydrate loading diet (CHO); Follicular menstrual cycle phase (F); Luteal menstrual cycle phase (L).

2.5.1 Physiologic and Substrate Measures During Testing

Breath by breath analysis was obtained during the treadmill run to determine submaximal oxygen consumption (VO_2) values, and the participant's Respiratory Exchange Ratio (RER) was calculated on-line every 20 seconds. The RER values were averaged for statistical analysis. The RER values obtained were assumed to represent the ratio of carbon dioxide production and oxygen consumption during exercise (VCO_2/VO_2) measured from the expired gas analysis indirect calorimetry (Robergs and Roberts, 1997). It was assumed that under the condition of steady state

exercise RER was equivalent to the respiratory quoteint (RQ) which reflects carbohydrate and lipid catabolism in the muscle for energy during exercise (the ratio of CO2 to O2 consumption during cellular respiration from the catabolism of food). (Robergs and Roberts, 1997). In both measurements it is assumed that protein contribution to these processes are negligible (Tarnopolsky et al., 1990; Robert and Robergs). Also for the term RER it is assumed that the measurement is not affected by the conditions of acidosis (e.g lactic acid build up with high intensity exercise where excess CO_2 results from buffering of the acid), hyperventilation (excessive exhalation of CO_2 unrelated to cellular metabolism) or non-steady state exercise (i.e. ATP production under anaerobic conditions which does not reflect ratio of VCO_2/VO_2 (Robergs & Roberts, 1997). Total carbohydrate and fat oxidation values were calculated for each trial from the endurance treadmill run VO_2 and VCO_2 data using the formula below (Peronnet & Massicotte, 1991).

$$CHO = 4.585(VCO_2) - 3.226(VO_2)$$

$$FAT = 1.695(VO_2) - 1.701(VCO_2)$$

Venous blood substrates were obtained in a fed state at rest (baseline), and at 30 (midpoint) and 60 (termination of endurance run) minutes of exercise. A 20 ml sample was drawn at each time point (5 ml each for serum glucose, lactate, glycerol, and free fatty acids). These samples assisted with determining the substrate utilized during the endurance tests. Serum progesterone was drawn at rest to assist with confirmation of luteal menstrual cycle phase. All samples were collected into sterile vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, New

Jersey, USA) and were placed immediately on ice until later centrifugation at the phlebotomy lab. Vacutainers treated with SST gel and a clot activator were used for blood glucose samples, potassium oxalate sodium flouride treated vacutatiners were used for blood lactate, and vacutainers with EDTA for blood lipids. Untreated vacutainers were used for the collection of progesterone sample. A registered nurse, physician, or phlebotomist drew the samples from the cephalic vein (wrist) in the non-dominant arm. Initially, a saline lock was used in all subjects, but this technique was abandoned in most subjects due to consistent difficulty drawing off the lock after 30 minutes of exercise. This was replaced by a simple venipuncture technique while the participant continued to work at a lower intensity on the treadmill for a 1 – 5 minute period.

2.5.2 Blood Substrate Analyses

Due to the financial constraints of the study, blood samples were drawn for four randomly selected participants (n = 4) and used in the determination of glucose (G), lactate (L), free fatty acid (FA), and glycerol (GL) concentrations at rest and during exercise. Serum progesterone (P) values were analyzed at rest only and used for confirmation of luteal cycle phase. Serum samples for progesterone were initially frozen at –20 °C and stored for up to three months then thawed to room temperature for batch analysis. Samples were analyzed using a solid-phase ligand-labeled, competitive chemiluminescent immunoassay (IMMULITE 2000 model analyzer, Diagnostic Products Corporation, Los Angeles, California, USA, 1998). Serum

glucose levels were determined by using a glucose oxidase colorimetric assay (Beckman model LX20, Beckman Coulter Products, 1996). Plasma lactate levels were measured using a lactate oxidase colorimetric assay (Beckman model LX20, Beckman Coulter Products, 1996). Portions of the blood samples were frozen at − 80°C and later analyzed for free fatty acids and glycerol. Blood free fatty acid (non-esterified fatty acid) samples were measured by an enzymatic oxidase colorimetric assay method as described by Elphick (1968). Blood glycerol samples were measured using a glycerol dehyrogenase/peroxidase oxidation reduction method as described by Kelly, & Christian (1984).

2.6 Statistical Analyses

Time to volitional exhaustion for the four running trials at 80% VO_2max values were compared using a 2 x 2 factor (diet treatment x menstrual cycle phase) repeated measures analysis of variance (ANOVA). When significant F values were observed, a one factor ANOVA was performed on the absolute and relative change scores between the menstrual cycle phases to determine the direction of the change.

RER measurements for both the 60 minute run at 70% VO_2max and for the timed run to exhaustion at 80% VO_2max were also analyzed using a 2 x 2 factor (diet treatment x menstrual cycle phase) repeated measures ANOVA. Carbohydrate and fat oxidation measures for the 60 minute run at 70% VO_2max were analyzed with a 2 x 2 factor (diet x menstrual cycle phase) repeated measures ANOVA.

In addition, each subject's total caloric intake and carbohydrate consumption for the four - day carbohydrate loading menu prior to testing were compared using a paired t-test with significance set at $p \leq 0.05$. The progesterone values are reported as means ± standard deviation (SD) for n = 4.

The blood substrates were to be analyzed using a 2 x 2 x 3 factor (diet treatment x menstrual cycle x time intervals) repeated measures ANOVA. However, due to financial restrictions, the sample was limited to a subset of four randomly chosen participants. Also, three of the four participants had missing data points for some of the substrates due to inadmissible lab samples thus, the blood substrate values have been expressed only as the group means to show the general trends throughout the 60 minute exercise bouts in each of the four trials.

Unless otherwise stated, statistical significance was accepted at $p < 0.10$ (Franks & Huck, 1986) and all values are reported as means ± standard deviation. Statistical analyses were performed with the SPSS statistical analysis program, version 10.5 (SPSS Inc., Chicago IL).

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Results

The purpose of this project was to compare the effects of carbohydrate loading on performance and physiologic substrates in the luteal and follicular menstrual cycle phases. The a priori hypotheses of this study were that, under the different hormonal milieu in the luteal menstrual cycle phase (progesterone and estrogen combined), trained females would respond positively to carbohydrate loading improving their performance (increased time to fatigue at 80% VO_2max) over normal mixed diet in the luteal phase, whereas, no performance enhancement was expected with CHO loading in the follicular phase (primarily estrogen itself), over NMD in the follicular phase. Also, RER and carbohydrate oxidation would be greater in the luteal phase compared to the follicular phase, and with carbohydrate loading over NMD.

3.1.1 Participant Characteristics

Data for two participants were omitted before the final analysis. Subject #9 (KC) completed three of four conditions but fell ill on her last test day and was unable to reschedule the test as she was moving from the province later that week. Subject #2 (LI) was found to have a protocol error in speed during one of the carbohydrate loading test

protocols at 85% VO$_2$max. The participant would not commit to rescheduling this test. Thus, only seven subjects were included in the final statistical analysis for performance and a subset of four individuals in the blood substrate analysis.

The descriptive characteristics are presented in Table 3.1. The mean age of the participants was 30.9 ± 9.6 years of age (yrs). All the participants individually were within an acceptable weight for height according to their calculated body mass index (kg/m^2) (Groff et al., 1995).The sum of five skinfold measurements included tricep, bicep, subscapular, iliac crest and medial calf. Five participants individually fell within the recommended range (sum of five skinfolds) for their age and gender category indicating good health and normal adiposity distribution (Canadian Physical Activity, Fitness and Lifestyle Appraisal, 1996), particularly once combined with body mass index measures. However, two individuals fell outside of the recommended range for their gender and age specific groups (CPAFLA, 1996). Participant number three (JP) scored 109.1 mm on the sum of five skinfold measurements, which was approximately 40.1 mm above the recommended range measuring highest at the iliac crest. This value may be skewed by the changed skin texture, along with stretch marks on the abdomen and iliac crest from past pregnancies. Participant number six (BMc) scored a sum of 85.6 mm for five skinfold measurements and fell 13.0 mm above the recommended range for her gender and age specific group.

Table 3.1. Summary of Participant Characteristics

	Mean ± SD	Range
Age (yrs)	30.9 ± 9.6	18 - 43
Height (cm)	172.4 ± 8.6	159 - 183
Weight (kg)	66.9 ± 8.4	57 - 79
Sum of 5 skinfolds (mm)	73.7 ± 18.8	51 - 109.1
Body Mass Index (kg/m^2)	22.3 ± 0.8	21 - 23
VO_2max (ml/kg/minute)	51.8 ± 2.3	49.5 - 54.4
Menstrual Cycle Length (days)	28.3 ± 2.4	24 - 32
CHO Intake:		
CHO g/kg body weight	7.1 ± 0.43	6.54 – 7.76
NMD g/kg body weight	4.5 ± 0.70	3.75 – 5.64

Note. All values are reported in means ± SD for 7 subjects. Normal Mixed Diet (NMD); Carbohydrate loading (CHO); Maximal oxygen consumption (VO_2max).

Participant six measured higher on both tricep (22.1 mm) and bicep (16.1 mm) skinfold measurements than other participants. Both participants recorded high levels of activity on training records and calculated BMI measurements indicated participants are within acceptable weight limits for height.

Baseline measurements and training history indicated that the group was moderately trained and all engaged specifically in running activities. Most participants also cross-trained using swimming, sprinting, soccer and basketball as other activities in conjunction with their running program.

The participants' overall menstrual cycle length ranged from 24 – 32 days, well within accepted normal cycle length of approximately 18 – 45 days (Berkow, 1992; Guyton, & Hall, 1996). The length of each individual participant's menstrual cycle remained relatively constant (within 2 – 4 days) throughout the duration of the study.

3.1. 2 Menstrual Cycle Phase Confirmation

The results of the urine test with the First Response Ovulation Predictor Test Kit (Carter-Wallace, Inc, 1999, San Diego, CA) revealed one participant was in the ovulatory phase of her menstrual cycle on a scheduled test day. As it was a carbohydrate loading trial, her test was rescheduled for the following month. All other participants tested negative for luteinizing hormone and completed their scheduled tests. The dose of estrogen and progesterone in the tri-phasic low dose oral contraceptives users (n = 3) are presented in Table 3.2. The measurement of resting serum progesterone levels was used to confirm menstrual cycle phase for four randomly chosen participants (n = 2 OC, n = 2 NOC) on their respective testing days. These results are summarized in Table 3.3. Blood progesterone levels greater than 7.5 nanamols/litre (nmol/L) are indicative of an ovulatory cycle and confirm the participant is in the luteal menstrual cycle phase for non-oral contraceptive users (Saskatchewan Provincial Laboratory, Regina, SK IN: Diagnostic Products Company Los Angeles, CA, 1998). Serum progesterone values of 2.2 nmol/L were considered indicative of luteal phase for oral contraceptive users as suggested by laboratory guidelines (Saskatchewan Provincial Laboratory, Regina, SK IN: Diagnostic Products Company, Los Angeles, CA, 1998). One non-oral contraceptive participant (participant #3 (NP)) was found to have an

unusually low resting progesterone value (2.60 nmol/L) for the luteal phase

carbohydrate loading trial despite careful anticipation of her menstrual cycle pattern.

However, this value was similar to the oral contraceptive users' level of progesterone

and her substrate responses to the exercise tests were similar to the overall group

responses so her values remained in the final analysis. According to Tarnopolsky and

Cortright (1999), the low dose oral contraceptive pill does not interfere with substrate

metabolism during exercise.

Table 3.2 Description of Triphasic Oral Contraceptives (OC) used by three participants

Participant	Name of OC and *Manufacturer*	Dose of Synthetic Hormone
1 & 8	Tri-cyclen *(Janssen – Ortho)*	Ethinyl Estradiol 35 mcg
		Norgestimate 180, 215, 250mcg
5	Triquilar *(Berlex)*	Ethinyl Estradiol 30, 40, 30 mcg
		Levonorgesterel 50, 75, 125mcg

Note. The composition of the oral contraceptive (OC) product is based on information
gathered from the company patient information leaflets and the incremental dose is a
typical characteristic of all triphasic OC compounds.

Table 3.3 Confirmation of resting progesterone levels in the luteal menstrual cycle phase per diet intervention.

Subject	Luteal Phase			
	NMD		CHO loading	
1 (OC)	2.8	nmol/L	2.4	nmol/L
3	32.6	nmol/L	2.6	nmol/L
4	28.7	nmol/L	36.4	nmol/L
5 (OC)	2.2	nmol/L	2.6	nmol/L
Means ± SD	16 ±16 nmol/L		11 ± 17 nmol/L	

Note. Actual values are reported for the 4 subjects involved in the blood substrate analyses. (OC) indicates the individual was an established oral contraceptive user. Values >7.5nmol/L for eumenorrheic females and >0.6- 4.5 nmol/L for (OC) indicate luteal phase progesterone levels. Group means ± standard deviation (SD) for n = 4.

3.1.3 Diet Analysis

The results showed that the four day carbohydrate loading menus (CHO) and the reported normal mixed diet (NMD) intake of participants differed significantly on total kilocalorie intake (CHO Loading 2420 ± 234 kcal, NMD 1980 ± 397 kcal, p = 0.017), total grams of carbohydrate (CHO Loading 469 ± 42 grams, NMD 302 ± 53grams, p = 0.001) and fat intake (CHO Loading 35 ± 9 grams, NMD 53 ± 16 grams, p = 0.016). This change was anticipated due to the adjustments in calories for either under-reporters or to compensate for their energy expenditure with the depletion exercise routine. There were no significant differences in total grams of protein between normal mixed (NMD

71 ± 22 grams) and carbohydrate loading diets (CHO Loading 80 ± 12 grams). Results

are summarized in Table 3.4.

Table 3.4 Summary of Dietary Analysis for Normal Mixed Diet (NMD) and Carbohydrate Loading Menus (CHO L)

Units	NMD	CHO L
Kcals	1980 ± 397*	2420 ± 234
CHO g/day	302 ± 53*	469 ± 42
CHO g/kg	4.5 ± 0.7*	7.2 ± 0.4
% CHO	63%	78%
% Protein	15%	13%
% Fat	24%	13%

Note. All values are reported in means ± SD for 7 subjects. Kilocalories (Kcals);

Normal Mixed Diet (NMD); Carbohydrate (CHO) and Carbohydrate Loading (CHO L).

* Significant differences (p<0.05) found for kcals and CHO intake (total grams and

grams/kilogram body weight) and total fat intake between NMD and CHO L.

3.1.4 Performance Variables

Results of the performance run are depicted in Figure 3.1. A significant

interaction [F(1,6)= 14.01, p = 0.01] was found for performance time in relation to diet

and menstrual cycle phase (Table 3.5). A one way ANOVA of the absolute and relative

change in performance scores from NMD to CHO loading for each menstrual cycle

phase revealed a significant 35% increase (p = 0.16) in the performance time for the run

to volitional exhaustion at 80% VO_2max in the luteal phase. The timed run to

exhasution in the luteal phase (L) carbohydrate loading trial (L/CHO 12.09 ± 7.72

minutes) was significantly greater than the normal mixed diet (L/NMD 8.74 ± 5.10

minutes). Thus the CHO loading diet significantly improved performance in the luteal

phase. The change in performance scores in the follicular phase (F) between

carbohydrate loading (F/CHO 13.02 ± 9.7 minutes) and normal mixed diet (F/NMD

10.99 ± 7.31 minutes) was found to be an average of 11% (non-significant). This

increase in time to volitional exhaustion with CHO loading in the follicular phase was

not significantly different from the follicular phase NMD time to exhaustion.

Figure 3.1. Running time to exhaustion at 80% VO_2max after endurance exercise.
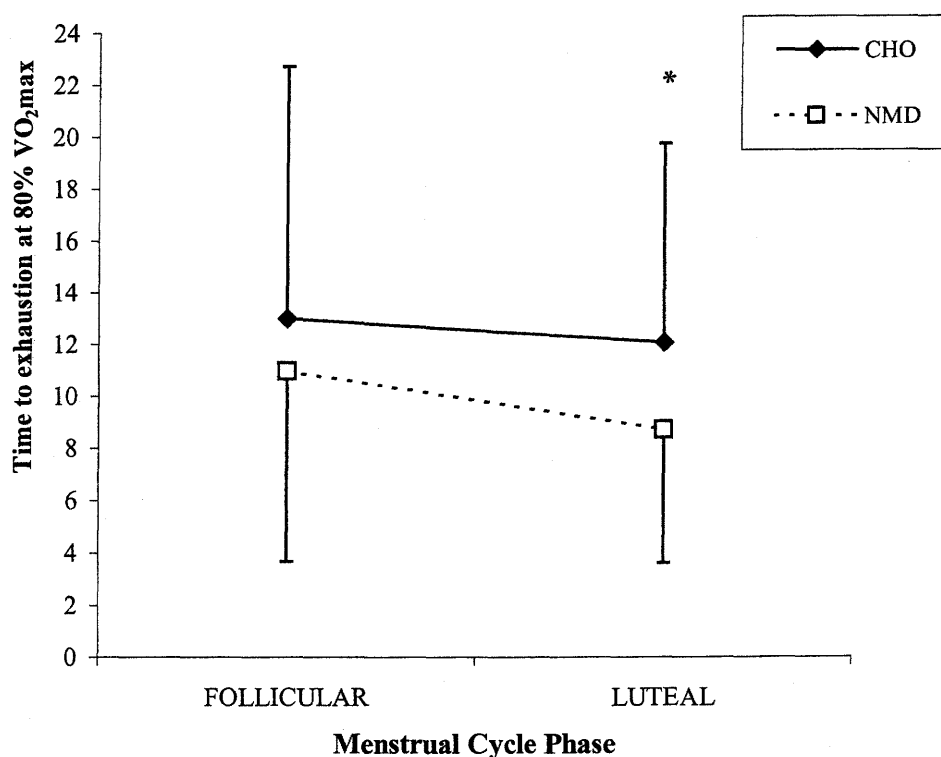
Note. Values are group means ± SD for 7 subjects. (*) Significant improvement in the luteal menstrual cycle phase with carbohydrate loading ($p<0.05$).

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3.1.5 Physiologic and Substrate Measures Analyses

There were no significant differences in VO_2 or heart rate among the four 60 minute running trials, or among the four timed runs to exhaustion at 80% VO_2max (Table 3.5). The average RER values were not significantly different for the high intensity run to exhaustion at 80% VO_2max between the four conditions (Table 3.5). However, a significant main effect for menstrual phase was found for RER during the 60 minute run at 70% VO_2max. RER values during the endurance runs at 70% VO_2max were significantly greater during the luteal menstrual cycle phase (0.926) compared to the follicular phase (0.915), irregardless of the diet intervention [$F(1,6)=19.20$, p $=0.005$] (Figure 3.1). Additionally, there was a significant difference in the means between the CHO loading and NMD conditions [$F(1,6) = 5.39$, p $= 0.059$]. More specifically, RER was significantly greater under the carbohydrate loading diet compared to the RER in the normal mixed diet condition (0.930 CHO loading vs 0.911 NMD), irrespective of menstrual cycle phase (Figure 3.2).

Also, there was a significant menstrual cycle phase difference in CHO oxidation, where the luteal phase oxidized more carbohydrate (2.48g/min) than the follicular phase (2.32 g/min) [$F(1,6)=10.05$, p $= 0.019$] (Figure 3.3 and Table 3.6). These findings suggest that the luteal phase utilized more carbohydrate than the follicular phase during prolonged moderate intensity exercise. Furthermore, estimated fat oxidation was significantly different for menstrual cycle phase [$F(1,6)= 15.584$, p$= 0.008$], where the follicular phase relied on a greater proportion of fat as fuel during the 60 minute run (0.34 g/min) compared to the luteal phase (0.29g/min) (Figure 3.4 and Table 3.6). Again, diet itself was found to significantly different between the CHO loading

intervention and NMD condition [$F(1,6) = 5.97$, $p = 0.05$], where the participants under the NMD condition oxidized more fat for fuel (0.36 g/min) than in the CHO loading diet (0.27 g/min).

Due to the low sample size (n = 4) and to missing data points within the four trial for all the blood variables there was insufficient data to run an ANOVA. The response patterns for blood glucose, and lactate values during the four trials at 70% VO$_2$max are summarized in Figures 3.5 and 3.6, respectively. As expected the blood glucose level initially increased with the onset of exercise in order to meet the energy demand of the working muscles. During the CHO loading diets blood glucose reached a plateau between 30 and 60 minutes, while in the NMD conditions blood glucose continued to rise until the termination of exercise. Blood lactate levels decreased with the onset of exercise, then proceeded to rise until the termination of exercise (60 minutes) under the CHO loading diet condition. Under NMD blood lactate levels increased with the onset of exercise to 30 minutes and then decreased in the luteal phase (NMD) a plateau occurred in the follicular phase (NMD). Blood lactate increases with normal mixed diet were on average less pronounced compared to the carbohydrate loading trials. The response pattern of the free fatty acids during exercise at 70%VO$_2$max in the four trials are summarized in Figure 3.7 and glycerol responses are summarized in Figure 3.8. There was a great amount of individual variation in free fatty acid and glycerol levels with the small sample. Free fatty acid levels increased with the initiation of exercise and continued to increase throughout the 60 minutes of exercise in all four trials. Glycerol levels rose in the luteal CHO loading trial until 30 minutes then decreased to pre-exercise levels by the end of the 60 - minute run. However, in the follicular CHO

loading trial glycerol declined with the onset of exercise until 30 minutes then rose to

near pre-exercise measures at 60 minutes. In the NMD condition glycerol increased

slightly in both follicular and luteal menstrual phases until 30 minutes. The glycerol

levels in the follicular phase continued the gradual rise until the end of exercise,

whereas, in the luteal phase it declined to baseline measures.

Figure 3.2. Respiratory Exchange Ratio (RER) values for 60 minute run at
70%VO$_2$max.
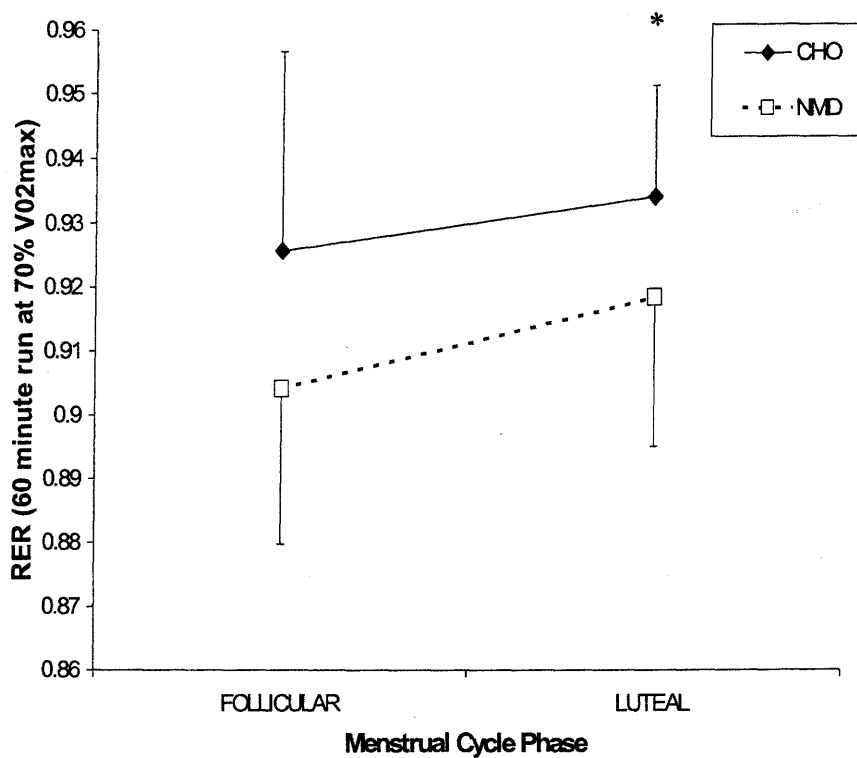
Note. Carbohydrate Loading (CHO); Normal mixed diet (NMD). Values are means $\pm$
SD for seven participants. (*) Significant menstrual cycle phase difference ($p<0.05$).

Table 3.5 Summary of Physiologic Measures During the Timed Run to Exhaustion at 80%VO$_2$max

	Mean ± SD	F ratios, P values MENSES	DIET	MENSES X DIET
Time to Exhaustion	F/CHO 13.02 ± 9.71 F/NMD 10.99 ± 7.31 L/CHO 12.09 ± 7.72 L/NMD 8.74 ± 5.01	F(1,6) =3.35, p = 0.117	F(1,6) = 6.601, p = 0.049	F(1,6) = 14.012, p = 0.01 *
RER	F/CHO 0.97 ± 0.053 F/NMD 0.95 ± 0.044 L/CHO 0.97 ± 0.040 L/NMD 0.95 ± 0.034	F(1,6) = 0.016, p = 0.904	F(1,6) = 1.604 p = 0.252	F(1,6) = 0.014, p = 0.910
VO$_2$ (ml/kg·min)	F/CHO 40.44 ± 3.33 F/NMD 40.66 ± 3.07 L/CHO 41.29 ± 2.81 L/NMD 41.07 ± 2.37	F(1,6) = 1.53, p = 0.262	F(1,6) = 0.00, p = 0.999	F(1,6) = 0.167, p = 0.697
Heart rate (bpm)	F/CHO 168.29 ± 11.58 F/NMD 163.00 ± 12.88 L/CHO 167.85 ± 11.42 L/NMD 168.00 ± 12.15	F(1,6) = 0.547, p = 0.487	F(1,6) = 3.25, p = 0.121	F(1,6) = 2.67, p = 0.153

Note. Values are group means ± SD for 7 subjects. Normal Mixed Diet (NMD); Carbohydrate Loading (CHO). * Significant difference. * Significant difference.

Table 3.6 Summary of Physiologic Measures During 60-minute run at 70% VO_2max

	Mean ± SD	F ratios, P values		
		MENSES	DIET	MENSES X DIET
RER	F/CHO 0.93 ± 0.031 F/NMD 0.90 ± 0.024 L/CHO 0.93 ± 0.017 L/NMD 0.92 ± 0.023	$F(1,6) = 19.20, p = 0.005*$	$F(1,6) = 5.39\ p = 0.059$	$F(1,6) = 0.168, p = 0.696$
CHO_{ox} (g/min)	F/CHO 2.42 ± 0.359 F/NMD 2.22 ± 0.333 L/CHO 2.57 ± 0.251 L/NMD 2.37 ± 0.279	$F(1,6) = 10.05, p = 0.019*$	$F(1,6) = 3.11\ p = 0.128$	$F(1,6) = 0.001, p = 0.980$
FAT_{ox} (g/min)	F/CHO 0.29 ± 0.12 F/NMD 0.38 ± 0.13 L/CHO 0.25 ± 0.09 L/NMD 0.33 ± 0.13	$F(1,6) = 15.58, p = 0.008*$	$F(1,6) = 5.97, p = 0.05$	$F(1,6) = 0.05, p = 0.830$
VO_2 (ml/kg min)	F/CHO 35.58 ± 2.42 F/NMD 36.99 ± 2.32 L/CHO 36.23 ± 2.27 L/NMD 36.16 ± 1.80	$F(1,6) = 0.41, p = 0.841$	$F(1,6) = 1.35, p = 0.289$	$F(1,6) = 2.13, p = 0.198$
Heart rate (bpm)	F/CHO 155.71 ± 12.77 F/NMD 156.14 ± 15.61 L/CHO 157.85 ± 13.69 L/NMD 155.14 ± 13.68	$F(1,6) = 0.094, p = 0.769$	$F(1,6) = 0.408\ p = 0.546$	$F(1,6) = 0.551, p = 0.486$

Note. Values are group means ± SD for 7 subjects. Normal Mixed Diet (NMD); Carbohydrate Loading (CHO). * Significant difference. * Significant difference.

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Figure 3.3. Estimated Carbohydrate Oxidation values (grams/minute) during 60 minute run at 70%VO2max.
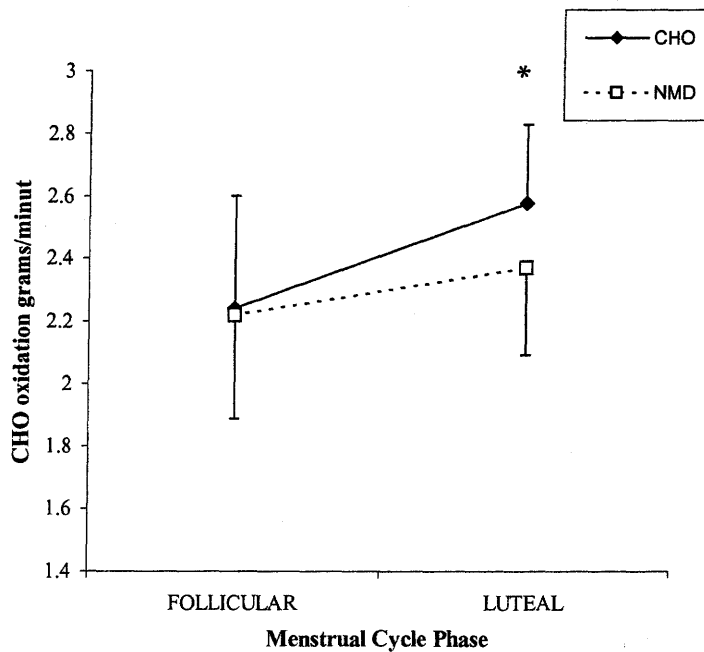
Note. Values are means ± SD for seven participants. (*) Significant menstrual cycle phase difference ($p<0.05$).

Figure 3.4. Estimated Fat Oxidation values (grams/minute) during 60 minute treadmill run at 70%VO2max.

Note. Values are means ± SD for seven participants. (*) Significant menstrual cycle phase difference ($p<0.05$).

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Figure 3.5 Blood glucose values during 60 minute run at 70%VO$_2$max
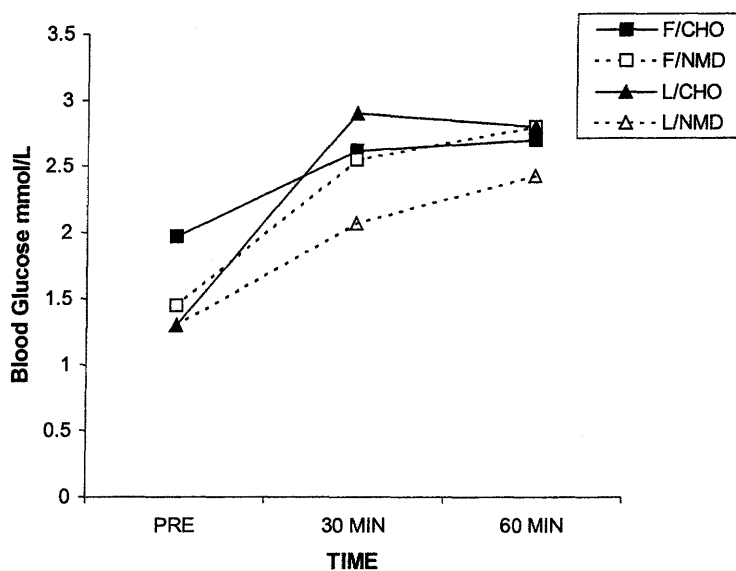
Note. All values are means for four participants. Time intervals: Pre-exercise (pre), 30 minutes (30min), 60 minutes (60min).

Figure 3.6. Blood lactate values for 60 minute run at 70% VO$_2$max.
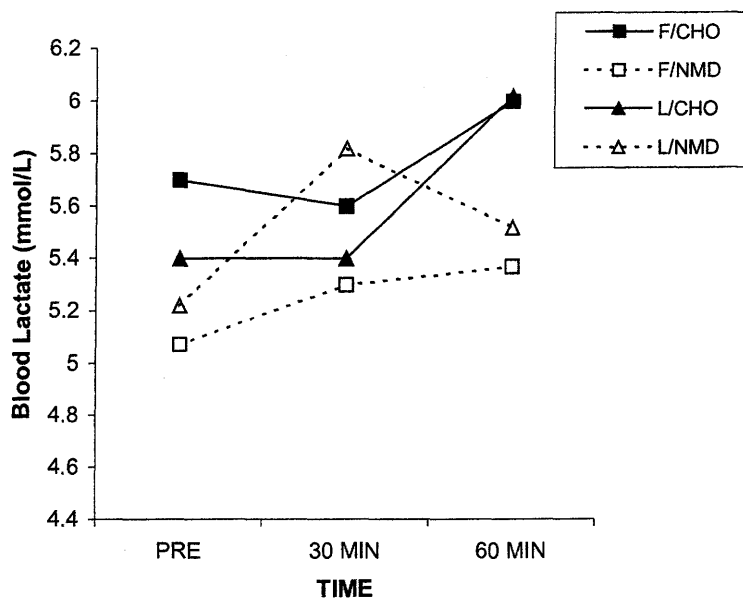
Note. All values are means for four participants. Time intervals: Pre-exercise (pre), 30 minutes (30min), 60 minutes (60min).

Figure 3.7. Free fatty acid values during the 60 minute run at 70% VO$_2$max.
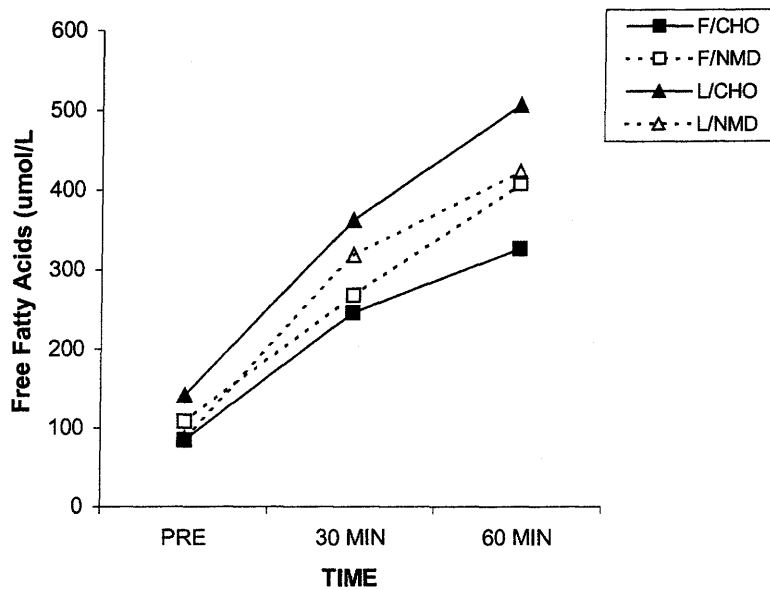
Note. All values are means for four participants. Time intervals: Pre-exercise (pre), 30 minutes (30min), 60 minutes (60min).

Figure 3.8. Glycerol values for the 60 minute run at 70%VO$_2$max.
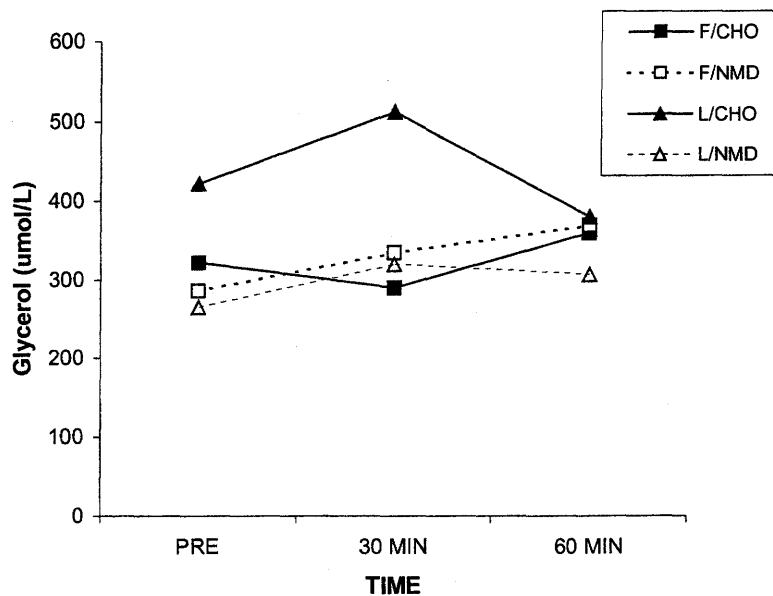
Note. All values are means for four participants. Time intervals: Pre-exercise (pre), 30 minutes (30min), 60 minutes (60min).

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3.2 Discussion

The present investigation examined the effect of carbohydrate loading (a high carbohydrate diet and exercise taper) on exercise performance and substrate utilization, in both the follicular and luteal menstrual cycle phases, in moderately trained females. The results of this study revealed a significant interaction between menstrual cycle phase and CHO loading for exercise performance (time to exhaustion at 80% VO_2max; $F(1,6) = 14.012$, $p = 0.01$). CHO loading in the luteal menstrual cycle phase significantly improved exercise performance by an average of 35% (time to exhaustion at 80% VO_2max) over performance with NMD in the luteal phase. A smaller benefit to exercise performance was observed when the women CHO loaded in the follicular menstrual cycle phase compared to performance with NMD in the follicular phase (11% increase). The results of the present study also revealed a significant menstrual cycle phase difference in substrate utilization, for RER [$F(1,6) = 19.20$, $p = 0.005$], CHO oxidation [$F(1,6) = 10.05$, $p = 0.019$] and fat oxidation [$F(1,6) = 15.58$, $p = 0.008$], irrespective of diet intervention. RER and carbohydrate oxidation values were greater in the luteal phase than in the follicular phase, for the same diet intervention. Also, RER values were greater with carbohydrate loading compared to the normal mixed diet in both menstrual cycle phases. Conversely, the fat oxidation values were greater in the follicular menstrual cycle phase than in the luteal phase for the same diet intervention, and they were also greater in the normal mixed diet condition compared to the carbohydrate loading condition.

Recently, a few well-controlled trials have established that with sufficient total energy intake, and a high carbohydrate diet/exercise taper regimen (CHO loading), females are able to increase muscle glycogen stores in both the follicular and luteal menstrual cycle phases (Walker et al., 2000; James et al., 2001; Paul et al., 2001; Tarnopolsky et al., 2001). However, only two of the studies above have investigated the effect of carbohydrate loading on exercise performance, and each of these studies have examined a single menstrual cycle phase (Walker et al., 2000; Paul et al., 2001). The present study is thought to be the first to investigate the effect of carbohydrate loading on performance in both menstrual cycle phases, in the same female participants.

The direction of the performance changes with CHO loading in both the follicular phase and luteal menstrual cycle phases are similar to previously reported results from the single menstrual cycle phase investigations (Walker et al., 2000; Paul et al., 2001). Walker et al.(2000) found a significant increase (8%) in exercise performance with CHO loading in the luteal menstrual cycle phase, over NMD in the luteal phase; while Paul et al., (2001), revealed that CHO loading did not enhance exercise performance over NMD in the follicular menstrual cycle phase. However, as this study examined both menstrual cycle phases, it is important to note that baseline exercise performances (NMD) in each menstrual cycle phase were different. Therefore the absolute performance results for the CHO loading intervention, in each menstrual cycle phase, should be cautiously compared to one another.

Interestingly, our performance increase with CHO loading in the luteal phase (35%) is larger than that found by Walker and colleagues (2000) (8%). The larger performance benefit in our study occurred despite utilizing a lower level of dietary CHO

at only 7.1g/kg body weight (range, 6.5 – 7.9g/kg body weight), compared to 8.2g/kg body weight in the study by Walker et al., (2000). The difference in the change for exercise performance with carbohydrate loading in the luteal phase of this study, compared to that of Walker et al., (2000), is more likely a result of the different exercise testing protocols, rather than a reflection of the dietary CHO intake.

The present study employed a 60 - minute fixed intensity exercise bout ($70\%VO_{2max}$) followed by a high intensity time trial ($80\%VO_{2max}$). In men, a similar exercise testing protocol (60 minute fixed intensity exercise at $75\%VO_2max$, followed by a high intensity time trial to exhaustion at 85% VO_2max) resulted in a large (45%) increase in performance with CHO loading (Tarnopolsky et al., 1995). Walker et al.(2000) used a 90 - minute steady state exercise testing protocol. In men, generally up to a 20% increase in exercise performance is found using steady state exercise protocols (≥ 90 minutes) with carbohydrate loading (Hawley, Schabort, Noakes & Dennis, 1997b). Thus, in men the degree in the magnitude of performance changes depends on the exercise testing protocol chosen (Tarnopolsky et al., 1995; Hawley et al., 1997b). Therefore, the different exercise testing protocol chosen in this study could account in part for the larger performance increases seen in the present study than in those seen in the investigation by Walker et al. (2000).

A major limitation to the interpretation of the performance results in the present study is that muscle glycogen, pre- and, post- exercise, was not measured to ensure that, with the 23% increase in total energy and the dietary carbohydrate intake of 7.1g / kg body weight (or >450g/day total CHO intake), the participants did indeed super-compensate muscle glycogen stores in both phases. In our study, the performance results

would lend themselves to the assumption that muscle glycogen stores were increased with the carbohydrate loading routine in the luteal menstrual cycle phase. However, it is also plausible that the positive performance changes in the luteal phase with carbohydrate loading were the result of higher hepatic glycogen stores as liver glycogen stores are very responsive to diet changes (Hultman & Nilsson, 1971). For example, in a 70 kg person, the liver glycogen stores provide typically 490 mmol (70g) glycogen but can increase up to 900 mmol (128g) with a high carbohydrate diet (Sherman & Wimer, 1991). This might provide between 200 – 500 kcal of energy from liver glycogen stores alone (Bonen, McDermott, & Hubtner, 1989). Theoretically, liver glycogen may contribute a substantial portion of the energy needed for our modified testing protocol. Thus, the usefulness of RER in this context is limited as it cannot distinguish between the contributions of these body stores of glycogen (Hultman & Nilsson, 1971). This is a major limitation in the present study and prevents any conclusive statements regarding the effect of carbohydrate loading on muscle glycogen stores in this study.

The results of this study also revealed a significant menstrual cycle phase difference in substrate utilization. In general during the luteal phase, RER and carbohydrate oxidation values were greater than in the follicular phase, signifying a greater reliance on carbohydrate as fuel during exercise in the luteal phase. At this time, there are no known studies that have compared substrate utilization measured by RER and/or carbohydrate or fat oxidation with carbohydrate loading, in both menstrual cycle phases. Moreover, it must be noted that the RER and oxidation values were also significantly influenced by diet, and the RER values become more alike under the carbohydrate loading condition within each menstrual cycle phase. During the luteal

phase, the findings of increased RER and carbohydrate oxidation values with carbohydrate loading are supported by Walker et al.(2000), who also reported higher RER and carbohydrate oxidation values with CHO loading in the luteal phase over normal mixed diet, during steady state exercise. However, both Tarnopolsky et al.(1995) and Paul et al. (2001), reported no difference in RER with carbohydrate loading compared to normal mixed diet, in the follicular phase. Furthermore in the present study, fat oxidation was greater in the follicular phase, than in the luteal phase, though again this measure was also influenced by diet. Whitley et al.(1998) also found an attenuated rate fat oxidation with a high carbohydrate diet which lead to a greater reliance on glycogen stores during exercise, though the study did not look at menstrual cycle phase differences.

In contrast to our finding of greater RER and carbohydrate oxidation in the luteal phase (irrespective of diet intervention), Hackney (1990; 1999) reported greater lipid oxidation at rest and during exercise (70%VO_2max) in the luteal phase with NMD than in the follicular phase, in both trained and untrained women. Also, Nicklas et al., (1989) found lower RER values in the luteal phase over the follicular phase (NMD) during prolonged exercise (70%VO_2max). Hackney, McCracken-Compton and Ainsworth (1994) reported, that during low – moderate intensity exercise (35 – 65% VO_2max) that lipid utilization and oxidation were significantly greater in the midluteal phase compared to the midfollicular phase, but there were no significant differences at 75%VO_2max. Plausible explanations for the discrepancy in RER values for the luteal phase (normal mixed diet) reported in the current study compared with the previous work discussed above, may be attributed to timing or type of dietary intake, timing of

measurements within a given menstrual cycle phase (e.g. early, mid or late), and possibly the inclusion of oral contraceptives users in this study.

The timing and type of dietary intake prior to exercise is known to influence RER values (Bergstrom et al., 1967; Bonen et al., 1983). For example, an investigation by Bergstrom et al. (1967) reported that after consuming a high carbohydrate meal prior to exercise, blood glucose and RER values during prolonged exercise were higher than after consuming a low carbohydrate meal (Bergstrom et al., 1967). In the present study participants consumed a pre-determined light meal 2-3 hours, primarily of CHO (80-97% Ein from CHO) before testing in both control and intervention trials. For example, a banana + cup of orange juice, or a half banana + ½ cup of rice krispies + ½ cup skim milk were common breakfast choices on the menu. This exogenous source of carbohydrate prior to exercise may have lead to slightly higher RER values compared to the investigations by Hackney (1990; 1999) and Nicklas et al., (1989) where subjects were tested in a fasted state, which typically promotes greater lipolysis (Bonen et al., 1983). Moreover, Bonen et al. (1983) found that a pre-exercise glucose load given to moderately trained females significantly decreased FFA utilization in the luteal phase, compared to the follicular phase, suggesting that the pre-exercise meal (given in both NMD and CHO Loading trials) itself may have influenced the greater RER findings in the luteal phase in this study.

Also, the differences in substrate utilization in the present study, compared to previous investigations in the luteal phase, may be a result of the unexpected variations in progesterone levels found in the mid-late luteal phase testing in the current study, and/or the inclusion of participants taking oral contraceptives. The level of sex-steroid

hormones, both estrogen and progesterone, fluctuates throughout a normal menstrual cycle (see Figure 1.1). The mid - luteal phase is characterized by the highest levels of both hormones, while towards the end of the luteal phase, a decline in both hormones to baseline levels is expected, signaling the near onset of the menses (Guyton and Hall, 1996). The hormonal analyses in this study revealed there was a great deal of intra- and inter-individual variation in the level of progesterone found in the mid-late luteal phase (Table 3.3). The progesterone level of the three participants on low dose oral contraceptives were expected to be low (2.2 – 2.6 nmol/L), but a fourth participant (no oral contraceptive use) was also found to have a very low progesterone level (2.6 nmol/L). This may have been the result of an anovulatory cycle, or perhaps a shortened luteal phase. Unfortunately, the progesterone levels of the other five participants in this study could not be obtained due to financial restrictions. However, according to Bunt (1990) this variation in a single hormone can be expected if testing over more than one menstrual cycle. This variation in hormone levels from cycle to cycle has potential to skew the interpretation of the data in relation to RER, oxidation or blood substrate utilization.

In general, the greater RER and high carbohydrate oxidation findings in the mid-late luteal phase of the present study are not supported by previous investigations in females, with or without oral contraceptives (Nicklas et al., 1989; Bonen et al., 1991; Bemben et al., 1992; Kanaley et al., 1992; Hackney et al., 1994). The majority of studies reviewed found either no differences in substrate utilization and RER values throughout the menstrual cycle phases or found greater lipid utilization and lower RER values in the luteal phase. For example, Kanaley et al., (1992) found that there were no menstrual

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cycle phase differences in substrate utilization or RER during 90 minutes of exercise at 60 % VO_2max in either trained eumenorrheic or amenorrheic females in either the early, or late follicular, or mid-luteal menstrual cycle phases. However, Bemben et al.(1992) found that oral contraceptive users utilized less carbohydrate and more lipid during 90 minutes of moderate intensity exercise in either early or mid luteal phase. Bonen et al., (1991) reported that during mild exercise (50%VO_2max), substrate utilization patterns (glucose, lactate, free fatty acids, glycerol) were not altered by menstrual cycle phase or oral contraceptive usage, though the oral contraceptive groups utilized more free fatty acids and less glucose than the non-oral contraceptive users (Bonen et al., 1991). Nicklas, Hackney and Sharp (1989), found that during prolonged exercise at 70%VO_2max in moderately trained individuals, RER values were lower in the mid-luteal phase than in the mid-follicular phase, and concluded that females oxidized more lipid in the mid- luteal phase compared to mid-follicular phase. Also, Hackney et al., (1994) found greater lipid oxidation and lower RER in the mid-luteal phase during exercise at 35-65%VO_2max compared to the mid-follicular phase. Moreover, they reported no differences at 75%VO_2max. The studies reviewed above suggest that, during low to moderate intensity exercise, there is greater fat oxidation in the luteal phase, but that at higher exercise intensities (75% VO_2max and up) there is no difference. Nonetheless, the above studies tested their subjects in a fasted state, which may account for the differences compared to the present study where subjects were tested in a fed state.

The observed performance changes and higher carbohydrate oxidation during exercise in the luteal menstrual cycle phase could occur for several potential reasons. In the luteal phase both estrogen and progesterone are elevated compared to primarily estrogen in the follicular phase (Guyton & Hall, 1996). This change in hormone milieu may account for the differences in substrate utilization and exercise performance observed between the two menstrual cycle phases. Progesterone was shown to antagonize the glycogen sparing actions of estrogen (increased lipolysis) in cardiac and skeletal muscle tissue in rats (Matute & Kalkhoff, 1973; Kendrick et al., 1987; Kendrick & Ellis, 1991; Bunt, 1990). Thus, the combination of progesterone and estrogen may promote a greater reliance on carbohydrate as substrate during exercise as evidenced by greater RER values in the luteal menstrual cycle phase compared to the follicular phase (estrogen alone). Whether this interference with the estrogen's glycogen sparing effect occurred in the muscle or liver cannot be determined from our indirect measures.

An alternate explanation may be that elevated progesterone levels during the luteal phase promoted greater protein catabolism and net protein utilization (Lebrun, 1993) as it is considered to have an overall catabolic effect on the body (Guyton & Hall, 1996). The increased protein utilization as energy (i.e., gluconeogenesis) may have contributed to the changes in RER and CHO oxidation values found in the luteal menstrual cycle phase of our study. A recent review by Tarnopolsky (2000) suggests that women oxidize less protein (1.6%) in the follicular phase than similarly trained men (2.8%) during moderate intensity endurance exercise. However, the area of protein utilization between the menstrual cycle phases has not been extensively explored.

The blood substrate findings observed in the present study can neither support, nor refute, the evidence for differences in substrate utilization between the menstrual cycle phases, or diet intake (CHO vs NMD) during the 60 minute run at 70% VO_2max. Admittedly, the interpretation of systemic substrates in relation to muscle metabolism is limited in the current study as there were a number of missing data points, which resulted from samples that could not be analyzed by the lab. Also, from the available data we cannot distinguish the rate of appearance or disappearance of these blood substrates during exercise progression, which limits our ability to draw any conclusions about how the substrate was utilized during exercise (Friedlander, Casazza, Horning, Huie, & Brooks, 1997). It is recognized that substrate turnover (i.e., isotopic tracers) would have provided a clearer picture of blood substrate utilization and is recommended for any future research in this area. Finally, both the small sample size and large degree of variation in "normal" blood substrate values also may have contributed to the lack of sensitivity of the aforementioned measures to detect substrate changes between the diet/exercise intervention, or menstrual cycle phase.

Another limitation of the study is that estrogen levels were not measured along with pre-exercise progesterone values. Bunt (1990) stated that menstrual status cannot be clearly established through single or even serial hormone assays of one sex-steroid. Instead, the ratio of estrogen/progesterone may be equally important, particularly in their effect on substrate metabolism (Bunt, 1990). This may be one plausible explanation of why, despite the great divergence of progesterone values, all participant responses were in the same direction.

It is interesting to note that in the present study under normal mixed diet conditions, exercise performance in the luteal phase looks as if it was worse than the follicular phase for six out of seven participants, though this effect was not as pronounced for those taking oral contraceptives. A number of potential mechanisms may explain this poor performance in the luteal phase. The 10 - fold increase in progesterone (P_4) during the luteal phase may alter the complex interaction of the aldosterone renin-angiotensin system and lead to net fluid retention and minor variations in serum electrolytes, osmolalitiy and hemoglobin (Lebrun, 1993). Some investigations have suggested that luteal phase plasma volume changes result in altered heart rate and body core temperature up to 0.6 degrees Celsius, thus increasing cardiovascular strain and leading to higher perceived exertion during exercise, but these studies were unable to demonstrate significant performance changes (Pivarnik et al., 1992; Schoene et al., 1981). In addition, during the luteal phase there is some evidence to suggest that there is an increase in ventilatory drive (increased VE/VO_2) causing mild hyperventilation (Schoene et al., 1981). Progesterone is thought to mediate ventilatory drive through a central neural mechanism, which in turn, affects the output of the respiratory system (Schoene et al., 1981). However, a great deal of variation among individuals from month to month was noted in eumenorrheic athletic women (Schoene et al., 1981). However, these differences in exercise performance between menstrual cycle phases are not supported by all the literature in this area (Lebrun, 1993).

The present study found that carbohydrate loading produced an 11% increase in time to exhaustion during a high intensity run in the follicular menstrual cycle phase. A previous investigation by Tarnopolsky et al. (1995) also found a non-significant

performance increase (5%) in the follicular phase with a similar carbohydrate loading and test protocol. From a performance perspective, an 11% difference may be substantial. For example, during the 2001 Boston Marathon, there was a 9% difference (2:23:58 to 2:40:00 minutes) between the 1st and 16th place winners in the women's marathon. Thus, the effectiveness of carbohydrate loading in the follicular phase should not be completely discounted.

CHAPTER 4

SUMMARY AND CONCLUSIONS

4.1 Summary

Female participation in athletics has increased significantly in recent years. This phenomenon may be best illustrated by the female presence on the recent (2002) winter Olympic podium in Salt Lake City, USA, where Canadians witnessed medal achievements by females in speed skating, cross - country skiing, ski aerials, curling and hockey. However, the scientific community offers few gender specific recommendations in regards to nutrition, or training, to female athletes. Carbohydrate loading is one ergogenic technique that is commonly recommended to both male and female endurance athletes, despite equivocal evidence of performance improvement in different menstrual cycle phases (Tarnopolsky et al., 1995; Walker et al., 2000; Paul et al., 2001).

The purpose of this study was to examine and compare the effects of carbohydrate loading on running performance, and selected physiologic and substrate variables (i.e., RER, blood glucose, lactate, free fatty acids and glycerol), in moderately trained females during both the follicular and luteal menstrual cycle

phases. The major hypotheses were that:

1) Carbohydrate loading in the luteal menstrual cycle phase would increase time to exhaustion during a high intensity treadmill run (preceded by a moderate intensity endurance run) compared to exercise performance under normal mixed diet conditions

2) Carbohydrate loading would not significantly improve time to exhaustion in the follicular menstrual cycle phase over performance with normal mixed diet in the follicular phase.

3) RER and carbohydrate oxidation values would be greater in the luteal menstrual cycle phase over the follicular phase and would also be greater with carbohydrate loading compared to the normal mixed diet, during the 60 minute run at 70% VO_2max.

Also, the secondary hypotheses predicted during the moderate intensity exercise that the luteal phase blood glucose would increase, and blood lactate levels increase to a greater extent in the luteal phase than the follicular phase. Also, in the follicular phase it was predicted that glycerol and free fatty acids would be greater than in the luteal phase. Finally, it was predicted that during the moderate intensity exercise (70% VO_2max) blood glucose would increase to a greater extent and blood lactate levels increase with carbohydrate loading compared to NMD, and that under NMD there would be greater glycerol and free fatty acids levels.

The results revealed that the luteal phase responded positively to carbohydrate loading increasing time to exhaustion by an average of 35% over the time to exhaustion under normal mixed diet treatment in the same menstrual cycle

phase. Also, RER, and carbohydrate oxidation were greater in the luteal phase compared to the follicular phase. This would suggest a greater reliance on carbohydrate as substrate for endurance exercise at 70% VO_2max in the luteal phase. Furthermore, though no group differences were examined by our statistical analysis, an observation was made from the raw data, which may suggest that performance and RER/oxidation responses were blunted with oral contraceptives. Also, it was observed that there was a trend towards differences in RER and oxidation values with diet treatment in the follicular phase. Finally, it was noted that the participants performed better in the follicular phase than in the luteal menstrual cycle phase with normal mixed diet. This difference in baseline performance between the menstrual cycle phase with normal mixed diet was not predicted. Moreover, the additional dietary and exercise changes with carbohydrate loading in the luteal phase improved exercise performance only to the point where it matched performance in the follicular phase.

Major limitations to this study include small sample size, lack of confirmation of glycogen super-compensation and utilizing those on oral contraceptives in the group analysis. Power calculations were performed and it was determined that approximately 50 or more subjects would be necessary to reach significance for the blood substrate variables. It is likely that sample size has affected some of the observations and there is potential for a type II error.

4.2 Conclusions

According to the results from this study, the following conclusions can be made:

1. Carbohydrate loading is an effective ergogenic aid in the luteal menstrual cycle phase.

2. Performance in the follicular menstrual cycle phase (11%) is less responsive to carbohydrate loading than the in the luteal phase (35%).

3. A menstrual cycle phase difference was found for RER and oxidation values. The luteal menstrual cycle phase RER and carbohydrate oxidation values were significantly greater, during moderate intensity endurance exercise compared to the follicular phase, where greater fat oxidation was evident.

4. Diet independently influences RER and oxidation values. A high carbohydrate diet promotes greater RER values, which reflects the greater proportion of carbohydrate utilized and the attenuated fat oxidation, during steady state exercise.

5. Menstrual cycle phase, diet intervention, or their interaction had no significant influence on VO_2 or heart rate in this study with either moderate intensity or high intensity exercise.

4.3 Recommendations for Future Research

In the present study, it was demonstrated that a carbohydrate loading regime might offer a performance advantage to females in the luteal menstrual cycle phase over a normal mixed diet in the same phase. The present study monitored training status of the participants, habitual diet intake, controlled for menstrual cycle phase

and timing of testing (to control for diurnal variations in hormone levels). Also, the caloric intake of the carbohydrate loading menus was adjusted to ensure the participants were eucaloric, during the extra exercise training. However, tighter controls and different methodology would have strengthened the findings in this study. The following are recommendations for future research in this area:

1. A sample size of 50 or more subjects would be necessary for most variables in this type of study to achieve adequate statistical power (60%) with alpha set at $p < 0.10$.

2. The menstrual cycle of participants should be monitored in a number of different ways (basal temperature changes, luteinizing hormone, or serum hormone levels of both hormones at rest) as mentioned in a review on female hormone status by Bunt (1990). Also, using these monitoring tools two to three months in advance of interventions (Hackney et al., 1994) would assist the investigator in understanding each participants' fluctuating hormone levels and would likely reveal any inconsistent patterns in ovulation or a shortened luteal phase that may exist in highly trained female athletes.

3. The use of basal thermometers to detect ovulation.

4. Monitoring blood levels of both estrogen and progesterone levels throughout the menstrual cycle in all participants would provide a clearer picture of their hormonal status and would assist with determining critical testing days.

5. Improve dietary controls by having participants replicate their normal mixed diet records in both NMD trials and monitor dietary intake for both treatment and

control (normal mixed diet) in a laboratory setting or by providing subjects with actual food hampers.

6. Improve exercise controls by having the participants replicate precisely their usual training routine in each NMD trial for one to three days prior to testing.

7. Some participants were thought to be under-reporting dietary intake for NMD. This could indicate restrictive eating behaviors related to eating disorders or body image dysmorphia, thus screening participants using a standardized tool prior to participation is recommended.

8. The addition of a muscle biopsy to determine muscle glycogen changes before and after endurance exercise at 70% VO$_2$max would assist with evaluation substrate metabolism and effect of carbohydrate loading diet in each menstrual cycle phase.

9. The use of isotopic tracers would enhance the ability to detect minor changes in substrate utilization versus standard blood analysis.

10. Protein utilization needs to be determined in both menstrual cycle phases.

11. Study participants taking exogenous hormones (oral contraceptives) separately from those under their own endogenous regulation.

One future research question that arose from this study, is why performance was better in the follicular menstrual cycle phase under normal mixed diet conditions than in the luteal menstrual cycle phase. Also, the results from the present study represent only the mid-late follicular and mid-late luteal menstrual cycle phases and those using triphasic oral contraceptives. Future research in this area may also want

to investigate the specific estrogen and progesterone ratios throughout different time points in the menstrual cycle phases (i.e., early, mid, or late phases), or investigate different exogenous hormonal preparations (oral contraceptives) and their influence on carbohydrate loading performance and substrate utilization.

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APPENDICES

APPENDIX A

RECRUITMENT LETTER

The Effect of Carbohydrate Loading in
Female Endurance Athletes

This study will investigate the response of the female endurance athlete to carbohydrate loading during both phases of the menstrual cycle. As we know it, carbohydrate loading have been positively linked to increased endurance performance. To date this knowledge has been developed on the basis of data collected using primarily male subjects. Recent research shows that the males and females respond differently to carbohydrate loading. Also, these gender studies have focused on female athletes in primarily one phase of their menstrual cycle. Our purpose is to determine the response to carbohydrate loading during both phases of the menstrual cycle.

Benefits to the participants:
1) VO$_2$max test
2) Full anthopometric measurements (including skinfold measurements)
3) Complete nutrition analysis by a registered dietitian
4) Ability to test your personal response to carbohydrate loading in a supervised environment before competition

All information you volunteer for the purpose of recruitment for this study will be considered confidential. The tentative start dates are February, 22-26, or March 8-12.

Participants will be required to adopt a high carbohydrate diet for a week and to modify their exercise routine (running only) according to the protocol below (see carbohydrate loading regimen below). A registered dietitian will be involved in determining the parameters of the high carbohydrate diet for each participant. On the test days an endurance run and a performance test will occur where we will be measuring a number of different experimental variables. Some of the experimental variables will be measured from blood samples to be taken during the endurance run. This entire process will be completed *twice* by each participant; once in the *follicular phase*, and once in the *luteal phase* of the menstrual cycle.

The estimated time commitment for each participant will be:
i)   One hour for initial VO2max and descriptive measurements (Kinesiology lab, University of Saskatchewan.
ii)   Two non-consecutive weeks for the carbohydrate loading regimens
iii)   Three hours for each endurance run and performance tests, and measurements (Kinesiology Lab, U of S).

The actual co-ordination of these events will depend on your personal schedules and the availability of the testers. Please contact me at   with questions, or I can be reached by phone at 966-2688 (U of S) or   (cell).

Example of the Modified Carbohydrate Loading Regimen:

Modified Carbohydrate Loading Regimen:

<u>**Day 1**</u>
High carbohydrate Diet (eucaloric menu + carbohydrate supplement totaling 8-9g carbohydrate/kg body weight)
Exercise (moderate intensity, long duration)

<u>**Day 2**</u>
High Carbohydrate diet
Exercise (moderate intensity, moderate duration)

<u>**Day 3**</u>
High carbohydrate diet
Exercise (moderate intensity, short duration)

<u>**Day 4**</u>
High carbohydrate diet
Rest

<u>**Day 5**</u>
Test Protocol:
Endurance Run and Performance Test

APPENDIX B

CONSENT FORM
and
ETHICS CERTIFICATE OF APPROVAL

CONSENT FORM

Title of Study:
The Effect of the Follicular and Luteal Menstrual Cycle Phases on Glycogen Super Compensation (Carbohydrate Loading) in Female Endurance Athletes

Names of Researchers:

Christina Glassford
College of Kinesiology at the University of Saskatchewan
Phone: 966-2688 or

Karen Chad
College of Kinesiology at the University of Saskatchewan
Phone: 966-6511

The purpose of the study:
To examine the influence of menstrual cycle phase on substrate utilization during endurance exercise when practicing glycogen super-compensation (carbohydrate loading).

The possible benefits of the study to the participants include:
1) Assessment of your fitness level, body composition and nutritional status

2) In addition you will see how carbohydrate loading will affect your performance
     in a supervised setting.

The procedures of the study are as follows:

On the first day of the study you will be required to report to the Kinesiology Laboratory for measurement of initial maximal oxygen consumption (a VO_2max test), body composition (sum of seven skinfolds, height, and weight), self-report of menstrual cycle length and birth control use, and a self-report of current endurance training. On this same day you will be asked to begin keeping a 3-day food record which will be used to determine your normal energy intake and will assist a dietitian in developing a high carbohydrate menu. A personalized high carbohydrate menu will be set for you to follow during the carbohydrate loading phase of this study. You will be required to follow the menu precisely and refrain from drinking caffeinated

beverages during carbohydrate loading period. The menu will consist of foods that you normally eat and a high carbohydrate supplement.

Once your menstrual cycle history has been determined you will be asked to complete a carbohydrate loading trial (Days 1-4) and exercise performance trial (Day 5), during two non-consecutive weeks. One carbohydrate loading trial will correspond with your follicular menstrual cycle phase and the with the luteal menstrual cycle phase.

The carbohydrate loading trials will consist of adopting a high carbohydrate diet and treadmill running as outlined below.

Modified Carbohydrate Loading Regimen:
Day 1
High carbohydrate Diet (eucaloric menu + carbohydrate supplement totaling 8-9g carbohydrate/kg body weight)
90 minute treadmill run at 65% VO2max

Day 2
High Carbohydrate diet
60 minute treadmill run at 65% VO2max

Day 3
High carbohydrate diet
30 minute treadmill run at 65% VO2max

Day 4
High carbohydrate diet
Rest

Day 5
Test Protocol:
60 minute treadmill run at 75% VO2max, 30 minute rest
Treadmill run to volitional fatigue at 85% VO2max

Heart rate will be the only measurement during the treadmill runs on Days1-3.

Measurements during the test protocol (Day 5) will include respiratory exchange ratio measured using a metabolic cart and various blood variables (plasma glucose, plasma insulin, plasma free fatty acids, plasma glycerol, blood lactate, hematocrit and hemoglobin and serum progesterone level in the luteal phase only) drawn from a saline lock inserted into the non-dominant hand. The blood will be drawn pre-exercise 15 minutes, during exercise (30 and 60 minutes) and post-exercise 30 minutes. An estimated total of 50 ml of blood will be drawn.

Potential Risks of the study:

You may experience discomfort during the run due to the saline lock. Bruising in the hand area may occur during the insertion of the saline lock needle.

Risks of carbohydrate loading include intestinal cramping and discomfort from the high carbohydrate intake may occur and possibly muscle soreness from the high intensity endurance runs.

There may be unforeseen risks during the study and after it is completed.

You are free to withdraw from the study at anytime and this withdrawal will not affect your access to services at the university or at the hospital.

All data received and recorded will be kept completely confidential and stored in locked filing cabinets at the College of Kinesiology. Data will be used to complete a thesis project for a Masters of Science degree and may be published in a journal article. Only group (aggregate) data will be reported. Your identify will remain confidential.

You will be advised of any changes in procedures that will have a bearing on your decision to continue in the study.

You will be given feedback on your results at the end of the study.

If you have any questions in regard to the study they should be directed to Christina Glassford at 966-2688 or   6, or Dr. Karen Chad at 966-6511.

I acknowledge that the study and contents of the consent have been explained to me, that I understand the contents, and that I have received a copy of the consent for my own records.

Subjects' Signature _____

Researchers Signature_____

Witness _____

Date _____

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC#
K. Chad	Kinesiology	1999-75

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT

College of Kinesiology

CO-INVESTIGATORS

C. Glassford

SPONSORING AGENCIES

None

TITLE:

The Effect of the Follicular and Luteal Menstrual Cycle Phases on Glycogen Super-Compensation in Female Endurance Athletes

APPROVAL DATE	TERM (YEARS)	AMENDED:	MODIFICATION OF:
April 22, 1999	3		

CERTIFICATION:

> The protocol and consent form (if applicable) for the above-named project have been reviewed by the Committee and the experimental procedures were found to be acceptable on ethical grounds for research involving human subjects.

APPROVED.

H.E. Emson MA MD FRCPC
Chair
University Advisory Committee on
Ethics in Human Experimentation

This Certificate of Approval is valid for the above term
provided there is no change in experimental procedures,
subject to annual re-approval.

Please send all correspondence to:

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University of Saskatchewan
Room 210 Kirk Hall, 117 Science Place
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Phone: (306) 966-4053   Fax: (306) 966-8597

APPENDIX C
TRAINING HISTORY FORM

<u>Training History</u>

List in short form your current training schedule. Be sure to include the type(s) of exercise(s), how long you perform the exercise(s), how often you perform the exercise, and estimate of the intensity of *each* bout of exercise. Be as specific as possible. (Also, include the time of day that you usually train)

Monday
Tuesday
Wednesday
Thursday
Friday
Saturday
Sunday

On average how many hours do you sleep every night?

APPENDIX D
MENSTRUAL CYCLE HISTORY FORM

Menstrual History

List the date of the first day of your last menses?

How many days does your period typically last?
(3, 5, 7 etc.)

How many days is your entire menstrual cycle (menses included)?
(i.e. 24, 28, 35 days)

Are you taking oral contraceptives? _____

If yes, what type of oral contraceptive?

How long have you been on this type?

If no, do you have any history of irregular menses, amenorrhea, endometriosis or other menstrual complications?

Menstrual cycle tracking calendars:

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY

APPENDIX E
ANTHROPOMETRIC MEASUREMENTS

Name:_____   Subject # _____

Birth Date (mm/dd/yy):_____   Date of Test (mm/dd/yy):_____

Decimal Date: __ __ . __ __ __   Gender: M   F

Tester :__ .   ___   Recorder ._____

Measurement	Test 1	Test 2	Test 3	Final Value
Stature (height), cm	__ __ __ . __ __	__ __ __ . __ __	__ __ __ . __ __	__ __ __ . __ __
Weight, kg	__ __ __ . __ __	__ __ __ . __ __	__ __ __ . __ __	__ __ __ . __ __
SKINFOLDS (mm)				
① Triceps	__ __ . __ __	__ __ . __ __	__ __ . __ __	__ __ . __ __
② Subscapular	__ __ . __ __	__ __ . __ __	__ __ . __ __	__ __ . __ __
③ Biceps	__ __ . __ __	__ __ . __ __	__ __ . __ __	__ __ . __ __
④ Iliac Crest	__ __ . __ __	__ __ . __ __	__ __ . __ __	__ __ . __ __
⑤ Medial Calf	__ __ . __ __	__ __ . __ __	__ __ . __ __	__ __ . __ __
GIRTHS (cm)				
Arm (flexed)	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Waist (minimum)	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Calf	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Gluteal	__ __ . __	__ __ . __	__ __ . __	__ __ . __
BREADTHS (cm)				
Humerus	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Femur	__ __ . __	__ __ . __	__ __ . __	__ __ . __

04/24/92

Anthropometric Proforma

APPENDIX F

THREE DAY FOOD RECORD

College of Physical Education
University of Saskatchewan

THREE-DAY FOOD RECORD

NAME: _____

DATES: _____

SPORT: _____

AGE: _____

HEIGHT: _____

WEIGHT: _____

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INTRODUCTION

This booklet is used to record your detailed daily food intake. It is meant to give the researchers some idea of your <u>usual</u> dietary intake. Therefore, it is very important that you do not alter your eating habits while taking part in this study. In other words, do not let the fact that you are writing down what you eat influence your choice of foods. The names of the participants in this study will be kept confidential.

<u>The usefulness of the results of this study depends on the accuracy with which you record your daily food intake. Please write down full details on all the food and drink that you consume each day.</u>

INSTRUCTIONS

1) The purpose of this diary is to record <u>all</u> the food (including drinks) which you eat for a three day period. The three day period should include 2 weekdays and 1 weekend day.

2) Two pages are provided for each day of the three day period.

3) <u>After each meal or snack</u> that you eat, please write down <u>in detail</u> each separate food item you consumed - including the <u>type</u> of food (e.g. <u>processed</u> cheese) and the <u>amount</u> of food in household measures (e.g. <u>1 cup</u> of cooked spaghetti). A meal will have to be listed by its separate parts (e.g. fried steak - 8 oz., french fries - 1 cup, coleslaw - 3 tbsp.)

4) The best way to record the information is by carrying this diary around with you wherever you go. Before going to sleep, you should look over the diary to check that you have not missed anything. Remember to include snacks!

5) If you eat fast food, you can just list the type of food you ate (e.g. 1 Big Mac, 1 large fires, 1 chocolate milkshake).

6) The following pages explain the use of household measures, and the description of foods. A sample day's diet sheet is given. Please take the time to read these pages as it will help to make your diet record more accurate.

RECORDING IN THE DIARY

1)   Please use household measures. For example:

cup: vegetables, cereal, fruit, milk, beverages
tablespoon: sauces, fats
teaspoon: sugar, honey, drink mix
slices: bread, bacon
fractions: 1/6 pie.

2)   State the _type_ of food eaten. For example:

Milk: homo, 2%, 1%, skim, goat's
Cheese: processed, Swiss, spread
Bread: enriched white, 60% whole wheat, sweet cinnamon
        bun, bran muffin
Cereal: Sugar Pops, Miniwheats, granola
Meat: hamburger, fried chicken - breasts, scrambled eggs,
        cod fillets
Others: strawberry jam, Becel margarine, Caesar
        dressing, oatmeal cookies.

3)   State the _amount_ of food eaten. For example:

Cheese: 1" cube cheddar
        3 tbsp lite cream cheese
        1/4 cup 2% creamed cottage cheese
Fruit: 1/2 cup canned peaches (in heavy syrup)
        12 grapes
        1 medium banana
Bread: 2 slices 100% whole wheat
        1 large kaiser
Cereal: 3/4 cup corn flakes
        1 shredded wheat biscuit

Meat: 1 cup baked beans with pork
       2 cups tuna casserole (tuna, cream of mushroom soup,
       noodles, peas)
       4 thin slices roast beef

Vegetables: 2 slices cucumber
            1/2 cup boiled cabbage

4)   Include manner of cooking: fried, boiled, raw.

5)   Remember all alcoholic drinks.

Here is a Sample:

Date: <u>Sat., Dec. 14th (Day 3)</u>

Time	Food Description	Amount	Code
9:30a.m.	Waffles-white flour	3, 8"x4"	ea.
	syrup-Aunt Jemima	1/2 cup	
	yogurt-peach	125ml	
	coffee, 1 tsp. sugar	1 cup	
	milk (2%)	1/4 cup	
10:30	Chocolate chip cookies	3	
	coffee, 1 tsp. sugar	1 cup	
	milk (Half & Half-10%)	1/4 cup	
12:30	Sandwich		
	-2 slices whole wheat bread	2 slices	
	-mozarella cheese (3"x1/4"x2")	2 slices	
	-salami	4 slices	
	-lettuce	1 leaf	
	-butter	1 tsp.	
	-mayonaise	1 tsp.	
5:30	Spaghetti	1 cup	
	meat sauce	1/2 cup	
	garlic toast	2 slices	
	(Continue on the next page if your need		
	it) Leave Code column blank.		

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Date: _____

Time	Food Description	Amount	Code

APPENDIX G

EXAMPLE of CARBOHYDRATE LOADING MENU

Date : 1999/05/27

Menu

Day 2 : Tuesday   S.D. : 0   Extras : 1

Breakfast (7h)	Recommended	Portion	Mass	ml
Eggs and dairy foods	Milk, fluid, skim	½ x glass	130g	125ml
Breakfast cereal	Cereal, rtc, cheerios, general mills	1½ x 175 ml	45g	263ml
Appetizer and sweets	Sweets, sugars, granulated	1 x 5 ml	4g	5ml
25 Beverage, Breakfast	Tea	1 x 250 ml	250g	250ml
Fruit and fruit juice	Bananas, raw	½ x fruit 22x6.3dm	58g	58ml

Snack AM (10h)	Recommended	Portion	Mass	ml
Bakery and pastry products	Muffins, wheat bran, from recipe, made from 2% milk	1 x 1.0	60g	
Fruit and fruit juice	Apple juice, frozen concentrate, diluted, no added vit c	2 x 125 ml	252g	250ml

Lunch (12h-13h)	Recommended	Portion	Mass	ml
Legumes	Soy sauce (made from soy), tamari	1 x 15 ml	18g	46ml
Vegetables	Carrots, raw	1 x 125 ml	111g	125ml
Vegetables	Broccoli, boiled, drained	1 x 1 spear	180g	125ml
Vegetables	Peppers, sweet, green, raw	½ x 125 ml	60g	63ml
Cereal products and pasta	Pasta, noodles, egg, cooked	2 x 125 ml	160g	249ml
Beverage	Carbonated drinks, lemon-lime soda	1 x 250 ml	260g	250ml
Fruit and fruit juice	Apples, raw, with skin	1 x 1 fruit 7cm dm	138g	164ml

Snack PM (15h30)	Recommended	Portion	Mass	ml
Cereal bars	Boost sport bar fruit and honey	1 x 65 g	65g	

Dinner (18h)	Recommended	Portion	Mass	ml
17 White rice	Rice - white	1 x 125 ml	108g	125ml
Cereal products and pasta	Grain, rice, wild, raw	1 x ½ cup	85g	125ml
Legumes	Soy sauce (made from soy), tamari	1 x 15 ml	18g	46ml
Dressing	Salad dressing, commercial, italian (low calorie)	1 x 15 ml	15g	15ml
Vegetables	Carrots, raw	¾ x 125 ml	83g	93ml
Green salad	Green salad	1 x 250 ml	100g	250ml
Vegetables	Mushrooms, raw	¼ x 125 ml	21g	32ml
Vegetables	Peppers, sweet, red, raw	½ x 125 ml	27g	63ml
Beverage	Cranberry-apple juice drink, + vitamin c, bottled	1 x 250ml drink box	258g	250ml

Snack eve (21h)	Recommended	Portion	Mass	ml
Beverage	Tea, herb, brewed	1 x 1 glass	250g	250ml

You should drink between meals : 1 liter of water.   Prot./kg :   Carb./kg :

Activities: 2726 kcal	Consumption: 2095 kcal	Carbohydrate: 81%	Protein: 12%	Fat: 7%	Alcohol: 0%

Breakfast	Kcal	C	P	F	TeFb	SugT
Milk, fluid, skim	46.6	6.2	4.4	0.3	0.0	5.7
Cereal, rte, cheerios, general mills	163.6	33.6	6.0	3.5	3.3	1.2
Sweets, sugars, granulated	15.5	4.0	0.0	0.0	?	4.0
Tea	2.5	0.7	0.0	0.0	?	?
Bananas, raw	93.4	13.6	0.6	0.3	1.0	9.0
Totals :	300.4	58.1	11.0	4.0	4.3	20.0

Snack AM	Kcal	C	P	F	TeFb	SugT
Muffins, wheat bran, from recipe, made from 2% milk	169.6	26.1	4.3	7.3	?	?
Apple juice, frozen concentrate, diluted, no added vit c	118.4	28.0	0.3	0.3	0.3	?
Totals :	288.2	54.1	4.5	7.6	0.3	0

Lunch	Kcal	C	P	F	TeFb	SugT
Soy sauce (made from soy), tamari	10.8	1.0	1.9	0.0	?	?
Carrots, raw	47.7	11.2	1.1	0.2	2.7	7.3
Broccoli, boiled, drained	60.4	9.2	5.4	0.5	4.3	?
Peppers, sweet, green, raw	16.2	3.6	0.5	0.1	0.6	1.5
Pasta, noodles, egg, cooked	224.6	41.9	8.1	2.5	3.7	?
Carbonated drinks, lemon-lime soda	104.0	27.0	0.0	0.0	?	26.5
Apples, raw, with skin	61.4	21.0	0.3	0.6	2.6	16.4
Totals :	535.3	115.2	17.3	4.0	14.2	53.7

Snack PM	Kcal	C	P	F	TeFb	SugT
Boost sport bar fruit and honey	219.7	38.0	10.0	2.7	0.0	0.0
Totals :	219.7	38.0	10.0	2.7	0.0	0.0

Dinner	Kcal	C	P	F	TeFb	SugT
Rice - white	140.4	30.6	2.9	0.3	0.6	?
Grain, rice, wild, raw	303.5	63.7	12.5	0.9	4.4	?
Soy sauce (made from soy), tamari	10.8	1.0	1.9	0.0	?	?
Salad dressing, commercial, italian (low calorie)	7.5	0.9	0.0	0.5	0.0	?
Carrots, raw	35.7	8.4	0.8	0.2	2.0	5.5
Green salad	16.0	2.4	1.8	0.2	1.7	2.0
Mushrooms, raw	6.3	1.0	0.4	0.1	0.3	?

	*=mg **=µg					
Peppers, sweet, red, raw	7.3	1.7	0.2	0.1	0.4	?
Cranberry-apple juice drink, + vitamin c, bottled	172.9	44.1	0.3	0.0	?	?
Totals :	699.2	153.7	20.7	2.2	9.2	7.5

Snack eve	Kcal	C	P	F	TeFb	SugT
Tea, herb, brewed	2.5	0.5	0.0	0.0	?	0.0
Totals :	2.5	0.5	0.0	0.0	0	0.0

Sub Totals : 2045 419.6 63.6 20.5 27.0 81.7

Polycose ⟶ + 160 40.0
Total ⟶ 2205 459.6 g

Total Kcal = 2205
CHOg = 459.6

@ 64 kg = 7.18 g CHO/kg
= 83 % CHO Ein

APPENDIX H

RECORDING FORMS:

SUMMARY SHEET

DEPLETION TAPER

TESTING AT 70%VO_2MAX

TESTING AT 85 % VO_2MAX

Summary Sheet

Subject (name/number: _____

Completion Checklist (list date of completion)

- ❑ _____ Consensus Forms completed; explanation of procedures
- ❑ _____ VO$_2$max test
- ❑ _____ Anthropometric measurements
- ❑ _____ Menstrual History
- ❑ _____ Training History
- ❑ _____ Diet portion and 3 day record training
- ❑ _____ Diet records submitted
- ❑ _____ Menus completed and returned with instructions

Inclusion/Exculsion Criteria

VO2max test result _____

Regular menstrual cycle _____

Oral contraceptive use _____

Descriptive Data

Anthropometric measurements: *Menstrual cycle length:*

    Height _____   Start of next menses

_____

    Weight _____

    Sum of 7 Skinfolds _____   *Menstrual cycle phase:*

                                        Estimate date Follicular ph

                                        Estimate date Luteal phase

Dietary intake:

    Average Habitual Caloric Intake _____

    Average intake CHO (grams/day) _____

    (simple ____; Complex_____; fibre _____)

    Average intake Protein (g/day) _____

    Average intake Fat (g/day) _____

Training History:

    Average Caloric Expenditure per day _____

Test Results (Running):

75 minute run @75%VO2max:

	Run 1(F)	Run 2 (L)	Run 3 (Fcho)	Run4 (Lcho)
60 minutes	_____	_____	_____	_____
Blood cmplt	_____	_____	_____	_____

Run to Exhaustion @ 85% VO2max:

Started	_____	_____	_____	_____
Ended	_____	_____	_____	_____

Depletion Taper Monitoring Sheet:

Subject Name	Target VO$_2$ max	Corresponding Target Heart Rate Range
Start Time:		End Time:
Heart Rate	Speed/Miles per hour	Time

Test Day Protocol Record of Events:

Subject Name: _____ **Date of Test** _____
Test No: _____ *(control / intervention)*

☐ _____ Hr - Pre-Exercise Blood Sample Drawn

☐ _____ Hr - 60 minute Run Start Time

☐ _____ mph - Treadmill Speed(s) _____Time

☐ _____ mph - Treadmill Speed(s) _____Time

☐ _____ mph - Treadmill Speed(s) _____Time

☐ _____ mph - Treadmill Speed(s) _____Time

☐ _____ mph - Treadmill Speed(s) _____Time

☐ _____ mph - Treadmill Speed(s) _____Time

☐ _____ mph - Treadmill Speed(s) _____Time

☐ _____ Hr - 60 minute Run End Time

☐ _____Breaks for Water

☐ _____ Completed Run Successfully

☐ _____ Reported Problems (Equipment / blood samples / Mental fatigue / physical fatigue) _____

Temperature _____ Humidity _____ Pressure _____

Total Amount of Water Consumed _____

Consumed Breakfast _____

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Test Day Protocol Record of Events:

Subject Name: _____ **Date of Test** _____
Test No: _____ *(control / intervention)*

85% VO 2max Timed Performance Run to Exhaustion

❑ _____ Hr Run Start Time

❑ _____ mph - Treadmill Speed(s) _____Time

❑ _____ mph - Treadmill Speed(s) _____Time

❑ _____ mph - Treadmill Speed(s) _____Time

❑ _____ mph - Treadmill Speed(s) _____Time

❑ _____ mph - Treadmill Speed(s) _____Time

❑ _____ mph - Treadmill Speed(s) _____Time

❑ _____ mph - Treadmill Speed(s) _____Time

❑ _____ Hr Run End Time

❑ _____ Completed Run Successfully

❑ _____ Reported Problems (Equipment / blood samples / Mental fatigue / physical fatigue)_____

_____

Temperature _____ Humidity _____ Pressure _____

Notes:

APPENDIX I

RAW DATA

Data for the Timed Run to Exhaustion at 80% VO2max

	Participant	Menstrual Phase	Diet Treatment	Weight kg	Room Temperature (Celsius)	Humidity	Barometric Pressure	VO2 ml/kg/min	Percent VO2max	Heart Rate bpm	RER (VCO2/VO2)	Time to Exhaustion minutes
Follicular/ CHO loading (1,1)	CT	1	1	70	25	58	710	41.3	76%	165	0.94	8.38
	NP	1	1	76	21	60	710	33.96	69%	166	0.95	22
	JP	1	1	56.7	22	23	719	41.15	81%	164	0.95	28.17
	SK	1	1	.	22	42	721	42.4	86%	174	1.06	6.57
	HW	1	1	79	19	68	721	39.16	77%	167	0.89	3.38
	BMC	1	1	60	22	26	721	44.65	83%	190	0.97	18.19
	TM	1	1	67	22	65	720	40.47	74%	152	1	4.46
Follicular/ Normal Mixed Diet (1,2)	CT	1	2	.	25	52	712	42.43	78%	167	0.92	8.34
	NP	1	2	74.5	22	61	723	35.15	71%	168	0.95	17.17
	JP	1	2	56	19	.	714	43.81	87%	153	0.91	23.4
	SK	1	2	62.5	23	40	722	40.25	81%	173	1	6.26
	HW	1	2	78.5	18	53	713	42.62	84%	148	0.99	4.11
	BMC	1	2	58	18	45	724	42.3	79%	182	0.99	13.49
	TM	1	2	64	20	43	716	38.1	70%	150	0.89	4.2
Luteal/ CHO loading (2,1)	CT	2	1	.	22.5	23	719	39.98	74%	164	0.9	8.22
	NP	2	1	75	22	39	721	37.9	77%	172	0.94	18.41
	JP	2	1	.	22	33	720	41.21	82%	153	0.96	24.6
	SK	2	1	65	21.5	55	722	42.38	86%	170	1.01	6.52
	HW	2	1	79	20	64	726	40.57	80%	176	0.96	4.21
	BMC	2	1	57.5	20	55	717	46.9	88%	185	0.97	16.44
	TM	2	1	68.5	18	54	727	40.15	73%	155	1.02	6.23
Luteal/ Normal Mixed Diet (2,2)	CT	2	2	71	21	46	719	42.65	78%	159	0.89	8.28
	NP	2	2	.	22	.	.	36.5	74%	174	0.93	11.58
	JP	2	2	57.5	24	32	724	42.73	85%	155	0.95	18.28
	SK	2	2	64	19	66	714	39.28	79%	172	1	6.06
	HW	2	2	79.5	22	22	728	41.76	83%	176	0.93	3.17
	BMC	2	2	59	24	.	714	41.56	78%	186	0.96	9.37
	TM	2	2	68	18	53	724	43.02	79%	154	0.97	4.45

Data for the 60 minute treadmill run at 70%VO2max

	Participant	Menstrual Phase	Diet Treatment	Weight kg	Room Temperature (Celsius)	Humidity	Barometric Pressure	VO2 ml/kg/min	Percent VO2max	Heart Rate bpm	RER (VCO2/VO2)
Follicular/ CHO loading (1,1)	CT	1	1	70	25	58	710	36.28	67%	154	0.91
	NP	1	1	76	21	60	710	31.57	64%	152	0.9
	JP	1	1	56.7	22	23	719	34.3	68%	138	0.93
	SK	1	1	.	22	42	721	34.6	70%	163	0.94
	HW	1	1	79	19	68	721	37.66	74%	161	0.88
	BMC	1	1	60	22	26	721	39.01	73%	177	0.95
	TM	1	1	67	22	65	720	35.64	66%	145	0.97
Follicular/ Normal Mixed Diet (1,2)	CT	1	2	.	25	52	712	37.95	70%	157	0.86
	NP	1	2	74.5	22	61	723	33.6	68%	160	0.91
	JP	1	2	56	19	.	714	35.7	71%	128	0.9
	SK	1	2	62.5	23	40	722	35.91	73%	162	0.93
	HW	1	2	78.5	18	53	713	36.7	73%	164	0.91
	BMC	1	2	58	18	45	724	40.94	76%	177	0.93
	TM	1	2	64	20	43	716	38.18	70%	145	0.89
Luteal/ CHO loading (2,1)	CT	2	1	.	22.5	23	719	39.4	72%	164	0.91
	NP	2	1	75	22	39	721	34.6	70%	165	0.93
	JP	2	1	.	22	33	720	34.7	69%	139	0.93
	SK	2	1	65	21.5	55	722	37.01	75%	156	0.95
	HW	2	1	79	20	64	726	35.5	70%	163	0.92
	BMC	2	1	57.5	20	55	717	38.93	73%	177	0.94
	TM	2	1	68.5	18	54	727	33.5	62%	141	0.96
Luteal/ Normal Mixed Diet (2,2)	CT	2	2	71	21	46	719	36.5	67%	142	0.89
	NP	2	2	.	22	.	.	33.85	68%	165	0.89
	JP	2	2	57.5	24	32	724	35.02	69%	138	0.92
	SK	2	2	64	19	66	714	34.73	70%	159	0.95
	HW	2	2	79.5	22	22	728	36.1	71%	162	0.91
	BMC	2	2	59	24	.	714	38.22	71%	175	0.94
	TM	2	2	68	18	53	724	38.72	71%	145	0.93

Blood Work

	Participant	Menstrual Phase	Diet Treatment	Resting Blood Glucose mmol/L	30 min Blood Glucose mmol/L	60 min Blood Glucose mmol/L	Resting Free Fatty Acids umol/L	30 min FFA umol/L	60 min FFA umol/L
Follicular/ CHO loading (1,1)	CT	1	1	4.4	6.6	6.4	91	315	451
	NP	1	1	6.5	5.8	5.5	114	412	425
	JP	1	1	4.6	5	5	51	111	119
	SK	1	1	7.1	5	7	84	143	312
Follicular/ Normal Mixed Di (1,2)	CT	1	2	4.6	6.1	5.8	58	396	438
	NP	1	2	4.3	5.2	4.7	.	.	.
	JP	1	2	4.5	5.1	5.3	42	179	279
	SK	1	2	6.9	4.8	5.7	227	230	511
Luteal/ CHO loading (2,1)	CT	2	1	.	4.7	7.5	174	410	.
	NP	2	1	5.8	6.2	5.7	139	472	603
	JP	2	1	4.9	5.5	5.5	113	207	411
	SK	2	1	5.5	5.2	5.4	.	.	.
Luteal/ Normal Mixed Di (2,2)	CT	2	2	5.1	6.1	5.7	39	195	305
	NP	2	2	5.8	6.3	6.6	208	552	763
	JP	2	2	5.7	5.5	5.5	74	236	331
	SK	2	2	4.3	5.4	4.3	29	291	298

Blood Work

	Participant	Menstrual Phase	Diet Treatment	Resting Glycerol umol/L	30 min Glycerol umol/L	60 min Glycerol umol/L	Resting Blood Lactate mmol/L	30 min Blood Lactate mmol/L	60 min Blood Lactate mmol/L	Luteal Phase Progesterone nmol/L
Follicular/ CHO loading (1,1)	CT	1	1	325	375	425	0.9	1.3	1.1	na
	NP	1	1	364	316	340	1	0.9	0.9	na
	JP	1	1	308	224	336	3.5	2	1.8	1.8
	SK	1	1	292	243	340	2.5	6.3	7	2.6
Follicular/ Normal Mixed Di (1,2)	CT	1	2	267	316	462	1.1	1.4	1.2	na
	NP	1	2	275	425	300	1.3	1.1	1.4	na
	JP	1	2	275	225	364	1.8	2.3	1.9	2
	SK	1	2	325	375	350	1.6	5.4	6.7	na
Luteal/ CHO loading (2,1)	CT	2	1	.	.	.	1.3	3	.	2.4
	NP	2	1	725	900	550	1.5	1.3	1.1	2.6
	JP	2	1	292	364	316	1.2	3	2.5	36.4
	SK	2	1	250	275	275	1.2	4.3	4.8	2.6
Luteal/ Normal Mixed Di (2,2)	CT	2	2	219	316	340	0.7	0.9	0.9	2.8
	NP	2	2	316	389	340	1.4	1.3	.	32.6
	JP	2	2	250	250	300	1.3	2.7	2.2	28.7
	SK	2	2	275	325	250	1.8	3.4	4.2	2.2

Carbohydrate Oxidation Data - Individual Averages   (grams/minute)

Subject	F/CHO (1,1)	F/NMD (1,2)	L/CHO (2.1)	L/NMD(2,2)
1	2.477	1.910	2.660	2.100
2	2.124	2.430	2.650	2.230
3	2.024	1.749	2.060	1.972
4	2.939	2.200	2.760	2.477
5	2.116	2.649	2.730	2.723
6	2.458	2.534	2.440	2.457
7	2.824	2.074	2.720	2.642
Group Ave	2.423	2.221	2.574	2.372

Fat Oxidation Data - Individual Averages   (grams/minute)

Subject	F/CHO (1,1)	F/NMD (1,2)	L/CHO (2.1)	L/NMD(2,2)
1	0.3480	0.3860	0.6040	0.4850
2	0.4060	0.3080	0.3450	0.4700
3	0.2210	0.2010	0.3460	0.2640
4	0.2100	0.1740	0.2310	0.1820
5	0.4850	0.3700	0.4470	0.4260
6	0.2480	0.2050	0.2490	0.2070
7	0.1360	0.1350	0.4440	0.2780
Group Ave	0.2934	0.2541	0.3809	0.3303

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Normal Mixed Diet Macronutrients and % of Total Energy Intake   (n=7)

Subject	Kcal	CHO g/day	% CHO	Fat g/day	%Fat	rotein g/da	% Protein
1	2015	267	52.9%	72	32.2%	84.5	16.7%
3	2402	337	62.7%	60	22.3%	95.0	15.8%
4	1780	322	72.3%	36	18.3%	58.4	13.1%
5	1467	251	68.5%	35	21.1%	30.2	16.4%
6	2549	373	58.4%	73	25.9%	78.9	12.3%
7	1606	230	57.3%	51	28.7%	64.3	16.0%
8	2036	334	65.5%	43	19.1%	84.9	16.6%
Avergage	1979	302	62.5%	53	23.9%	70.9	15.3%
Standard Deviation	398	53	6.8%	16	5.2%	21.9	1.8%

CHO Loading Menu Macronutrients (g/day) and % Total Energy Intake   (n=7)

Subject	Kcal	CHO g/day	% CHO	Fat g/day	%Fat	rotein g/da	% Protein
1	2348	465	79%	29	11%	80	14%
3	2754	525	79%	44	14%	94	14%
4	2287	443	77%	34	13%	78	14%
5	2284	458	80%	26	10%	73	13%
6	2591	481	74%	45	16%	84	13%
7	2077	409	79%	25	11%	60	12%
8	2585	502	78%	37	13%	89	14%
Average	2430	470	78%	35	13%	80	13%
Standard Deviation	254	42	2%	9	2%	12	1%

Table 1 Summary of Participants (n=7)

SUBJECT NUMBER	AGE (years)	HEIGHT (centimeters)	WEIGHT (kilograms)	BODY MASS INDEX (kilograms/meters squared)	SUM of 5 SKINFOLDS (millimeters)	Maximal Oxygen Consumption VO2 max (ml/kg/minute)
1	28	173.8	71	23	72.4	54.4
3	18	182	75	22.6	109.1	49.5
4	43	159	57	22.4	66.8	50.5
5	26	172.7	64	21.3	68.4	49.5
6	24	183	79	23.5	85.6	50.6
7	43	165	57.5	21.3	51	53.8
8	34	171	65	22.2	62.6	54.4
Average	30.9	172.4	66.9	22	73.7	51.8
Standard Deviation (+/-)	9.6	8.6	8.4	1	18.8	2.3

SUBJECT NUMBER	AGE (years)	MENSTRUAL CYCLE (days)
1	28	28
3	18	32
4	43	30
5	26	28
6	24	24
7	43	28
8	34	28
Average	30.9	28
Standard Deviation (+/-)	9.6	2