INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

The Effect of Gustatory Stimulation on Endurance Performance

A Thesis Presented
to the
Department of Kinesiology
Lakehead University

In Partial Fulfillment
of the Requirements for the
Degree of Masters of Science
in
Applied Sports Science and Coaching

by
Katharine Lise Sodek ©

Abstract

The purpose of this study was to determine whether the ingestion of an energy-free aspartame beverage (ASP) (99 mg aspartame/L) would influence endurance performance relative to a water (WAT) trial. Nine trained male cyclists underwent two cycle ergometer trials to exhaustion at 75% VO_2 max. Experimental beverages were ingested every 15 minutes beginning 45 minutes prior to cycling. Mean (± SD) exercise times to exhaustion were 100 ± 28 minutes for the ASP trial and 99 ± 34 minutes for the WAT trial ($p>.05$). Respiratory exchange ratio and heart rate did not differ in response to the treatment condition. Ratings of perceived exertion were significantly elevated in the ASP trial but only at the initiation of exercise. It was concluded that the ASP beverage did not impair endurance performance relative to WAT.

Acknowledgements

I would like to express my gratitude to the following people who were each responsible for a critical part of this thesis. First and foremost, thanks to Dr. Teresa Socha for her time, patience and expertise. The guidance and advice provided by Dr. Jim McAuliffe and Dr. Bob Thayer was greatly appreciated. Thanks to Dr. Moira McPherson for her generous loan of equipment. Finally, a special thanks to all of the participants who compromised their schedules and volunteered their time (sweat and tears) in order to make this study possible.

Table of Contents

List of Tables

List of Figures

CHAPTER 1. Introduction

Endurance athletes may consume low-calorie beverages containing aspartame, an "artificial" (non-nutritive) sweetener, in order to maintain the low weight necessary for their optimal competitive performance. Aspartame has also been used to sweeten the standard "placebo" beverage in experiments designed to determine the potential ergogenic effect of carbohydrate (CHO) ingestion on endurance performance (Devlin, Calles-Escandon, and Horton, 1986).

Palatable substances stimulate two vagally-mediated responses: a cephalic phase (pre-absorptive) insulin release (CPIR), and an orohepatic reflex (OR), which mediate physiological responses that oppose the metabolic conditions associated with an improved endurance performance. At rest, the CPIR and OR serve to inhibit lipolysis, and to increase the deposition of blood glucose and free fatty acids (FFA), effectively lowering the availability of blood-borne energy substrate. The net result is a shunting of energy substrate away from oxidative pathways, toward storage (Tordoff and Friedman, 1989).

Endurance athletes benefit from the opposite metabolic state. An increased availability of blood-borne substrate, particularly plasma FFA, allows a sparing of the muscle's endogenous glycogen stores, which promotes an improved endurance performance since muscle glycogen depletion is the main factor limiting endurance exercise performance (Foster, Costill, and Fink, 1979). Thus the metabolic consequences following the ingestion of palatable substances would conceivably be detrimental to the performance of endurance exercise. Although previous investigators (Devlin et al., 1986; Hargreaves,

Costill, Fink, King, and Fielding, 1987; Hasson and Barnes, 1987) have assumed that the ingestion of beverages sweetened non-nutritively would elicit metabolic effects identical to those of water, the effect of non-nutritive sweeteners on endurance exercise performance has not actually been evaluated.

Purpose

The purpose of this study was to determine whether the consumption of an energy-free beverage, sweetened with aspartame, would affect endurance performance.

Hypothesis

Since aspartame does not provide substrate for energy metabolism, but has been evidenced to elicit physiological alterations in substrate metabolism which are adversely associated with endurance performance, it was hypothesized that the ingestion of an aspartame-sweetened beverage both before and during exercise at an intensity of 75% VO_2 max would result in a decreased time to exhaustion, and would be associated with an elevated respiratory exchange ratio (RER) due to an increased reliance on muscle glycogen stores.

Significance of Study

The practical significance of this study was two-fold. Firstly, in an attempt to maintain a low body weight, many endurance athletes routinely ingest "diet", or "sugar-free" beverages, containing non-nutritive sweeteners. If this practise has a detrimental effect on performance, then it is important that this phenomenon be revealed so

that athletes can make the appropriate dietary modifications prior to competition (ie. hydrate with water).

Secondly, investigators have routinely used a non-nutritive sweetener to disguise the "placebo" or "control" treatment when studying the effects of CHO ingestion on endurance performance Devlin et al., 1986). The assumption is that the placebo is comparable to water, since negligible energy is provided. However, it was hypothesized that the placebo would impair performance relative to the ingestion of water, since the negative metabolic effects associated with "sweet" taste are not offset by the provision of oxidizable substrate. Thus it is possible that results which indicate an ergogenic effect of CHO ingestion are actually exaggerated, due to a comparison with a performance-impairing "control". This study attempts to evaluate the efficacy of the routine use of artificially sweetened beverages as the standard "control" treatment.

Limitations

Muscle glycogen levels were not directly measured. Therefore, a two-day period of controlled exercise and diet was implemented prior to each experimental trial in attempt to equalize pre-trial muscle glycogen levels. The mechanisms underlying any observed differences in endurance performance could not be determined since muscle glycogen utilization and blood concentrations of insulin, glucose, and free fatty acids were not measured. However, the RER measurements estimate the proportion of substrates undergoing oxidation and may provide clues to some metabolic alterations. Additionally, this study lacks a double blind design because taste is the independent variable. In order to motivate the subjects to exert

a maximal effort under each treatment condition, a monetary award was offered to the subject who was able to produce the longest time to exhaustion for both treatment conditions combined. However, it is possible that this external source of motivation may have introduced a psychological component which, in turn, could potentially influence the physiological response.

Delimitations

Delimitations of the study preclude generalizations to gender and all age ranges as well as to other types of exercise modalities. Random sampling was not possible, so all participants were recruited volunteers. Subjects were trained male cyclists, aged 19-29, with a minimum VO_2 max of 55.0 $ml \cdot kg^{-1} \cdot min^{-1}$. Diet was limited to the pre-trial composition. Trials were performed in the morning following a minimum fast of 4 hours and a maximum fast of 10 hours (overnight). Finally, any effect on performance was delimited to the combination of gustatory stimulation and exercise protocol employed in this study.

CHAPTER 2. Review of Literature

Metabolic Regulation

Both endocrine and neural events help to satisfy the organism's need for metabolic fuels. The endocrine and neural systems mediate bi-directional biochemical reactions through their control of substrate availability and enzyme activity. The autonomic nervous system complements the actions of the endocrine system, and in part, modulates endocrine secretions (Friedman and Stricker, 1976).

In response to the sympathetic stimulation concomitant to exercise (or its anticipation), the adrenal medulla releases the hormone epinephrine. Epinephrine increases glycogenolysis in muscle by activating phosphorylase. This hormone also stimulates lipolysis in adipose tissue by activating hormone-sensitive lipase. Both phosphorylase and hormone-sensitive lipase are activated via a cAMP-dependent phosphorylation event (Houston, 1995). The endocrine hormones, insulin and glucagon, exert a coordinated control over liver and adipose tissue, directing the deposition and mobilization of fuels, respectively (Friedman and Stricker, 1976). Insulin mediates metabolic effects that are opposite to those of epinephrine, stimulating glucose uptake by skeletal muscle and adipose tissue, increasing CHO oxidation, decreasing blood glucose, and promoting lipogenesis (Houston, 1995; McArdle, Katch, and Katch, 1994). Glucagon stimulates hepatic glycogenolysis in much the same way that epinephrine stimulates glycogenolysis in muscle (Houston, 1995).

In addition to the aforementioned endocrine events, neural events play a role in the regulation of metabolism. When a muscle fiber is stimulated to contract, the increased Ca^{2+} activates phosphorylase,

stimulating glycogenolysis. Sympathetic and parasympathetic nerves regulate the activities of separate enzymes implicated in glycogen metabolism in the liver (Shimazu, 1967). Excitation of the sympathetic nerves results in the release of the neurotransmitter norepinephrine. Similar to the mechanism of the hormone epinephrine, norepinephrine stimulates lipolysis in adipose tissue by activating hormone-sensitive lipase (Houston, 1995) and stimulates glycogenolysis in the liver by activating phosphorylase and glucose-6-phosphate phosphatase (Shimazu, 1967). Additionally, as in muscle, hepatic glycogenolysis can be stimulated through a neurally-stimulated Ca^{2+}-dependent pathway. Parasympathetic stimulation causes glycogenesis in liver by increasing the activity of glycogen synthetase (Shimazu, 1967).

Although hepatic metabolism is regulated through both hormonal and autonomic influences, the autonomic regulation occurs much more rapidly than hormonal regulation (Shimazu, 1967). The insulin response is an aspect of endocrine control, whereas the OR is under direct neural control, occurring through the parasympathetic innervation of the liver. The response of hepatic glycogen synthetase to vagal stimulation occurs within 5 minutes compared to the 40 minutes required under insulin stimulation (Shimazu, 1967). It would seem that the OR is responsible for the immediate metabolic changes with insulin contributing at a later time.

Metabolic Regulation During Exercise

The proportion of substrate oxidized is determined by the relative intensity and duration of exercise. Generally, the reliance on CHO and the proportion of CHO derived from muscle glycogen increases as the intensity is increased from 50 to 70% VO_2 max (Coyle and Coggan, 1984). The

increased energy demands of exercising muscle are met through an accelerated rate of muscle glycogenolysis, and an increased uptake and oxidation of circulating FFA and glucose. The uptake of blood-borne substrate is matched by increased rates of lipolysis and hepatic glycogenolyis. It is the increased sympathetic stimulation concomitant to exercise that stimulates the aforementioned substrate mobilization (Houston, 1995).

The catecholamines, epinephrine and norepinephrine, are released from the adrenal medulla in response to stress/exercise. The magnitude of the increase in catecholamine output is directly related to the intensity of exercise (Coyle and Coggan, 1984). The release of insulin from the pancreas is blunted following sympathetic stimulation (McArdle et al., 1994). This is important during exercise because elevated insulin levels can cause hypoglycaemia due to the synergistic effects of insulin and muscle contraction on glucose uptake. The decrease in insulin levels also facilitates lipolysis in adipose tissue, a favourable effect since an increased FFA oxidation allows a decreased reliance on muscle glycogen (Coyle and Coggan, 1984).

Cephalic Phase Response

The sight, smell and taste of food can profoundly affect an organism's physiology (Renwick, 1993). Cephalic phase responses stimulate the first metabolic adjustments upon exposure to food. Cephalic phase responses prepare the body to deal with the anticipated influx of nutrients, and buffer large deviations in the regulated concentrations of plasma nutrients (Powley and Berthoud, 1985).

Oral stimulation, particularly by sweet-tasting substances, induces a series of neurally-mediated cephalic phase changes in metabolism that divert fuel away from oxidation and toward storage (Tordoff and Friedman, 1989). Evidence for such metabolic alterations includes a drop in plasma FFA (Penick, Prince, and Hinkle, 1966; Rodin, 1990; Tordoff and Friedman, 1989), a drop in blood glucose levels (Jorgensen, 1949; Kun and Hovarth 1947; Louis-Sylvestre, 1976; Teff, Mattes, and Engelman, 1991), and the activation of hepatic glycogen synthetase (Shimazu, 1967). These responses are mediated by two parasympathetic components of the autonomic nervous system: the OR and the CPIR, which act independently but stimulate the same metabolic effect, reallocating substrate from oxidative pathways toward nutrient storage (Shimazu, 1967; Tordoff and Friedman, 1989).

Orohepatic reflex

Tordoff (1988) has proposed that sweet taste initiates a vagally-mediated OR which directs ingested and endogenous fuels toward storage and away from oxidation. The OR involves a direct neural parasympathetic stimulation of the hepatic vagus, which shifts the liver into "storage mode", increasing lipogenesis and glycogenesis. Electrical stimulation of the hepatic vagus has been demonstrated to result in an activation of glycogen synthetase, the enzyme responsible for the storage of glucose as glycogen (Shimazu, 1967). Thus, hepatic output of substrate via glycogenolysis and lipolysis are diminished following the OR. Shimazu, (1967) demonstrated that the effect of vagal stimulation on glycogen synthetase activity of the liver is not dependent

on insulin stimulation, since a similar activation of glycogen synthetase was observed in both pancreatectomized and intact rabbits.

Cephalic phase insulin release

Insulin is a hormone that is released by the pancreas in response to high blood glucose levels. The release of insulin in response to glucose occurs in two phases. The cephalic phase insulin release is a neurally-stimulated pre-absorptive release of insulin from the pancreas that occurs in response to gustatory stimulation. The cephalic or pre-absorptive phase is fast, peaking about 5 minutes after exposure to the taste stimulus, preceding the absorption of glucose. A decrease in blood glucose in response to the CPIR has been observed 20 to 60 minutes following exposure to the taste stimulus (Jorgensen, 1949; Kun and Hovarth, 1947). The CPIR is stimulated by the organoleptic properties of food, such as taste, sight and smell (Renwick, 1993). Its physiological purpose is to prepare the body for the anticipated rise in blood glucose concentration. The second and main phase of insulin secretion is post-absorptive, occurring after about 15 minutes, and is stimulated by elevated blood glucose or amino acid concentrations (Renwick, 1993).

Insulin serves to mediate glucose uptake mainly by muscle cells (75%-95%) but also by the liver and adipose cells (Baron and Bretchel, 1993). Insulin directs hepatic fat and CHO metabolism away from oxidation, toward storage (Geiselman, 1988; Newsholme and Start, 1973) by activating lipoprotein lipase and glycogen synthetase (Sakaguchi and Yamaguchi, 1979; Shimazu, 1967; Shimazu, 1983), thus impairing the mobilization of fat and glucose. Insulin retards

hepatic glycogenolysis and lipolysis of adipose tissue, thereby lowering the circulating levels of metabolic fuels (Friedman and Stricker, 1976). When plasma insulin levels are high, hepatic fuel oxidation is likely to be low (Friedman and Ramirez, 1987; Friedman, Ramirez, Wade, Siegel, and Granneman, 1982). Thus, the OR and the CPIR together direct an increased reliance on glucose as a substrate for oxidation, due to an inhibition of lipolysis and subsequent fatty acid oxidation (Tordoff and Friedman, 1989).

Effect of Non-Nutritive Sweeteners on the Cephalic Phase Response

A non-nutritive sweetener is a substance that tastes sweet but does not provide energy, either because the substance cannot be oxidized, or because it is consumed in such minute quantities that the energy provided is negligible. Aspartame (L-aspartyl-L-phenylalanine methyl ester), saccharin and acesulfame-K are examples of non-nutritive sweeteners. Aspartame, a dipeptide composed of phenylalanine, aspartic acid and a methyl group is the sweetener that is most commonly consumed in "diet" beverages and foods (Whitney and Rolfes, 1993).

Beverages that are sweetened with non-nutritive sweeteners present a unique physiological situation in which sweet taste is uncoupled from the provision of oxidizable substrate. Expecting to receive substrate, the CPIR and OR serve to shunt the available fuel toward storage resulting in a lowered availability of blood-borne substrates. However, there is not an accompanying compensatory influx of fuel from the gastrointestinal tract. Thus artificial sweeteners cause a dissociation between the body's normal response to a glucose

load, and the provision of substrate, resulting in a state of lowered substrate availability (Tordoff and Friedman, 1989).

Decreased plasma free fatty acid levels

The sight or taste of food might be a sufficient stimulus to some hungry people to cause FFA mobilization to decrease "in anticipation" of a CHO load (Penick et al., 1966). This decrease in FFA is part of a physiological reaction comparable to events such as increased salivation, gastric tone and secretion (Penick et al., 1966). When food is ingested FFA levels may fall prior to absorption in anticipation of the substrate load, an effect which occurs as a direct response to CPIR (Bruce, Storlein, Furler, and Chisholm, 1987). This anticipatory fall in FFA, mediated by the central nervous system, occurs even when a CHO load is not ingested at all (Penick et al., 1966). Indeed, artificial sweeteners have been evidenced to cause a decrease in plasma FFA in both human subjects (Devlin et al., 1986; Koivisto, Karonen, and Nikkila, 1981; Penick et al., 1966; Rodin, 1990) and in rats (Tordoff and Friedman, 1989) under resting conditions.

Penick et al. (1966) designed experiments to test the effect of seeing and tasting food on plasma FFA. In a series of trials, subjects were presented with food and allowed a small bite (roast beef sandwich). The experimenters reported that FFA fell in association with the sight and taste of food. Tordoff and Friedman (1989) reported an elevated in vitro hepatic lipogenesis in rats, after they drank small volumes of a solution sweetened with saccharin. Rodin (1990) reported that plasma FFA fell to a greater extent following ingestion of a lemon flavoured beverage sweetened with aspartame than following ingestion of water.

Although it was not the intention of the investigators to study the metabolic effects consequent to the ingestion of a sweet placebo, the pre-exercise administration of a sweet placebo was shown to impair the exercise-induced increase in plasma FFA (Devlin et al., 1986; Koivisto et al., 1981). Koivisto et al. (1981) compared the ingestion of glucose, fructose and an artificially sweetened (saccharin) placebo beverage, administered 45 minutes prior to exercise at 70% VO_2 max. The investigators reported that the ingestion of the sweet placebo "surprisingly" caused an 18% fall in plasma FFA levels ($p<.05$) during the 45 minute period preceding exercise. An accompanying increase in plasma insulin concentration was not observed, likely because these measurements were not made until 45 minutes after ingestion, and although this time period was appropriate for the detection of the post-absorptive phase of insulin release, it would have been much too late to detect a pre-absorptive (cephalic phase) insulin release.

Devlin et al. (1986) compared the ingestion of a candy bar, and a sweet placebo (containing aspartame), administered 30 minutes prior to exercise at 70% VO_2 max. Both the candy bar and the sweet placebo caused a significant decline in plasma FFA concentration. Additionally, FFA levels did not differ between the two trials throughout exercise or during recovery. Similar to the study by Koivisto et al. (1981), the lack of an increased insulin concentration following placebo ingestion was to be expected, since the CPIR peaks 5-15 minutes after ingestion, and in the current study, blood was not sampled until 30 minutes after feeding.

Hyperinsulinaemia and decreased blood glucose levels

Non nutritive sweeteners have been demonstrated to provoke a CPIR and concomitant hypoglycaemia (Jorgensen 1949; Kun and Hovarth 1947; Louis-Sylvestre, 1976; Teff et al., 1991). Kun and Hovarth (1947) administered a saccharin solution to subjects and observed a 12-16% decrease in blood glucose concentration. They suggested that this decrease occurred as a consequence of sweet taste stimulating the vagus nerve. Jorgensen (1949) conducted an experiment to determine whether the decrease in blood sugar occurred due to taste or to some other reaction. During one trial subjects drank a saccharin sweetened solution, and during a second trial they rinsed their mouths with the solution. He found that 5 out of 12 subjects experienced a fall in blood sugar of over 10 mg% when they drank the saccharin solution, whereas 6 out of 8 subjects experienced this fall after rinsing their mouths. In another trial, saccharin was introduced directly into the duodenum, through a duodenal tube, so that the gustatory nerves were not influenced. Under these circumstances, a decrease in blood glucose concentration did not occur. In addition, two subjects were given a different sweetening agent (1-n-propoxy-2-amino-4-nitrobenzene). When they rinsed with the 1-n-propoxy-2-amino-4-nitrobenzene solution, the subjects' blood sugar fell by 12 and 16 mg%, whereas drinking this solution produced a fall of 11 mg%. In all cases the decline in blood glucose levels reached its peak approximately 30-40 minutes after the treatment was administered. Jorgensen (1949) stated that the exposure time of the sweet taste on the gustatory nerves was a contributing factor to the decline in blood sugar. Jorgensen thus demonstrated that sweetening agents of different

chemical structures cause this cephalic phase reflex, indicating that the effect was produced by the gustatory stimulation rather than by a particular chemical reaction.

Louis-Sylvestre (1976) used non-nutritive sweeteners to demonstrate a consistent CPIR in animals. The resultant cephalic phase insulin secretions reached amplitudes of +200% from the basal insulin concentration. Teff et al. (1991) reported a reliable CPIR in "normal-weight" males following the ingestion of a dessert sweetened with aspartame. Hyperinsulinaemia was observed 4 minutes after ingestion, and a concomitant hypoglycaemia followed. In order to demonstrate the involvement of the parasympathetic nervous system, Tordoff and Friedman (1989) fed rats a saccharin-sweetened solution and measured plasma insulin levels 15 minutes prior to treatment, and at 15 and 45 minutes post-ingestion. Plasma insulin levels were elevated 15 minutes post-ingestion relative to the other two samples. The insulin response was attenuated, but not prevented, in rats that had undergone a celiac vagotomy (operative procedure to sever the celiac vagal nerve) or a hepatic vagotomy (partial sympathetic liver denervation), relative to rats that were sham-fed (control condition), indicating the role of the parasympathetic nervous system in the cephalic phase response.

In contrast to the aforementioned observations, some studies have not demonstrated a CPIR after the ingestion of aspartame-sweetened drinks (Bruce et al., 1987; Carlson and Shah, 1989; Rodin, 1990; Wolf-Novak, Stegink, and Brummel, 1990). However, a number of these studies used a solution of aspartame in water, a beverage that was noted to taste "unpleasant" by the subjects in the study of Bruce et al. (1987), which may have resulted in the lack of findings (Renwick, 1993). Based on these

results, it would seem that an agreeable flavour in combination with an aspartame-sweetened solution is necessary to elicit a cephalic phase response. Additionally, in many of the aforementioned experiments the blood sampling was obtained at a time which would have been too late (greater than 5-10 minutes) to detect the cephalic phase response (Renwick, 1993).

Factors Limiting Endurance Performance

Muscle glycogen depletion is the primary cause of fatigue during moderately intense exercise (60-80% VO_2 max) particularly when the duration exceeds two hours (Coyle and Coggan, 1984). Although exercise-induced hypoglycaemia was thought to cause exhaustion, it probably only coincides with exhaustion due to the depletion of body CHO stores (Coyle and Coggan, 1984). Muscle glycogen levels have been reported to drop to low levels (3-8 $mmol \cdot kg^{-1}$) at the time of exhaustion (Gollnick, Pernow, Essen, Jasson, and Saltin, 1981).

Endurance performance is limited by the initial muscle glycogen stores and their rate of depletion (Bergstrom, Hermansen, Hultman, and Saltin, 1967). Initial muscle glycogen stores are influenced by previous (two to three day) diet and activity, while the rate of muscle glycogenolysis is influenced by such factors as the relative intensity of exercise, the blood insulin and FFA concentrations, intramuscular triglyceride utilization, and the fitness level of the subject (Hasson and Barnes, 1989; Hickson, Rennie, Conlee, Winder, and Holloszy, 1977; Philips, Green, Tarnopolsky, Heigenhauser, Hili, and Grant, 1996). The rate of muscle glycogenolysis increases with an increasing exercise intensity, expressed as a function of VO_2 max (McArdle et al., 1994). Due to their increased ability to oxidize

fat at a given submaximal exercise intensity, trained subjects demonstrate a lower rate of muscle glycogenolysis compared to their untrained counterparts (Gollnick and Saltin, 1982). Endurance training allows an increased oxidation of intramuscular triglycerides, and a decreased reliance on glycogen utilization (Philips et al., 1996). Muscle glycogenolysis occurs at an accelerated rate when blood insulin levels are elevated and the availability of external fuel sources (blood-borne FFA and hepatic glucose) is reduced (Costill, Coyle, Dalsky, Evans, Fink, and Hoopes, 1977; Coyle and Coggan, 1984; Hasson and Barnes, 1989; Levine, Evans, Cadarette, Fisher, and Bullen, 1983). Therefore, it is feasible that any substance which lowers the availability of blood-borne substrate, thus accelerating muscle glycogenolysis, may impair endurance performance.

Free fatty acid concentrations

An elevation in plasma FFA concentration has been shown to be associated with an improved endurance performance (Hickson et al., 1977; Ivy, Costill, Fink, and Lower, 1979). With an increased supply of exogenous substrate (FFA), the muscle does not need to rely as heavily on its endogenous muscle glycogen stores. A decreased rate of muscle glycogenolysis follows (Costill et al., 1977; Hickson et al., 1977), and allows the onset of exhaustion to be delayed (Costill, Dalsky, and Fink, 1978; Costill et al., 1977; Hickson et al., 1977; Rennie, Winder, and Holloszy, 1976). The sparing of muscle glycogen has been demonstrated through a manipulation of substrate availability.

Costill et al. (1977) combined a fatty meal with a heparin injection in order to elevate plasma FFA. Subjects were seven trained males, who cycled for 30 minutes at 70% VO_2 max. Pre- and post-exercise muscle

biopsies were taken, so that the rates of muscle glycogenolysis could be quantified. The investigators reported that when subjects underwent the elevated FFA trial, their muscle glycogen was spared by 40% relative to the placebo trial.

Hickson et al. (1977) demonstrated that elevated FFA levels improved endurance performance by slowing the rate of muscle glycogen depletion. These investigators combined a corn oil feeding with a heparin injection to elevate plasma FFA in endurance-trained rats. The rats were subsequently run to exhaustion. It was reported that rats with the elevated plasma FFA levels were able to run for an hour longer than their paired control rats (3 hours compared with 2 hours). At the time of exhaustion for the control rats, the rats with elevated plasma FFA concentrations had higher muscle and liver glycogen levels than the exhausted control rats. When the elevated FFA rats reached exhaustion (an hour later) their glycogen concentrations were comparable to those of the control rats, which indicated a slower rate of glycogenolysis in rats with elevated FFA. Therefore, the investigators reported that it was the CHO-sparing effect of increased plasma FFA which allowed the improvement in endurance performance.

Ivy et al. (1979) used a caffeinated beverage to elevate plasma FFA levels in 9 trained subjects. Caffeine is a potent stimulator of lipolysis and beta-oxidation of fatty acids (Davis, 1968). The subjects were instructed to cycle for two hours and to produce as much work as possible in this time. The investigators reported an improved performance (7.4% greater work production) in the elevated FFA trial relative to the control condition. Although muscle biopsies were not taken, the authors speculated that the improved performance occurred as a result of a sparing of muscle

glycogen, enabled by the elevated FFA concentration. This speculation was supported by the lowered RER values reported in the caffeine trial.

Insulin

It has been suggested that elevated insulin levels have negative effects on endurance performance (Costill et al., 1977; Foster et al., 1979). Insulin increases lipoprotein lipase (LPL) activity, fostering the uptake of circulating lipids into storage, and redirecting the flux of fat away from oxidative pathways (Friedman and Ramirez, 1987). Since it impairs lipolysis (Ahlborg and Felig, 1976) and inhibits hepatic glucose output (Hasson and Barnes, 1987), insulin lowers the blood-borne/exogenous supply of substrate to the muscle (Friedman and Stricker, 1976). This effect is detrimental to endurance performance, because when exogenous substrate availability is decreased, a greater reliance is placed on the muscle's endogenous stores, thus muscle glycogen becomes more rapidly depleted (Costill et al., 1977; Coyle and Coggan, 1984; Hasson and Barnes, 1989; Levine et al., 1983). Insulin has a residual effect on muscle tissue which persists for some hours following the return of plasma insulin to basal levels (Coyle, Coggan, Hemmert, Lowe, and Walters, 1985).

Hawley, Bosch, Weltan, Dennis, and Noakes (1995) demonstrated that an increased reliance on CHO occurred as a result of elevated insulin levels. Rates of CHO oxidation were compared under conditions of elevated insulin levels (resulting from glucose ingestion) and low insulin levels (glucose infusion). Blood glucose levels were maintained at comparable levels in both trials. Subjects were 10 trained male cyclists, who rode for 125 minutes at 70% VO_2 max. The elevated insulin condition resulted in concomitant increases in the rate

of plasma glucose oxidation, and total rate of CHO oxidation, whereas FFA oxidation was decreased. Fat oxidation contributed only 18% to total energy expenditure when insulin was elevated, compared to 51% when insulin remained low. The authors speculated that conditions which elevate plasma insulin concentrations during the first 90-120 minutes of exhaustive exercise would be detrimental to endurance due to an inhibition of fat metabolism and an increased reliance on CHO oxidation.

Glucose ingestion 15 to 60 minutes preceding exercise leads to an elevation of insulin at the onset of exercise which has adverse effects on metabolism (and consequently performance) during moderate to high intensity exercise (Costill et al., 1977; Foster et al., 1979; Hargreaves, Costill, Katz, and Fink, 1985; Koivisto et al., 1981). Such adverse metabolic effects include a decline in blood glucose, and an impairment of fat oxidation. Blood glucose levels decline due to the synergism between insulin and muscular contraction in their ability to accelerate glucose uptake, and because an adequate rise in hepatic glucose output is prevented by elevated insulin levels (Ahlborg and Felig, 1977). Fat oxidation is depressed due to an insulin-mediated impairment of lipolysis (Coyle et al., 1985). The reduced availability of blood-borne substrate increases the reliance of the exercising musculature on its endogenous glycogen stores (Costill et al., 1977; Hargreaves et al., 1985; Levine et al., 1983), accelerating the onset of exhaustion (Foster et al., 1979). The muscle's increased reliance on glucose as a source of oxidizable substrate following CHO ingestion is evidenced by an increased RER (Gleeson, Maughan, and Greenhaff, 1986; Hawley et al., 1995; Horowitz and Coyle, 1993; Koivisto

et al., 1981; Sherman, Brodowicz, Wright, Allen, Simonsen, and Dernbach, 1989; Sherman, Peden and Wright, 1991).

Numerous studies have reported an impairment of endurance performance occurring as a result of elevated insulin levels. Costill et al. (1977) reported that glucose (75 g) ingested 30 minutes prior to exercise elevated plasma insulin, and increased muscle glycogen utilization by 17% relative to the placebo. The increased rate of CHO oxidation was supported by an elevated RER. Subjects were seven trained males, who cycled for 30 minutes at 70% VO_2 max. Although this study did not incorporate a measure of "performance", the authors speculated that the (observed) accelerated rate of muscle glycogenolysis would be detrimental to endurance exercise performance. Hargreaves et al. (1985) confirmed the results of Costill et al. (1977). Subjects were 8 males who ingested 50 g of glucose prior to cycling at 45 minutes prior to 30 minutes of cycling at a workload corresponding to 75% VO_2 max. Pre- and post-exercise muscle biopsies revealed an accelerated muscle glycogen utilization in the glucose trial relative to the placebo trial.

Foster et al. (1979) conducted a follow-up study to that of Costill et al. (1977) in order to determine the practical effects of CHO feedings on endurance performance. Subjects cycled to exhaustion at a workload corresponding to 80% VO_2 max. Foster et al. (1979) reported that the pre-exercise ingestion of glucose elevated plasma insulin levels and was associated with an endurance performance (measured as time to exhaustion) that was impaired 19% relative to the placebo condition. Although muscle glycogen levels were not measured, the investigators attributed the reduced performance to an

accelerated muscle glycogen depletion. An increased reliance on CHO was supported by an elevated RER. The investigators concluded that the results supported earlier findings that glucose feedings increase the rate of CHO oxidation and impair the mobilization of FFA, thereby reducing exercise time to exhaustion.

Coyle et al. (1985) demonstrated that the physiologic effect of insulin on peripheral tissue is prolonged, and the resultant metabolic effects which impair endurance performance occur even when basal plasma insulin levels have been restored prior to exercise. In this study a CHO feeding (2 g CHO per kg body weight) was administered 4 hours prior to 105 minutes of cycling at 70% VO_2 max. Relative to the control condition (fasted), an impaired fat mobilization and increased CHO oxidation were evident in the CHO trial, despite a return of insulin and blood glucose levels to basal state an hour prior to the initiation of exercise. The altered substrate availability was attributed to the persistent effects of insulin on muscle tissue.

In contrast, two groups of investigators have reported an ergogenic effect of pre-exercise CHO ingestion on endurance performance (Gleeson et al., 1986; Sherman et al., 1991). Gleeson et al. (1986) observed an improvement of 13% in performance time when subjects consumed 1.0 g CHO per kg body mass 1 hour prior to exercise. The subjects were 6 untrained males, who cycled at 73% VO_2 max until exhaustion (approximately 1.5-2.0 hours). However, some aspects of the methodology make the validity of this study questionable. Pre-trial diet and activity were not controlled. These measures should have been taken in order to equalize pre-trial muscle glycogen levels, particularly since these investigators did not measure

muscle glycogen levels. In addition, the investigators did not use a double blind design. This would allow for experimenter bias, particularly since the definition of "exhaustion" can be quite subjective, particularly when verbal encouragement is given, and the subjects are untrained (and perhaps not as intrinsically motivated as competitive athletes).

Sherman et al. (1991) conducted a study to compare the ingestion of a low CHO beverage (1.1 g CHO/kg), a high CHO beverage (2.2 g/kg) and a placebo, which were ingested one hour prior to exercise. Subjects were 9 trained male cyclists, who rode for 90 minutes at 70% VO_2 max, followed by a time trial based on the work that would be accomplished in an additional 45 minutes. The investigators reported that despite elevated insulin concentrations during the first 60 minutes in the CHO trials, performance was improved. Blood glucose concentrations were significantly higher in the treatment groups for the first 45 minutes, which probably countered the effect of the elevated insulin levels. The researchers attributed the improvement in performance to the greater CHO availability during exercise, which likely resulted from the continued gastric absorption of CHO during exercise. The rate of CHO oxidation averaged 12% higher for the CHO treatment groups relative to the placebo group. The high CHO treatment allowed a glucose absorption rate during exercise that exceeded the whole-body glucose disposal, resulting in an elevation of blood glucose levels. Neither the elevated insulin, nor the transient hypoglycemia at the onset of exercise impaired performance of the CHO treatment groups. The investigators explained that the benefits of the provision of oxidizable substrate outweighed the performance-impairing effects of insulin under their particular experimental

conditions/protocol. However, they speculated that if plasma insulin levels were elevated without the accompanying provision of oxidizable substrate, a negative effect on endurance performance would result.

Some investigators have not found pre-exercise CHO feedings to have an effect on muscle glycogen utilization (Decombaz, Sartori, Arnaud, Thelin, Schurch, and Howald, 1985; Hargreaves et al., 1987; Koivisto, Harkonen, Karonen, Groop, Elovainio, Ferrannini, Sacca, and Defronzo, 1985), or performance (Decombaz et al., 1985; Hargreaves et al., 1987). Hargreaves et al., (1987) reported that the ingestion of glucose 45 minutes prior to cycling at 75% VO_2 max had no effect on either muscle glycogen utilization, or performance. Additionally, Decombaz et al. (1985) reported that the ingestion of 1 gram of glucose per kg body weight 1 hour prior to cycling did not alter performance or muscle glycogen utilization. However, the protocol featured a 45 minute ride at 61% VO_2 max, followed by a 15 minute performance trial. It is unlikely that muscle glycogen depletion would be a factor limiting performance under these conditions, since most of the exercise was of a moderate intensity and did not exceed an hour. Lactate accumulation would more likely be the factor limiting performance during the 15 minute time trial (Coyle and Coggan, 1984).

Koivisto et al. (1985) reported that the pre-exercise ingestion of 75 g of glucose 45 minutes prior to cycling at 55% VO_2 max for 2 hours did not alter the rate of muscle glycogen utilization. Performance was not assessed. The subjects in this study were untrained, and the intensity of exercise was relatively low, so these findings may not be generalizable to those conditions experienced by competitive endurance athletes. Devlin et al. (1986) reported that the ingestion of a candy bar (65 g) 30 minutes

prior to cycling at 70 % VO_2 max did not affect performance or muscle glycogen utilization. However, a candy bar does not elicit the same insulin peak as an equal bolus of glucose, due to the fat content (Horowitz and Coyle, 1993). In addition, the subjects were untrained males who reached exhaustion at approximately an hour and this duration was probably too short for muscle glycogen to have been the limiting factor. Local muscle fatigue and lactate accumulation are the factors which limit performance in exhaustive exercise lasting less than an hour (Coyle and Coggan, 1984).

Differences in results may be due to variations in the amounts of CHO administered, the glycemic index of CHO, the exercise intensity, the exercise duration, and the trained state of the subjects. The great variation in protocols between studies accounts for many of the inconsistencies in results (Costill, 1988). Investigators who used lower exercise intensities (50-75% VO_2 max) tended not to find an impairment of performance following pre-exercise ingestion of glucose, whereas protocols involving high intensity exercise (75-90% VO_2 max) found that such pre-exercise feedings resulted in an impairment of performance (Hasson and Barnes, 1989).

The effect of pre-exercise glucose ingestion on endurance performance is determined by the relative magnitudes of the adverse metabolic effects associated with insulin release, and the ergogenic effects resulting from the provision of substrate for oxidation (Costill and Hargreaves, 1992; Sherman et al., 1991). If the CHO absorbed from the gut is of sufficient quantity to offset the insulin-induced lowering of blood-borne substrate, then an improved performance may be expected. The occurrence of an insulin response independent of a

provision of oxidizable substrate should result in an impairment of performance (Sherman et al., 1991). Non-nutritive sweeteners elicit an (adverse) lowering of blood-borne substrate without providing (the ergogenic) substrate for oxidation. The net result should therefore be an impairment of performance, mediated through an increased reliance on muscle glycogen stores, and their accelerated depletion.

Summary

A reduction in exogenous/blood-borne substrate causes an increased reliance on muscle glycogen and may produce a reduced capacity for endurance performance (Costill et al., 1977; Foster et al., 1979). Elevated plasma FFA levels enhance endurance performance through a muscle glycogen sparing effect (Ivy et al., 1979). An impairment of the exercise-induced increase in plasma FFA has been evidenced subsequent to pre-exercise sweet placebo ingestion (Koivisto et al., 1981). Through the OR and CPIR, non-nutritive sweeteners may lower the availability of oxidizable substrate provided exogenously to the muscle. Therefore, the ingestion of a beverage sweetened with aspartame, both before and during exercise, may accelerate muscle glycogen depletion, leading to an impaired endurance performance.

CHAPTER 3: Methods and Procedures

Subjects

Subjects were 10 male volunteers, ranging from 19 to 32 years of age. These individuals were trained cyclists, had competed in endurance sport for a minimum of three years, and who each had a VO_2 max which exceeded 55.0 ml $\bullet kg^{-1} \bullet min^{-1}$. This pool of subjects was obtained from an initial recruitment of 13 subjects. A detailed explanation of the procedures and potential discomforts was provided. The subjects were informed that the purpose of the study was to compare the effect of "taste" on endurance performance and that the treatment beverages could contain the sweetener aspartame. Participants were aware that they were free to withdraw from the study at any time. Written informed consent was obtained from each subject prior to participation in the study. The study was approved by the Ethics Advisory Committee to the Senate Research Committee of Lakehead University.

Experimental Design

Subjects each performed two experimental trials (water and aspartame) so that the effects of the aspartame beverage on exercise time to exhaustion could be compared to the water trial. Treatment beverages were assigned in a randomized counterbalanced fashion and were served from an opaque water bottle. They were: (i) an aspartame-sweetened lemon beverage made with 6 g KoolAid crystals and 12 packs (0.9g) of Equal in 2000 ml of water (ASP) and (ii) water, coloured yellow with food dye (WAT). KoolAid crystals were not

added to the water because they would produce a bitter taste, altering the (desired) "neutral" taste of the control beverage.

Experimental Procedures

This study required each subject's participation for approximately three weeks. During the first week each participant performed a preliminary VO_2 max test so that the workload corresponding to 75% VO_2 max could be established. The first experimental trial was conducted during the second week of the study, and the second experimental trial was conducted one week later.

Cycling was performed on an electronically braked cycle ergometer with toe-stirrups (Ergometrics, by SensorMedics, California). Respiratory gases were measured using a Vmax 29 series metabolic cart by SensorMedics, California. The metabolic cart was calibrated prior to each test and was verified every 30 minutes. The cycle ergometer allowed the subjects to select a preferred cadence within a comfortable range (70-120 rpm). The subject's VO_2 max was determined using an incremental protocol. The initial workload was 200 watts, and it was increased 30 watts every 2 minutes for the first 4 levels, and every minute thereafter until volitional exhaustion. VO_2 max was indicated by a peak and/or plateau in VO_2 with increasing power output, and was recorded as the highest 30 second average. Maximum heart rate was also measured. All subjects achieved VO_2 max between 10 and 15 minutes, with respiratory exchange ratio (RER) > 1.0. The 75% VO_2 max workload was verified or adjusted during a steady state ride two days prior to the first experimental trial and was held constant thereafter.

The protocol for the experimental trials was as follows: Subjects reported to the lab 45 minutes prior to exercise. They consumed 150 ml of the treatment beverage every 15 minutes. After ingesting the fourth aliquot, subjects warmed up for 5 minutes on the cycle ergometer at a self-selected workload, followed by light stretching. Subsequently, subjects rode at their predetermined workload (75% VO_2 max) until volitional exhaustion. The treatment beverage (200 ml) was given every 15 minutes for the duration of the trial. Respiratory gases, heart rate (HR) (Polar Heart rate Monitor), and ratings of perceived exertion (RPE) (Revised Borg Scale, 10-point) (Nieman, 1990) were collected at the start of exercise, every 30 minutes throughout the trial, and within 15 minutes of exhaustion. When nearing exhaustion, the subject was repeatedly encouraged to increase pedalling cadence (which would lower the resistance). Subjects were asked to indicate when they thought they could continue for only another 5 minutes, so that metabolic measurements could be taken. Once the final collection was made, subjects were again encouraged to continue for as long as possible. If they continued for more than 15 minutes, another final collection of respiratory gases was made when they again felt that they could only continue for 5 minutes. Time to exhaustion was defined as the duration for which the subject was able to maintain the pre-determined (75% VO_2 max) cycling workload. Volitional exhaustion was attained when the subject was unable to keep pedalling, or when cadence dropped below 30 rpm. Performance was assessed against the water trial, with a decreased exercise time to exhaustion indicating an impaired performance.

Experimental Control

Exercise and diet were held constant for two days before each trial in an attempt to achieve comparable pre-trial muscle glycogen levels. Participants recorded their food intake prior to the first trial and, to the best of their abilities, replicated it prior to the second trial. A three-day dietary analysis (Food and Diet Analyzer software, Fitness Technologies Press, Ann Arbor Michigan) was performed prior to the trials in order to determine the subjects' average dietary composition. Pre-trial exercise was controlled, as subjects reported to the lab for a 30-minute ride at their 75% VO_2 max workload two days prior to each experimental trial. The day prior to each trial was designated as a "rest" day. Each subject performed his experimental trials at the same time of day. Subjects were non-smokers and were asked to refrain from eating and drinking (except water) for a minimum of 4 hours prior to testing, and to avoid any physical activity on the day of testing.

A financial incentive ($50) was offered to the subject with the longest performance time (both trials combined) in an attempt to promote a maximal effort during each trial, and to control for any preconceived notions of the participants. Subjects were informed of this "prize purse" following completion of the VO_2 max test. Subjects were blinded to time during their trials and did not receive feedback regarding their performance until all subjects had completed the study. In order to eliminate experimenter bias, the experimenter was blinded to the treatment condition. The treatment beverages (both coloured yellow) were made by an assistant, and subjects were asked to refrain from commenting on the taste.

Data Analysis

Descriptive statistics were calculated for the dependent variables. Performance time was analyzed using a paired *t* -test. A 2 (treatment) by 3 (time: 0, 30 and 60 minutes) repeated-measures analysis of variance was calculated for RER, HR, and RPE. Tukey's post-hoc tests were conducted following significant F-ratios. Alpha was set *a priori* at $p<.05$. The statistical software packages used were SPSS and Statistica.

CHAPTER 4. Results

The time to exhaustion, RER, HR and RPE were compared between the two treatment conditions in order to determine whether a beverage sweetened with aspartame would impair endurance performance and adversely affect substrate metabolism. An impaired endurance performance would be evidenced by a decrease in time to exhaustion. Adverse effects on substrate metabolism would be reflected by an increased RER.

Subject Characteristics

In the present study, 13 subjects were initially recruited. Two subjects withdrew as a result of injuries that were unrelated to this study, and a third withdrew because of time constraints, so that in total, 10 participants completed the study. The data of one of the 10 subjects was eliminated due to his lack of compliance to the control periods, in terms of both diet and training. Mean (± SD) characteristics of the 9 male subjects are reported in Table 1. The dietary compositions were analyzed for 8 of the 9 subjects. One of the participants could not provide enough detail to allow for an accurate analysis because his food was prepared in the residence cafeteria. The mean diet consisted of 60 (± 3) % carbohydrate, 15 (± 1) % protein, and 24 (± 4) % fat.

Performance (Time to Exhaustion)

A paired samples *t* -test was used to analyze the difference between exercise times to exhaustion. The mean (± SD) exercise times to exhaustion were 100 (± 28) minutes for the ASP trial and 99 (± 34)

Table 1. Subject characteristics

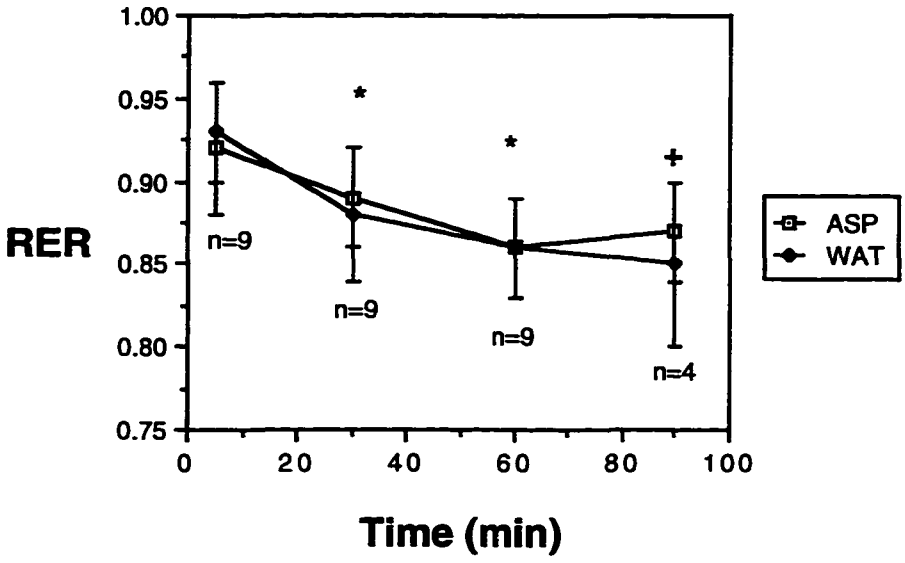
Characteristic	Mean	±SD	Minimum	Maximum
age (years)	23	3	19	29
height (cm)	177	7	164	188
weight (Kg)	74	6	62	83
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	66	3	61	69
VO_2 max ($L \cdot min^{-1}$)	4.8	0.3	4.1	5.3
workload (watts)	254	17	223	278
% VO_2 max	73	3	69	78

minutes for the WAT trial. Performance times were not significantly different between treatment conditions ($t(8)=0.12$, $p>.05$). A power test was performed in attempt to provide some insight into possible explanations for the non-significant treatment effect. The treatment effect size, d, was calculated to be 0.05 (Cohen, 1988) and eta squared was calculated to be 0.002 (Kiess, 1996), indicating a very small treatment effect.

RER, HR and RPE

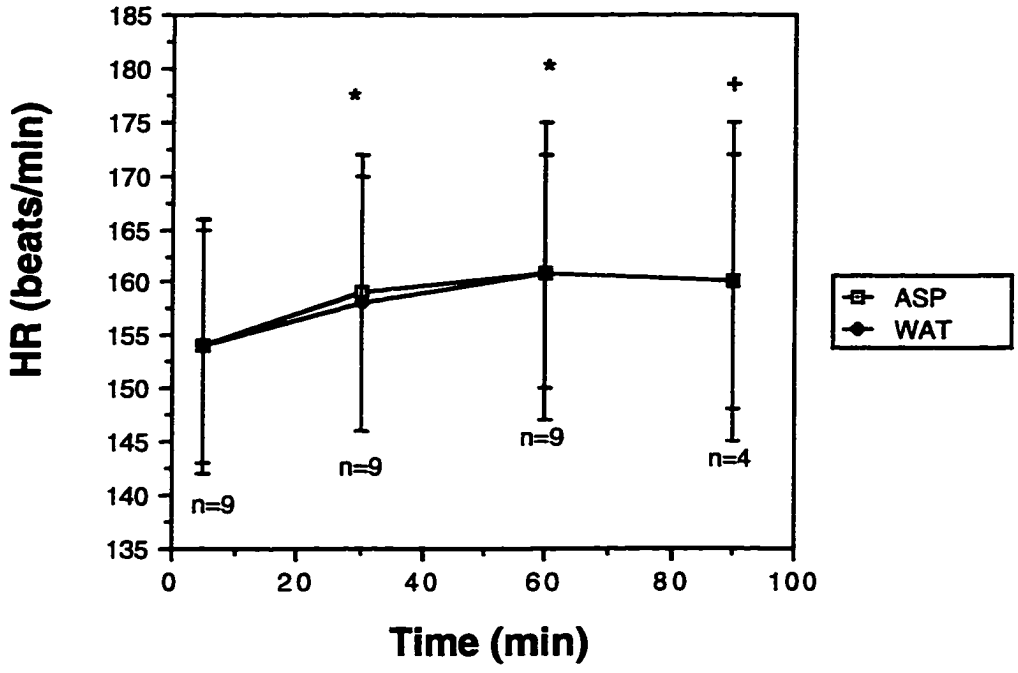
The effect of the treatment beverage on RER, HR and RPE were analyzed using repeated measures ANOVAs (for treatment and time). A significant main effect was found for the factor of time on RER ($F(2,14)=21.92$, $p<.05$). The main effect for treatment on RER, and the interaction effect between treatment and time on RER were not found to be significant ($p>.05$). A significant main effect was found for the factor of time on HR ($F(2,16)=14.87$, $p<.05$). The main effect for treatment on HR, and the interaction effect between treatment and time on HR were not found to be significant ($p>.05$). A significant interaction effect existed between treatment and time on RPE ($F(2,16)= 10.13$, $p<.05$).

Tukey's post hoc analyses were conducted for the main effect of time on RER, the main effect of time on HR, and the interaction effect between treatment and time on RPE. The results are shown in Figures 1, 2, and 3, respectively.



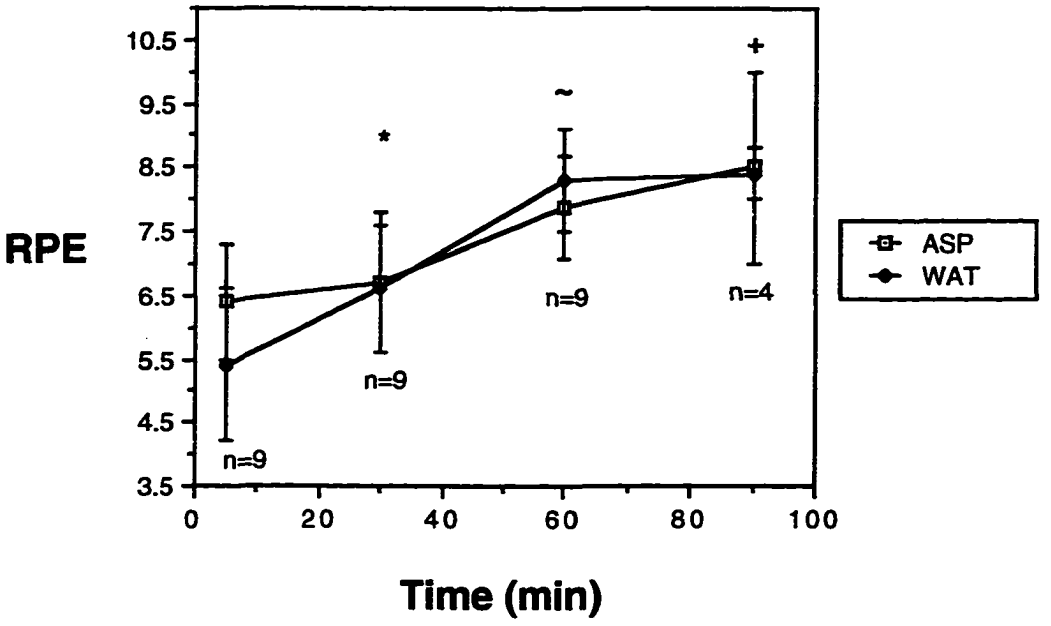
* significantly different from 5 min ($p<.05$) for the main effect of time
+ No statistical analysis performed due to the small number of subjects remaining.

Figure 1. The effect of treatment and time on respiratory exchange ratio (mean±SD).



* significantly different from 5 min ($p<.05$) for the main effect of time
+ No statistical analysis performed due to the small number of subjects remaining.

Figure 2. The effect of treatment and time on heart rate (mean±SD).



* WAT and ASP significantly different from WAT at 5 min ($p<.05$)

~ WAT and ASP significantly different from WAT and ASP at 5 and 30 min ($p<.05$)

+ No statistical analysis performed due to the small number of subjects remaining.

Figure 3. The effect of treatment and time on perceived exertion (mean±SD).

CHAPTER 5. Discussion

Previous studies have shown that palatable substances stimulate two vagally-mediated responses: a cephalic phase (pre-absorptive) insulin release (CPIR), and an orohepatic reflex (OR) (Tordoff and Friedman, 1989) which effectively lower the availability of blood-borne substrate and oppose the metabolic state that is known to benefit endurance performance (Foster et al., 1979). Aspartame and other non-nutritive sweeteners have been shown to elicit the (potentially adverse) aforementioned cephalic phase responses without a compensatory provision of substrate for energy metabolism (Louis-Sylvestre, 1976; Teff et al., 1991; Tordoff and Friedman, 1989). Therefore, it was hypothesized that the ingestion of an aspartame-sweetened, lemon-flavoured beverage would impair cycling performance compared to the water treatment in this study.

To date, the effect of non-nutritive sweeteners on endurance exercise performance has not been evaluated. Therefore, the present study can not be compared to any past findings, since it is the first to examine the effect of gustatory stimulation on substrate utilization and subsequent endurance performance. An emphasis was placed on the practical implications, and they were assessed through exercise time to exhaustion. The RER was measured to provide insight into the mechanisms underlying any differences in performance. Previous studies have used RER values to reflect substrate metabolism. Costill et al. (1977) used respiratory gases to estimate CHO and fat utilization, and found CHO utilization and muscle glycogen depletion to be

correlated (r= 0.64), supporting the contention that RER can be used as a valid indicator of substrate metabolism.

The results of the study revealed that neither performance, measured as exercise time to exhaustion, nor the physiological variables, RER and HR, differed as a result of the treatment condition. In this study, the RER values gradually declined over time. It has been previously shown that RER decreases over time, approaching a value of 0.7 as the body's CHO stores become depleted, and a greater reliance is placed on the oxidation of fat (Hermansen, Hultman and Saltin, 1967). The HR tended to gradually increase towards exhaustion in both treatment conditions. This phenomenon, known as cardiovascular drift, is associated with dehydration, and may have occurred in order to compensate for a decreasing stroke volume (McArdle et al., 1994). These observed trends are in agreement with previous reports (Bjorkman, Sahlin, Hagenfeldt, and Wahren, 1984; Coyle et al., 1983; Sherman et al., 1991).

The interaction between treatment and time had a significant effect on RPE (Figure 3). This interaction effect was due to an elevated RPE at the onset of exercise in the ASP trial. The reason for this increased perceived exertion at the onset of exercise in the ASP trial is unclear. A cephalic phase reflex, occurring in response to the ASP beverage prior to exercise could have lowered the availability of blood-borne substrate. This might have contributed to feelings of weakness, translating to a higher perceived exertion. However, the corresponding RER values did not reflect any differences in substrate metabolism at this time. Regardless of whether or not the increased RPE was due to a pre-exercise cephalic phase response, the differences

were diminished within 30 minutes of exercise, and performance was not affected. It would seem that this transient increase in RPE was of little practical significance.

Two potential explanations may contribute to the lack of significant findings. First, there may not be a treatment effect, meaning that either (a) the cephalic phase response did not occur in response to the ASP treatment, or, (b) if it did occur, it was of insufficient strength/magnitude to affect subsequent performance and substrate metabolism. Alternatively, it is possible that a treatment effect exists but was masked by a large variability in the performance measures and/or a small sample size, resulting in little power to detect the treatment effect.

It can not be established whether the cephalic phase reflex occurred in response to the ASP treatment beverage, since blood glucose, insulin and FFA levels were not measured. It is possible that the cephalic phase reflex occurred, but was of insufficient strength to offset the sympathetic stimulation experienced during exercise. The cephalic phase response is suppressed by adrenergic mechanisms (Powley and Berthoud, 1985) so it is feasible that the stress associated with the test situation might have blunted this parasympathetic reflex. Many investigators have reported that cephalic phase responses are labile, and may be disrupted by unspecified aspects of the test situation (Powley and Berthoud, 1985). It is well known that the insulin response is blunted during moderate to intense exercise because of the associated sympathetic stimulation (Costill, 1988). In light of this information, the present study was designed in order to maximize the potential of the cephalic phase response. The treatment beverage was

consumed for 45 minutes prior to exercise to allow the cephalic phase response to occur prior to sympathetic stimulation. However, it is possible that during this 45 minute period, the subject's anticipation of the upcoming trial, and/or the unfamiliar laboratory environment might have caused sufficient stress to stimulate a physiological response which would inhibit the cephalic phase response. The possibility of a dose-response relationship between the strength of the taste stimulus and the magnitude of the cephalic phase response cannot be discounted. Previous literature does not reveal whether or not such a relationship exists.

An alternate explanation for the lack of a significant finding is that confounding factors may have increased the variability in performance times to such an extent that subtle differences resulting from treatment effects were masked. A few confounding factors may have contributed to the variability. Although an attempt was made to control pre-trial muscle glycogen levels and motivation, outside influences including the amounts of stress and sleep could potentially have affected the subject's performance and contributed to the variability. Additionally, the large range in cadence selected by the subjects in the present study (70-120 rpm) could affect motor recruitment and therefore substrate utilization. This would potentially influence time to exhaustion. Two further sources of variability were those associated with the "time to exhaustion" protocol, and those resulting from an (observed) testing/learning effect. A retrospective analysis indicated that performance times to exhaustion were significantly increased ($t(8)=2.3$, $p<.05$) for the second trial compared to the first, which might indicate a testing/practise effect. Bjorkman et

al. (1967) noted a similar trend, but in addition to finding that exercise time to exhaustion increased from the first to second trial, these investigators found that performance was also significantly improved in the third trial relative to the second trial. Although the counterbalanced design used in this study was able to prevent this testing effect from interfering with treatment effects, it could not prevent the concomitant increase in variability.

McLellan, Cheung, and Jacobs (1995) examined the variability associated with the time to exhaustion protocol during exercise at 80% VO_2 max. They reported that a substantial range of intra-individual variability existed in the cycling time to exhaustion for males of average fitness. In fact, these investigators reported that a sample size of 40 would be needed to increase the power to 0.8, assuming a large treatment effect size. The coefficient of variability was reported to be 17.3%. It was observed that the coefficient of variability tended to increase with a decreasing intensity of exercise. The coefficient of variability increased from 12% for subjects cycling at 85% VO_2 max to 24% for participants riding at 80% VO_2 max. This may suggest that an even greater coefficient of variability would exist for subjects riding at 70-75% VO_2 max, as in the present study. However, in contrast to McLellan et al. (1995), the present study used trained subjects who were accustomed to exhaustive exercise, and who controlled their diet and exercise for the two days preceding each experimental trial. It is feasible that these factors might have reduced intra-individual variability somewhat in the present study.

Cohen (1988) emphasizes the importance of calculating the power of a statistical test when one fails to reject the null hypothesis.

The power of a statistical test is the probability that the effect of an independent variable will be detected when such an effect is present. The power is influenced by the predetermined alpha level, the sample size, and the effect size of the treatment. Power can be maximized either by increasing the difference between the means of the treatment conditions, or by decreasing variance. The present protocol was already designed in an attempt to maximize the difference between means, so this area can not be improved. A decreased variance could be achieved in the present study by (i) reducing the variability between the subject's performance times and (ii) increasing the number of subjects.

Since the *t* -statistic considers variance between the subjects (in addition to intra-individual differences) it follows that variability could be reduced if all subjects were of a very uniform fitness level, so that they produced similar exercise times to exhaustion. The variability between subjects in the present study is comparable to those previously reported by investigators who have used a similar protocol (exercise time to exhaustion) to test the practical effects of various nutritional manipulations. Variability of the ASP trial in this study will be compared to the placebo condition in previous studies. In the present study the standard deviation for the mean time to exhaustion was 28 minutes which accounted for 28% of the mean. This result is similar to the 29% reported by Calles-Escadon, Devlin, Whitcomb, and Horton, (1991). Standard error of the mean in the present study was 9.5%, which falls within the previously reported values of 21% (Foster et al., 1979), 6% (Coggan and Coyle, 1987), 7% (Coggan and Coyle, 1989),

11% (Bjorkman et al., 1984) and 4% (Coyle, Hagberg, Hurley, Martin, Ehsani, and Holloszy, 1983).

Variability can also be reduced by increasing the sample size. One can determine the number of subjects required to increase the power to 0.8 (an arbitrarily-defined optimal level, according to Cohen, 1988). This sample number is inversely related to the strength of the treatment effect, so that the stronger the treatment effect, the lower the number of subjects required to achieve a power of 0.8. In the present study, the treatment effect size (Cohen's d) was 0.05 which is extremely small considering that a d=0.2 is considered a "small" effect size and d=0.8 is considered to be a "large" effect size (Cohen, 1988). In the present study the number of subjects required to achieve a power of 0.8 was calculated to be in excess of 5000 (Cohen, 1988). The strength of a treatment can also be quantified by the eta value (Kiess, 1996). Eta values range from 0.00 (no treatment effect) to 1.0 (treatment accounting for 100% of the variance). The strength of the treatment effect (eta squared) was calculated to be 0.002, which means that only 0.2% of the variance in exercise time to exhaustion can be explained by the treatment beverage. Therefore, it would seem probable that the lack of significant findings may be attributed to a treatment effect so small that it has no physiological relevance, rather than to a lack of power.

It was hypothesized that the ASP treatment would impair endurance performance by accelerating the depletion of muscle glycogen. Therefore, it was essential that subjects were not riding at an intensity above their individual anaerobic thresholds. If lactate accumulation was the factor limiting performance (and not muscle

glycogen) then the treatment effect could not have been accurately assessed. A person is generally not able to exercise for longer than 50 minutes when working at an intensity above his/her anaerobic threshold (Loat and Rhodes, 1993). Since all subjects were able to cycle for over an hour, it is unlikely that any subject was exercising at an intensity above his anaerobic threshold. The shortest recorded time to exhaustion was 60 minutes. In addition, ventilatory thresholds were checked and each subject was found to have exercised at or below his ventilatory threshold.

In summary, although it can not be established whether a cephalic phase reflex occurred in response to the ASP treatment beverage, results from this study would suggest that the ingestion of a pallatable (sweet) non-nutritive beverage prior to and during exercise does not adversely affect endurance performance. If a cephalic phase response did occur, then it was probably overcome by the strong sympathetic stimulation concomitant to intense exercise. A lack of this response would provide further support of its lability. It would seem that if the stress associated with the upcoming trial was sufficient to disrupt the cephalic phase response, then the stress associated with a "real" race situation would also abolish this reflex. In either case, it would seem that athletes are safe to ingest artificially sweetened beverages prior to (and during) competition. As well, the results provided no evidence to refute the use of an aspartame-sweetened "placebo" beverage to disguise water.

CHAPTER 6. Summary, Conclusions and Recommendations

Summary

The purpose of this study was to determine whether the consumption of an energy-free beverage, sweetened with aspartame, would affect endurance performance relative to the control condition, water. Ten male cyclists each rode at a pre-determined workload (75% VO_2 max) to exhaustion on two occasions, ingesting either water or a lemon-flavoured aspartame-sweetened beverage both prior to and during exercise. Respiratory gases, HR and RPE were collected periodically (every 30 minutes) throughout exercise. It was established that the independent variable "taste" did not affect endurance performance or substrate metabolism (RER), although perceived exertion was initially elevated following pre-exercise ingestion of the ASP treatment beverage.

Conclusions

Under the conditions of the present study, the ingestion of a lemon-flavoured beverage sweetened non-nutritively with aspartame was not found to impair performance compared to water, nor were any alterations in substrate metabolism observed, as evidenced by RER values. An elevated RPE was observed following pre-exercise ingestion of the ASP treatment beverage, but had no practical significance in terms of performance, and was not supported by accompanying differences in RER or HR. It can not be established whether the cephalic phase response actually occurred in the ASP treatment condition, since blood insulin and substrate levels were not

measured. However, judging by the absence of a treatment effect on performance and substrate metabolism, it would seem that this reflex is of little practical significance. Therefore, the results provided no evidence to refute the use of an aspartame-sweetened "placebo" beverage to disguise water in studies testing the effects of CHO ingestion on endurance performance. Additionally, this study suggests that the ingestion of "diet" or "sugar-free" beverages, containing non-nutritive sweeteners would not impair endurance performance when consumed prior to or during exercise.

Recommendations

In the present study it could not be ascertained whether or not the cephalic phase response occurred. Blood insulin, glucose and FFA levels could be measured in order to provide evidence for a cephalic phase reflex, and muscle biopsies could be performed to provide an estimate of glycogen depletion. However, the size of the treatment effect, and the lack of the present practical findings should be noted when considering the justification for follow-up research using these invasive techniques.

The importance of a rigorous compliance to the 2-day control period is supported since massive discrepancies in exercise time to exhaustion were observed for the (excluded) subject who did not comply. In order to maximize compliance it would be ideal for the investigator to be able to provide the subjects with their food for the two days prior to each trial. This would ensure an exact replication of the dietary intake in terms of both energy and composition.

The observation that subjects performed better on the second trial than the first indicates the importance of using a counterbalanced design

for this type of study. It may be advisable for subjects to complete a practise/familiarization trial, without a treatment, so that the confounding effect of learning would be minimized in the subsequent test sessions. Calles-Escadon et al. (1991) had subjects perform two familiarization trials prior to their experimental trials and still noted large individual variations in exercise times to exhaustion that were not related to treatment. However, the "familiarization" trials consisted of only a 30-45 minute ride at the designated intensity, suggesting that it might be necessary for the subjects to actually experience cycling to "exhaustion" in the practise trials.

The 75% VO_2 max workload was predicted from the VO_2 max test. However, in all cases, the workload needed to be adjusted down approximately 20-30 watts in order to elicit the desired VO_2. This indicates that the exact workload should be checked or evaluated during a steady state ride, because the VO_2 does not plateau until 4 to 6 minutes into exercise.

In the present study, exercise intensity was assigned based on a pre-determined percentage (75%) of VO_2 max. Exercise intensity might better have been designated based on a pre-determined percentage of anaerobic threshold. This alternative technique might allow a more precise equalization of exercise intensity, and thereby could potentially reduce the between-subject variability in exercise time to exhaustion.

References

Ahlborg, G., & Felig, P. (1976). Influences of glucose ingestion on fuel-hormone response during prolonged exercise. Journal of Applied Physiology, 41, 683-688.

Ahlborg, G., & Felig, P. (1977). Substrate utilization during prolonged exercise preceded by ingestion of glucose. American Journal of Physiology, 233(2), E188-E194.

Baron, A.D., & Bretchel, G. (1993). Insulin differentially regulates systemic and skeletal muscle vascular resistance. American Journal of Physiology, 265, E61-E67.

Bergstrom, J.L., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen, and physical performance. Scandanavian Journal of Clinical and Laboratory Investigation, 19, 218-28.

Bjorkman, O., Sahlin, K., Hagenfeldt, L., & Wahren, J. (1984). Influence of glucose and fructose ingestion on the capacity for exercise in well-trained men. Clinical Physiology, 4, 483-494.

Bruce, D.G., Storlein, L.H., Furler, S.M., & Chisholm, D.J. (1987). Cephalic phase metabolism responses in normal weight adults. Metabolism, 36, 721-725.

Calles-Escadon, J., Devlin, J.T., Whitcomb, W., & Horton, E.S. (1991). Pre-exercise feeding does not affect endurance cycle exercise but attenuates post-exercise starvation-like response. Medicine and Science in Sports and Exercise, 23(7), 818-824.

Carlson, H.E., & Shah, J.H. (1989). Aspartame and its constituent amino acids: Effects on prolactin, cortisol, growth hormone, insulin and glucose in normal humans. American Journal of Clinical Nutrition, 49, 427-432.

Coggan, A.R., & Coyle, E.F. (1987). Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. Journal of Applied Physiology, 63(6), 2388-2395.

Coggan, A.R., & Coyle, E.F. (1989). Metabolism and performance following carbohydrate ingestion late in exercise. Medicine and Science in Sports and Exercise, 21(1), 59-65.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences (2nd ed.). New Jersey: Lawrence Erlbaum Associates, Publishers.

Costill, D.L. (1988). Carbohydrates for exercise: Dietary demands for optimal performance. International Journal of Sports Medicine, 9, 1-18.

Costill, D. L., Coyle, E., Dalsky, G., Evans, W., Fink, W., & Hoopes, D. (1977). Effects of elevated FFA and insulin on muscle glycogen usage during exercise. Journal of Applied Physiology, 43(4), 695-699.

Costill, D. L., Dalsky, G., & Fink, W. (1978). Effect of caffeine ingestion on metabolism and endurance performance. Medicine and Science in Sports and Exercise, 10, 155-158.

Costill, D.L., & Hargreaves, M. (1992). Carbohydrate nutrition and fatigue. Sports Medicine, 13(2), 86-92.

Coyle, E.F., & Coggan, A.R. (1984). Effectiveness of carbohydrate feeding in delaying fatigue during prolonged exercise. Sports Medicine, 1, 446-458.

Coyle, E.F., Coggan, A.R., Hemmert, M.C., Lowe, R.C., & Walters, T.J. (1985). Substrate usage during prolonged exercise following a preexercise meal. Journal of Applied Physiology, 59, 429-433.

Coyle, E.F., Hagberg, J.M., Hurley, B.F., Martin, W.H., Ehsani, A.A., & Holloszy, J.O. (1983). Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. Journal of Applied Physiology, 55(1), 230-235.

Davis, I. (1968). In vitro regulation of lipolysis of adipose tissue. Nature, 218, 349-352.

Decombaz, J., Sartori, D., Arnaud, M.-J., Thelin, A.-L., Schurch, P., & Howald, H. (1985). Oxidation and metabolic effects of fructose or glucose ingested before exercise. International Journal of Sports Medicine, 6, 282-286.

Devlin, J. T., Calles-Escandon, J., & Horton, E. S. (1986). Effects of pre-exercise snack feeding on endurance cycling exercise. Journal of Applied Physiology, 60, 980-985.

Foster, C., Costill, D.L., & Fink, W. J. (1979). Effects of preexercise feedings on endurance performance. Medicine and Science in Sports and Exercise, 11, 1-5.

Friedman, M. I., & Ramirez, I. (1987). Insulin counteracts the satiating effect of a fat meal in rats. Physiology and Behavior, 40, 655-659.

Friedman, M.I., Ramirez, I., Wade, G. N., Siegel, L. I., & Granneman, J. (1982). Metabolic and physiologic effects of of a hunger-inducing injection of insulin. Physiology and Behavior, 29, 515-518.

Friedman, M.I., & Stricker, E.M. (1976). The physiological psychology of hunger: a physiological perspective. Psychological Reviews, 83, 409-431.

Geiselman, P.J. (1988). Sugar-induced hyperphagia: is hyperinsulinemia, hypoglycemia or any other factor a "necessary" condition? Appetite, 11(Suppl), 26-34.

Gleeson, M., Maughan, R.J., & Greenhaff, P.L. (1986). Comparison of the effects of pre-exercise feeding of glucose, glycerol, and placebo on endurance and fuel homeostasis in man. European Journal of Applied Physiology, 55, 646-653.

Gollnick, P.D., Pernow, B., Essen, B., Jasson, E., and Saltin, B. (1981). Availability of glycogen and plasma FFA for substrate utilization in leg muscle of man during exercise. Clinical Physiology, 1, 27-42.

Gollnick, P.D., & Saltin, B. (1982). Significance of skeletal muscle oxidative enzyme enhancement with endurance training. Clinical Physiology, 2, 1-12.

Hargreaves, M., Costill, D.L., Fink, W.J., King, D.S., & Fielding, R.A. (1987). Effect of pre-exercise carbohydrate feedings on endurance cycling performance. Medicine and Science in Sports and Exercise, 19, 33-36.

Hargreaves, M., Costill, D.L., Katz, A.L., & Fink, W.J. (1985). Effect of fructose ingestion on muscle glycogen usage during exercise. Medicine and Science in Sports and Exercise, 17(3), 360-363.

Hasson, S.M., & Barnes, W.S. (1987). Blood glucose levels during rest and exercise: influence of fructose and glucose ingestion. Journal of Sports Medicine, 27, 326-332.

Hasson, S.M., & Barnes, W.S. (1989). Effect of carbohydrate ingestion on exercise of varying intensity and duration. Sports Medicine, 8(6), 327-334.

Hawley, J.A., Bosch, S.M., Weltan, S.M., Dennis, S.C., & Noakes, T.D. (1995). The effects of glucose ingestion and glucose infusion on fuel metabolism during prolonged cycling. Journal of Sport Sciences, 13(1), 31.

Hermansen, L., Hultman, E., & Saltin, B. (1967). Muscle glycogen during prolonged severe exercise. Acta Physiologica Scandinavica, 71, 129-139.

Hickson, R.C., Rennie, M.J., Conlee, R.K., Winder, W.W., & Holloszy, J. O. (1977). Effects of increasing plasma free fatty acids on endurance. Federation Proceedings (abstract), 36, 450.

Horowitz, J.F., & Coyle, E.F. (1993). Metabolic responses to preexercise meals containing various carbohydrates and fat. American Journal of Clinical Nutrition, 58, 235-241.

Houston, M.E. (1995). Biochemistry Primer for Exercise Science. Illinois: Human Kinetics.

Ivy, J.L., Costill, D.L., Fink, W.J., & Lower, R.W. (1979). Influence of caffeine and carbohydrate feedings on endurance performance. Medicine and Science in Sports and Exercise, 11(1), 6-11.

Jorgensen, H. (1949). The influence of saccharin on the blood sugar. Acta Physiologica Scandinavica, 20, 33-37.

Kiess, H.O. (1996). Statistical concepts for the behavioral sciences (2nd ed.). Toronto: Allyn and Bacon.

Koivisto, V.A., Harkonen, M., Karonen, S.-L., Groop, P.H., Elovainio, R., Ferrannini, E., Sacca, L., & Defronzo, R.A. (1985). Glycogen depletion during prolonged exercise: influence of glucose, fructose, or placebo. Journal of Applied Physiology, 58, 731-737.

Koivisto, V., Karonen, S-L., & Nikkila, E.O. (1981). Carbohydrate ingestion before exercise: comparison of glucose, fructose, and sweet placebo. Journal of Applied Physiology, 51, 783-787.

Kun, E., & Hovarth, I. (1947). The influence of oral saccharin on blood sugar. Proceedings of the Society for Experimental Biological Medicine, 66, 175.

Levine, I., Evans, W.J., Cadarette, B.S., Fisher, E.C., & Bullen, B.A. (1983). Fructose and glucose ingestion and muscle glycogen use during submaximal exercise. Journal of Applied Physiology, 55(6), 1767-1771.

Loat, C.E.R., & Rhodes, E.C. (1993). Relationship between the lactate and ventilatory thresholds during prolonged exercise. Sports Medicine, 15(2), 104-115.

Louis-Sylvestre, J. (1976). Preabsorptive insulin release and hypoglycemia in rats. American Journal of Physiology, 230, 56-60.

McArdle, W.D., Katch, F.I., & Katch , V.L. (1994). Essentials of Exercise Physiology. Philadelphia: Lea & Febiger.

McLellan, T.M., Cheung,S.S., & Jacobs, I. (1995). Variability of time to exhaustion during submaximal exercise. Canadian Journal of Applied Physiology, 20(1), 39-51.

Newsholme, E. A., & Start, C. (1973). Regulation in metabolism. London: Wiley.

Nieman, D.C. (1990). Fitness and sports medicine: an introduction. California: Bull Publishing Company.

Penick, S. B., Prince, H., & Hinkle, L. E. (1966). Fall in plasma content of free fatty acids associated with sight of food. New England Journal of Medicine, 275(8), 416-419.

Philips, S.M., Green, H.J., Tarnopolsky, M.A., Heigenhauser, G.F., Hili, R.E., & Grant, S.M. (1996). Effects of training duration on substrate turnover and oxidation during exercise. Journal of Applied Physiology, 81(5), 416-422.

Powley, T.L., & Berthoud, H.-R. (1985). Diet and cephalic phase insulin responses. The American Journal of Clinical Nutrition, 42, 991-1002.

Rennie, M., Winder, W. W., & Holloszy, J. O. (1976). A sparing effect of increased FFA on muscle glycogen content in exercising rat. Biochemistry Journal, 156, 647-655.

Renwick, A.G. (1993). Intense sweeteners, food intake and the weight of a body of evidence. Physiology and Behavior, 55, 139-143.

Rodin, J. (1990). Comparative effects of fructose, aspartame, glucose and water preloads on calorie and macronutrient intake. American Journal of Clinical Nutrition, 51, 428-435.

Sakaguchi, T., & Yamaguchi, K. (1979). Effects of electrical stimulation of the hepatic vagus nerve on the plasma insulin concentration in the rat. Brain Research, 164, 314-316.

Sherman, W.M., Brodowicz, G., Wright, D.A., Allen, W.K., Simonsen, J.C., & Dernbach, A. (1989). Effects of 4 h preexercise carbohydrate feedings on cycling performance. Medicine and Science in Sports and Exercise, 21, 598-604.

Sherman, W.M., Peden, M.C., & Wright, D.A. (1991). Carbohydrate feeding 1h before exercise improves cycling performance. American Journal of Clinical Nutrition, 54, 866-70.

Shimazu, T. (1967). Glycogen synthetase activity in liver: regulation by the autonomic nerves. Science, 156, 1256-1257.

Shimazu, T. (1983). Reciprocal innervation of the liver: its significance in metabolic control. Advances in Metabolic Disorders, 10, 355-384.

Teff, K. L., Mattes, R.D., & Engelman, K. (1991). Cephalic phase insulin release in normal weight males: Verification and reliability. American Journal of Physiology, 261(24), E430-E436.

Tordoff, M.G. (1988). How do non-nutritive sweeteners increase food intake? Appetite, 11, 5-11.

Tordoff, M. G., & Friedman, M. I. (1989). Drinking saccharin increases food intake and reference IV. Cephalic phase and metabolic factors. Appetite, 12, 37-56.

Whitney, E.N., & Rolfes, S.R. (1996). Understanding Nutrition (7th ed.). Minnesota: West Publishing Company.

Wolf-Novak, L. C., Stegink, L. D., & Brummel, M. C. (1990). Aspartame ingestion with and without carbohydrate in phenylketonuric and normal subjects: effect on plasma concentration of amino acids, glucose, and insulin. Metabolism, 39, 391-396.

Appendix 1. Revised Borg Scale for Rating of Perceived Exertion

0	Nothing at all
1	Very Weak
2	Weak
3	Moderate
4	Somewhat strong
5	Strong
6	
7	Very strong
8	
8	
10	Very very strong

Appendix 2. Subject Information Sheet

LAKEHEAD UNIVERSITY
955 [illegible] Road Thunder Bay, Ontario, Canada P7B 5E1
School of Kinesiology
Telephone (807) [illegible]
Fax (807) [illegible]

Dear Participant:

Thank you for agreeing to participate in this study on nutrition for endurance sport. Katharine Sodek and Dr. Teresa Socha are the principle investigators in this study entitled "The Effect of Gustatory Stimuli on Endurance Performance".

The purpose of this study is to determine the effect of "taste" on endurance performance. Through your participation in this research, you will help us to establish nutritional recommendations for athletes involved in endurance sport.

Subjects will be required to perform one VO2 max test, and two experimental trials. The VO2 max test will take about 15 minutes. The experimental trials require the subject to cycle at 75% VO2 max until exhaustion. Trials will be completed approximately one week apart, requiring subject participation for just under three weeks. Subjects are asked to report to the lab for a 30 minute control ride 2 days before the experimental trials, and to refrain from training on the day before each trial.

Dietary intake for the two days preceding the first experimental trial must be recorded and replicated for the two days preceeding the second experimental trial. Subjects must report to the two experimental trials after an overnight fast (ie. subjects will not eat breakfast before reporting to the lab). Beverages will be administered during the experimental trials. One or more of the beverages may be sweetened with aspartame, a non-nutritive sweetener that is used in low-calorie sodas.

The risks/side effects associated with this study are those discomforts associated with maximal/exhaustive exercise (to which competitive athletes are quite familiar). It is emphasized that participation in this study is voluntary, and the participant can withdraw from the study at any time.

After all trials have been conducted, an information session will be offered, so that subjects can receive a full explanation of the purpose, hypothesis, and the results/findings of the study.

I look forward to your participation in this exciting research endeavor. If you have any questions regarding this research, I (Katharine Sodek) can be reached at 767-1243.

Sincerely,

Katharine Sodek

Appendix 3. Informed Consent Form

The Effect of Gustatory Stimuli on Endurance Performance

I,_____consent to participate in a study which will examine the effect of "taste" on endurance exercise performance. The results from this study will aid in making nutritional recommendations for endurance athletes.

The principal investigators, Katharine Sodek (767-1243) and Dr. Teresa. Socha, have explained to me that I will be asked to exercise on a bicycle for an initial VO2 max test, then for two experimental trials, during which I will ingest a test beverage. Some of the beverages administered may contain the non-nutritive sweetener aspartame (contained in "diet" soda). Although aspartame has been approved by the FDA, there may be unknown side effects associated with its ingestion. During the two experimental trials, I will be asked to cycle to exhaustion at 70% VO2 max. In addition to these trials, I will report to the lab for control exercise two days prior to each experimental trial and will refrain from exercise on the day before each experimental trial. I will keep a record of my dietary intake during the two days preceding the first experimental trial, and I will replicate this diet to the best of my abilities for the second trial. I will report to the two trials in a fasted state, having not eaten for 10-12 hours (overnight).

The risks/side effects associated with this study are those discomforts associated with maximal/exhaustive exercise (and to which competitive athletes are accustomed).

I understand that there will be no direct benefit to me from participation in this study. I realize that my participation in this study is voluntary, and that I am free to withdraw from the study at any time, even after signing this form. Any information that is collected about me during this study will remain confidential, and should the results be published, I will not be identified in any way.

_____

Signature Of Participant Date

_____

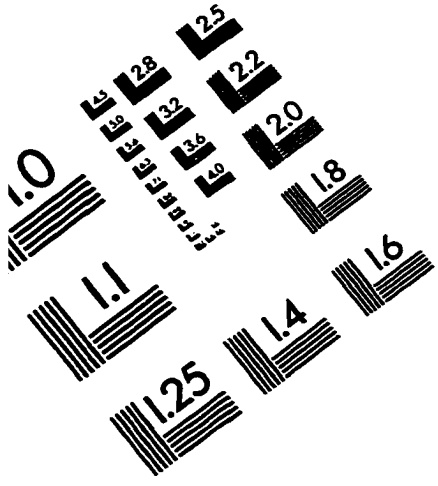
Signature Of Witness Date

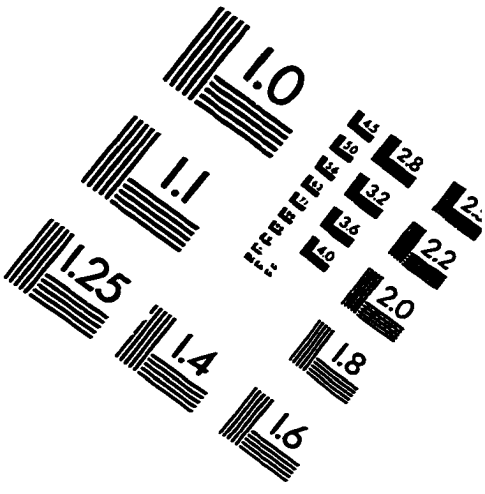
I have explained the nature of the study to the participant, and believe that he/she understands it.

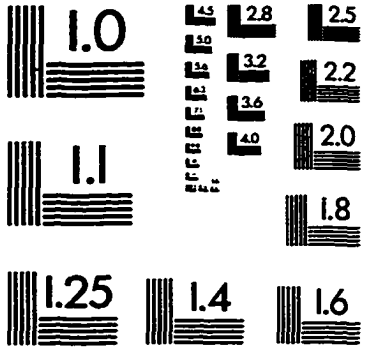
_____

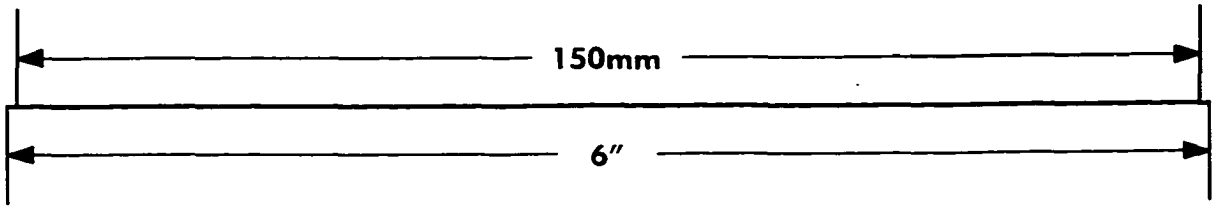
Signature Of Researcher Date

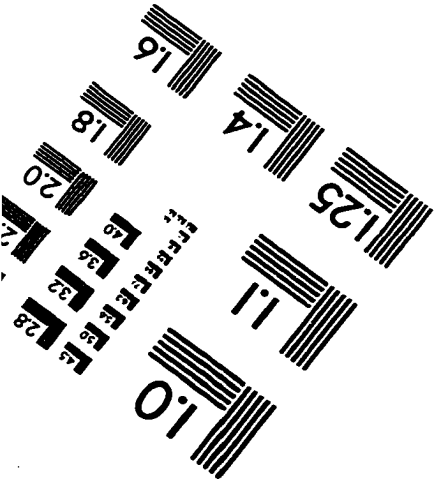
IMAGE EVALUATION
TEST TARGET (QA-3)

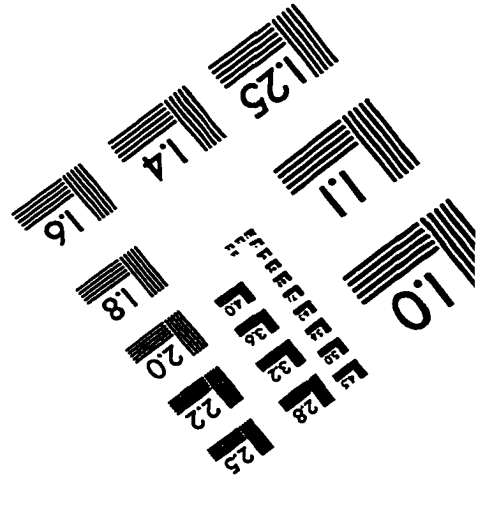












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989