Effects of repeat exercise under different prandial states and diet composition on glucoregulation and appetite

by

Po-Ju Lin

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Doctoral Committee:
Professor Katarina T. Borer, Chair
Professor Charles Burant
Professor Jeffrey F. Horowitz
Professor Karen E. Peterson

DEDICATION

This dissertation is dedicated to:

My father, Han-Fu Lin

My mother, Hsin-Hong Wu

My brother, Po-Yu Lin

My sister, Po-Chun Lin

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ABSTRACT

Glycemic responses to meal ingestion and exercise are important due to their relevance to type 2 diabetes. Studying the impact of a single exposure of exercise or meal on glucoregulation could not reveal the real life situation that meals are eaten, and exercise if necessary, more than once in a day. It requires a better understanding how exercise and meal interactions on glycemia through different prandial states from fasting, to early- then late-postprandial periods. Additional complexity is a circadian decline in glucose tolerance and insulin secretion in the afternoon/evening. This dissertation employs a repeat-event design, using two isocaloric meals and two bouts of moderate-intensity exercise to examine the effects of exercise performed in different prandial states on glucoregulation (Study 1), the influence of dietary composition in this process (Study 2), and the impact of satiating gut peptides, glucagon-like peptide-1 and peptide tyrosine tyrosine, associated with different prandial stages on exercise anorexia (Study 3). In Study 1, exercise significantly lowered blood glucose only when it was performed during late postprandial period, but not during early postprandial or fasting period. In Study 2, reducing carbohydrate content from 60% to 30% of energy intake reduced afternoon postprandial insulin response by 39% in parallel with a 48% reduction in glucose-dependent insulinotropic peptide. In Study 3, appetite suppression was associated with the late postprandial period regardless of the presence or absence of exercise. No consistent and specific association was seen between the two measured satiating gut peptides and exercise anorexia.

This dissertation provides scientific knowledge about the interactions between exercise and meals, including: (1) exercise performed during late postprandial period, but not other prandial states, leads to substantial declines in blood glucose concentration which could have negative impact on exercise performance but a positive impact in hyperglycemic insulin-resistant states; (2) reducing carbohydrate content of the meal to a half can reduce insulin response by 39% within a day, which could reduce the diabetogenic risk of postprandial insulin over-secretion; and (3) the repeat-event design of exercise and diet rather than the exclusive single-event design reveals the complexities of glucoregulation in the real life condition.

CHAPTER 1

Introduction

Glucose is an important metabolic fuel that is available in limited amounts in the liver and in the muscle but supplied to circulation only by the liver and the food. Circulating glucose concentrations reflect a dynamic state consisting of endogenous utilization by the muscle and other tissues, production by the liver glycogenolysis from the modest 70 to 90 g hepatic glycogen store and by gluconeogenesis, and periodic re-supply from food digestion and absorption. Maintenance of stable glucose in circulation is regulated by the brain and hormones at each of these junctures, utilization, production, and meal absorption. Exercise and meals present a special challenge by respectively increasing glucose withdrawal from the blood and its dietary re-supply, so it is important to understand how the intermittent episodes of exercise and meal taking that characterize real life events affect the glucose regulatory process. In the long-standing tradition of physiological investigation, a scientific question is typically addressed by focusing on a single variable with the exclusion of all others. This approach has produced a vast body of information on the effects of individual exercise events of different kinds or different sources of nutrient energy on the uptake, storage, and utilization of glucose and on the regulation of blood glucose concentration. However, human system of energy balance is to eat intermittent meals and to engage in intermittent bouts of physical activity. This real-life situation brings up the question of how exercise performed in different prandial states, fasting, early meal absorption, and late postprandial state, affects glucoregulation, and how different macronutrients, especially carbohydrates, affect glucoregulation in this more complex and interactive situation. The present dissertation addresses this issue by implementing a repeated-exercise and repeated-isocaloric-meal design to determine to what extent the prandial state at the time of exercise (examined in Study 1) and the carbohydrate content of the meals (examined in Study 2) affect the maintenance of blood glucose concentration. Digestive gut peptides, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) were measured in these studies because they are integral concomitants of nutrient

digestion. They stimulate insulin secretion (GIP and GLP-1), and they slow gastric emptying and increase satiation. These gut peptide properties were of interest to examine their possible role in glucoregulatory effects on the exercise-meal interactions (Studies 2 and 3) and their possible involvement in exercise anorexia, the poorly-understood temporary decline in hunger and increase in satiation during and immediately following exercise (Study 3).

As the repeat-event experimental paradigm allowed also implementation of alternate timing of meals and exercise, it was possible in the three studies to examine the effects of exercise on glycemic responses under three prandial states that characterize human intermittent meal taking: the fast, the early post-meal glucose absorption, and the late postprandial state of waning insulin and gut peptide concentrations.

Identification of the prandial state that is vulnerable to blood glucose declines during exercise, and of the contribution to this phenomenon of the macronutrient composition of the meals may be of use both to the athletes whose performance may be limited by declines in circulating glucose availability and to insulin-resistant hyperinsulinemic subjects who may benefit by such outcome.

The three studies in this dissertation examined:
(Study 1) Postprandial glucose responses to timing of repeat exercise and meals;
(Study 2) Postprandial glucose and insulin responses to dietary macronutrient content and exercise; and
(Study 3) Exercise anorexia: influence of gut peptides and dietary macronutrient composition.

CHAPTER 2

Review of literature

The purpose of this dissertation is to examine how the repeated events of exercise and meals that simulate the habitual intermittent pattern of eating and physical activity in humans affect glucoregulation and insulin and psychophysical responses to both meals and exercise. This is important because a significant proportion of people in developed countries exhibit hyperglycemia and insulin resistance [1] that can lead to type 2 diabetes [2], and because even healthy individuals display an afternoon decrease in glucose tolerance that may represent a predisposition for development of insulin resistance. It is therefore of particular interest to determine whether the diurnal decline in glucose tolerance may be a consequence of the current dietary recommendations and preferences for high-carbohydrate diet and whether reducing the carbohydrate (CHO) load of the meals and applying exercise can reduce glucose intolerance in the afternoon and evening and hyperglycemia in general. The approach in this dissertation to resolution of these questions has been to examine (1) the influence of the prandial state at the time of exercise on glucoregulation in Study 1, (2) the effectiveness of reducing the CHO load in both meals or the glycemic index (GI) in the morning meal on the postprandial insulin response and its possible dependence on insulinotropic gut peptides glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) in the context of exercise, in Study 2, and (3) determine whether exercise anorexia, a transient suppression of hunger and increase in the satiation during and immediately following exercise, is also influenced by the prandial state and the associated secretion of satiating gut peptides, GLP-1 and peptide tyrosine tyrosine (PYY), in Study 3.

Chief glucoregulatory hormone of insulin is glucagon, although epinephrine (E), norepinephrine (NE), cortisol, and growth hormone (GH) also oppose insulin actions but will not be considered in the dissertation studies. Insulin secretion is initiated by absorbed glucose and amino acids and is amplified by secretion of incretin hormones, gastric inhibitory polypeptide or glucose-

dependent insulinotropic peptide (GIP), and glucagon-like peptide-1 (GLP-1) which react to nutrients as they are being absorbed [3-5]. Among the absorbed nutrients, the primary trigger for insulin release from pancreatic β cells is a rise in plasma glucose concentration and to a lesser extent an increase in amino acid concentration. Insulin stimulates muscle and adipose tissue glucose uptake, inhibits hepatic glucose production (HGP) and glucose release into circulation, and stimulates utilization of carbohydrates as a fuel. On the other hand, a decline in plasma glucose to a threshold concentration of about 50 mg/dl [6] stimulates glucagon secretion from pancreatic α cells and triggers additional counterregulatory hormonal and autonomic reflexes that increase hepatic glycogenolysis and gluconeogenesis. When liver glycogen supplies are low, growth hormone and cortisol contribute to gluconeogenesis, but this role during exercise is largely carried out by glucagon [7]. Exercise, on the other hand, activates sympathetic outflow to the pancreatic β cells that, in turn, suppresses insulin secretion through norepinepherine action on α adrenergic receptors [8]. In addition to reducing insulin hypoglycemic action, sympathetic suppression of insulin secretion reduces inhibition by insulin of HGP. Thus, exercise in different prandial states may profoundly affect both postprandial glucose appearance and HGP during exercise. It is important to examine hormonal role in glucoregulation during and after exercise bouts in different prandial states.

Challenges to glucoregulation: Meal eating and exercise

Glucoregulation is challenged both by meal eating when insulin and gut peptide secretion predominate, and during exercise when insulin secretion is blocked and the counterregulatory responses are elicited. During nutrient absorption, secretion of incretin gut peptides is increased, and they enhance insulin response in anticipation and in conjunction with the appearance of absorbed glucose and amino acids in systemic circulation. With CHO-rich diet, incretin hormones, GIP (glucose-dependent insulinotropic peptide) and GLP-1 (glucagon-like peptide 1) stimulate approximately 50-70% of postprandial insulin secretion [9].

During exercise, two processes challenge glucoregulation. First, insulin secretion is inhibited during exercise due to sympathetic α adrenergic inhibition of insulin release from action via receptors on pancreatic beta cells [8]. Second, muscle glucose uptake increases through a translocation of the glucose transporter, GLUT4, to sarcolemma that creates a glucose drain

through an insulin-independent mechanism. Insulin-independent increase in glucose tolerance continues for several hours after exercise [10] and appears to be mediated by persistence of phosphorylated state of AS160, a protein substrate of Akt [11-13].

Exercise-associated hypoglycemia

Although glucose contributes only about one half of the metabolic fuel during moderate-intensity exercise [14], there has been considerable interest in circumstances that lead to declines in plasma glucose during exercise that may cause transient disturbance in glucoregulation. Here are three sets of exercise-linked and/or meal-associated circumstances that may affect glycemic responses. The first circumstance is exercise-associated hypoglycemia seen when glucose is ingested within one hour before the onset of exercise [15-18]. This hypoglycemic effect could be caused by increased postprandial insulin concentration stimulating insulin-dependent glucose uptake and the insulin-independent exercise effect on increased muscle glucose uptake. The time lag between stimulation of insulin secretion by glucose ingestion and sympathetic suppression of insulin secretion at the start of exercise contribute to the decline in blood glucose because both events lead to increased muscle glucose uptake. The second circumstance that leads to progressive lowering of blood glucose to the level of hypoglycemia is prolonged moderate-intensity exercise that depletes the liver glycogen and overcomes its gluconeogenic capacity [19, 20]. The third glucoregulatory deviation leading to lower postprandial or 12-hour overall blood glucose concentrations can be produced by repeated short, intermittent bouts of exercise rather than an energy-matched single bout of exercise [21, 22]. Three bouts of 15-minute postprandial exercise reduced postprandial hyperglycemia in older obese subjects to a greater extent than a single energy- and intensity-matched 45-min walk performed in the morning or in the afternoon [21]. Hourly 5-minute moderate-intensity exercise produced lower glucose concentrations over a 12-h period compared to a single 1-h bout of morning exercise in young obese individuals [22]. These two studies point to the importance of repeat events of exercise and meals on glycemic responses and raise the question about the relevance of prandial status during exercise on glucoregulation.

The influence of exercise in different prandial states on glucoregulation

Some studies suggest that the prandial state at the time of exercise could have a profound effect on plasma glucose concentration and glucose uptake. A moderate-intensity exercise bout performed after a CHO-rich meal acutely reduced the meal-induced hyperglycemia [23, 24] while exercise of the same intensity performed before the same type of meal did not [25]. Another study found that if a single bout of exercise was performed 2 hours after the meal, it produced a 15% greater hyperglycemic response to the following meal, but no such glucose intolerance was found if the first meal was omitted [26]. Different findings were found if two bouts of moderate-intensity exercise are separated by seven hours and carried out in fasted or post-absorptive state. Plasma glucose concentration declined between the two exercise bouts [27] and caused either a delayed and sustained blood glucose lowering after the second exercise bout [27] or deficient counterregulation to a hypoglycemic challenge [28-30].

The effect of the time interval between repeated exercise bouts on glucoregulation

Athletes often train more than once in a day, and hormonal regulation of plasma glucose during the rest periods between exercise bouts is not fully understood. When the rest periods are of short duration, for instance 30 min, then repeated 30-min exercise bouts over a 2-hour period elicit progressive elevation of counterregulatory hormones with a moderate decline in plasma glucose [31]. However, when 90 to 120 min exercise is repeated after 3- to 5-hour rest period, then plasma glucose declines after both exercise bouts if performed in fasted or postabsorptive state, but not if it is carried out in postprandial state [27]. Likewise, hormonal control of glucoregulation is impaired during a hypoglycemic challenge following two bouts of exercise separated by 3-hours if they are performed in fasted or postabsorptive state [28-30].
Clearly, additional study is warranted for a better understanding of the hormonal involvement in this post-exercise glucoregulatory disturbance caused by a temporal delay between exercise bouts when the meals precede, but not when they follow physical activity as well as other effects of different temporal application of the exercise stimulus. The change in counterregulatory hormonal response to two spaced bouts of exercise in postabsorptive state could reflect both reduced hepatic glycogen capacity and inadequate post-exercise hepatic glycogen repletion due to different affinities of muscle and liver phosphorylating enzymes. Liver accumulates only about 90 g of glycogen during the 5-hour postprandial period [32]. Liver glycogen declines at

about 4.5 to 5 g per hour overnight and during rest. If morning exercise is performed in fasted state, the liver may be already about 37% glycogen depleted [27]. Due to 100 times greater affinity for glucose of hexokinase (Km=100μM) than of hepatic glucokinase (Km= 10mM), muscle glycogen repletion takes precedence over liver glycogen re-synthesis during several hours after exercise [33, 34]. The sustained delay in hepatic glycogen re-synthesis and increased post-exercise muscle glucose uptake and re-synthesis may trigger changes in HGP and in counterregulatory hormones that lower plasma glucose and reduce responses to hypoglycemic challenges.

In addition to the effects of different timing of meals and exercise, duration of exercise and the duration of time interval after glycogen- depleting exercise will also affect glucose availability in circulation.

Plasma glucose concentration also is affected by the time of the day. Evening PP glucose concentrations are consistently higher than morning glycemia to the same nutrient stimulus. It is, therefore, of substantial interest to find out whether exercise, either before or immediately after the afternoon meal can reduce this apparent glucose intolerance.

Diurnal decline in glucose tolerance

Postprandial insulin and glucose responses may be influenced by the circadian clock as glucose tolerance and insulin sensitivity decline in the afternoon and evening. A diurnal decline in glucose tolerance has been reported after a single oral challenge of between 50 and 100 g of glucose [35-37] or twice daily as 25 g intravenous glucose infusions in the morning (7-9 am) and in the afternoon (3pm) or evening (7 pm) [35, 38], as well as continuous intravenous glucose infusion at a constant low rate [39, 40]. Repeat high-CHO meals providing 50% of total daily calories raise postprandial glucose and insulin concentrations in the afternoon compared to the morning [41]. Findings from these studies indicate that glucose tolerance is reduced in the afternoon over an extended period of time compared to the more rapid plasma glucose clearance in the morning.

Insulin responses also follow a pattern suggestive of declining insulin sensitivity throughout the day. Insulin response is greater within the first hour of glucose loading in the morning compared to its protracted elevation after glucose administration in the afternoon [35-37]. Insulin response is about 50% lower in the afternoon compared to the same treatment with an 100 g oral glucose load or 25 g intravenous glucose dose applied 12-hour earlier in the morning despite a protracted plasma glucose elevation in the afternoon [35]. Insulin concentration remains relatively stable but its secretory rate rises during the glucose infusion at 5g/kg/24h [39, 40]. Similar diurnal decline of insulin sensitivity is also seen in type-1 diabetic patients for whom insulin dose needs to be increased in the course of the day to maintain euglycemia [42].

Therefore, there is a need for a better understanding of the hormonal glucoregulatory responses to either temporal influences on blood glucose availability that result from different deliberate variations in the timing of meals and exercise or to the obligatory circadian timing effect on the glycemia. Using the study paradigm of two daily bouts of exercise, one in the morning and the other in the afternoon, applied in different prandial states with respect to repeat daily meals, offers the opportunity for a systematic study of the effect of exercise at three different prandial states: fast, early postprandial period (1-hour after the first meal), late postprandial period (4-hour after the first meal), on the glycemic responses.

The specific aim of Study 1 is to examine the effects of exercise in different prandial states: fast, early postprandial state, 1 hour after the meals, and late postprandial state, 4 hours after the first meal, on glucoregulation in a repeat-event experimental paradigm. The hypothesis is that that moderate-intensity exercise during both early postprandial periods will reduce the glycemic and insulinemic responses to high-CHO meals because of coincident actions of insulin and exercise and eliminate afternoon decline in glucose tolerance compared to the same exercise performed during fasting or late PP period and to sedentary state, where only one of the two variables is operating.

Influence of the glycemic index of carbohydrates on glucoregulation during exercise

The rate of postprandial glucose appearance in blood varies according to the properties of individual CHO sources. The glycemic index (GI) of foods influences the rate of glucose

absorption and is defined as the area under the two-hour blood glucose response curve following the ingestion of 50 g of CHO using 50 g of glucose as a reference. High GI of food leads to rapid increases in postprandial blood glucose and insulin levels [66] and stimulates appetite to promote overeating [67, 68]. GI can influence plasma glucose when simple sugars or mixtures of dietary CHOs are ingested 45 to 60 minutes before acute, higher-intensity exercise. A high-GI meal, compared to a low-GI meal, produces higher postprandial glycemic responses and leads to a greater decline in blood glucose concentrations in response to exercise [69]. That may be because a high-GI meal, relative to a low-GI meal, not only increases muscle glycogen storage but also leads to greater utilization of muscle glycogen during subsequent exercise [70]. High-GI meal leads to greater glycogen repletion and higher postprandial glycemia and insulin responses during the 24-hour recovery from glycogen-depleted exercise, than does the low-GI meal [71, 72]. Glucose solution ingested before exercise produces greater hypoglycemia during an exercise bout than the same amount of fructose solution [73, 74]. A possible explanation for the glucoregulatory disturbance to exercise performed in early postprandial state [27] may be due to a high-GI morning meal provided in the study. This is due to the combined hypoglycemic effects of increased insulin responses to the high-GI meal as well as increased insulin-independent, exercise-associated muscle glucose uptake during exercise. In contrast, low-GI diets elicit slower and smaller postprandial glucose and insulin responses [75]. These acute GI effects [66] extend to the postprandial stage of a subsequent meal many hours later [76]. Moreover, when food of low GI is eaten a short time before exercise, hypoglycemic response is reduced [77]. A low-GI meal eaten the night before the trial improved glucose tolerance to the next-morning meal [76]. Afternoon postprandial glycemia and insulin response are reduced after a morning meal of low GI [78, 79]. The knowledge of the effect of the glycemic load (a product of the quantity of ingested CHOs and their glycemic index) on glycemic and insulinemic responses to repeated exercise bouts is still needed. Therefore, the effect of GI in the morning meal on postprandial glycemic and insulin responses to exercise will also be examined in Appendix A with the same experimental paradigm as in Study 1.

Study 2: Postprandial insulin and glucose responses to dietary macronutrient content and exercise

Postprandial glycemic and insulin responses can be modified by macronutrient composition of the meals. Mixed meals containing 50-75% CHO stimulate greater glycemic and insulinemic responses than low-CHO (20-30%) meals [41, 43] during the 2-4 hour postprandial periods. Such high-CHO meals abolish diurnal decline in glucose tolerance when they are of moderate size (25% of daily total calories) but not if they are large (50% of total calories) [41, 43]. Previous studies also reported that long-term intake of low-CHO diets improves diabetes control as well as promotes the weight loss [44-46]. Johnsson et al. (2009) reported that 3-months of Paleolithic-type low-CHO diet lowered HbA1c more than the usual recommended low-fat diet for treatment and prevention of diabetes [45]. Tay et al. (2014) also reported better glycemic control on a very low-CHO and low-saturated fat diet than on a low-saturated fat, high-CHO diet [46].

Macronutrient composition of meals can affect the secretion of incretin peptides that, in turn, may affect insulin secretion and plasma glucose during exercise. Both incretin hormones, GIP [47-49] and GLP-1 [50] respond to both fat and CHO [51-56]. GIP is secreted from duodenal K cells and stimulates insulin biosynthesis and, less conclusively, secretion [53]. Exogenous GIP administration during a mixed meal does not increase insulin secretion; affect gastric emptying [56] or energy intake [57]. GLP-1 is secreted from L cells located in the ileum and colon. It plays a more critical role in enhancing glucose-induced insulin secretion than GIP [5, 56-58] and also inhibits glucagon secretion. In addition, high-fat meals [59, 60] or direct intra-duodenal infusion of lipid into the small intestine [61, 62] slowed gastric emptying, attenuated blood glucose and insulin responses, and stimulated incretin secretions, particularly of GLP-1 [63]. Thus as both GIP and glucagon-like peptide-1 (GLP-1) have insulinotropic incretin actions [4, 64], exercise could also affect insulin secretory response by altering the postprandial responses of these gut hormones. In support of incretin hormone responsiveness to exercise, a single bout of exercise has been reported to reduce postprandial GIP response to glucose [65]. In Study 2 of this dissertation, the effects of macronutrient composition of the meals and of exercise on glycemic and insulin responses are examined as is the proposition that any changes in glycemic

and insulin responses to exercise could be mediated by a dietary effect on incretin hormone secretion.

Since multiple daily meals of variable macronutrient composition are customarily eaten by healthy individuals as well as those at risk of pre-diabetes and diabetes, and some of them choose or need to engage in physical activity more than once in a day, Study 2 examines the effects of dietary manipulation of macronutrient composition and exercise on postprandial glucose and insulin responses. The effect of two large daily meals differing in CHO content by a factor of two with the absence (experiment 1) or presence (experiment 2) of preceding aerobic exercise on postprandial insulin and glucose responses will be examined. Two hypotheses are: (1) that large customary dietary CHO load is the cause of the afternoon glucose intolerance and of a large insulin response mediated, in part, by the insulinotropic incretin hormones GIP and GLP-1; and (2) that moderate-intensity aerobic exercise performed before each meal will reduce to a greater extent the afternoon glucose intolerance and high insulin responses in trials with high-CHO meals than with low-CHO meals.

Study 3: Exercise anorexia: influence of gut peptides and dietary macronutrient composition

Exercise is frequently used as a means of weight control or weight loss. The transient hunger suppression during and shortly after the exercise and absence of post-exercise compensatory food intake for energy expended during exercise has been characterized as "exercise anorexia", a reduction in the sensation of hunger, also usually accompanied by increased sensation of fullness [80-85]. The cause of exercise anorexia is not known, and as it not consistently elicited, the dietary and exercise conditions necessary for its elicitation also are not well understood. Exercise anorexia is not consistently elicited. In some studies, it is elicited when a threshold exercise intensity of about 65-70% of maximal effort is exceeded [81, 82, 85], and the magnitude of hunger suppression is proportional to duration of exercise above this threshold intensity [82].

Two recently published studies using isocaloric bouts of moderate and high-intensity exercise showed different effect of exercise intensity on appetite responses in normal weight vs. overweight/obese men [86, 87]. Deighton et al. [86] reported that normal-weight men showed a

more significant suppression of hunger if they performed high-intensity than moderate-intensity exercise. However, overweight/obese individuals showed no difference in appetite responses between moderate and high-intensity exercise [87]. In other studies, exercise anorexia was elicited at lower intensity (46% of VO_2max) in healthy postmenopausal women [27]. Despite its inconsistent manifestation, the phenomenon is of interest because it, along with absence of compensatory increase in post-exercise food intake, may contribute to weight loss and weight-loss maintenance [82, 88].

Exercise anorexia and gut peptides

The mechanism of exercise anorexia is not understood. One hypothesis is that it may be mediated by exercise-induced changes in the secretion of gut hormones, including ghrelin, cholecystokinin (CCK), GLP-1 and PYY [84, 89-93]. Among the gut hormones, ghrelin is secreted by the stomach [94, 95] and is viewed as an appetite-stimulating hormone as its concentration changes in parallel with hunger sensation [96-98].

The gut hormone hypothesis could be supported if exercise anorexia and concurrent increase in satiation could be found to be associated with increased satiating gut hormones. Two most frequently measured gut hormones, GLP-1 and PYY, secreted by L cells in the distal intestine and colon in response to food ingestion [99, 100], have been implicated in conscious sensations of fullness or satiation [101-105]. In some studies, both GLP-1 and PYY concentrations were reported to increase significantly during, and shortly after a half-, or one-hour bout of moderate-intensity exercise performed one hour after eating [84, 92, 93]. However, the changes in GLP-1 and PYY concentrations were not invariably associated with changes in appetite responses or subsequent energy intake. Ueda et al. (2009) reported reciprocal relationship between increases in GLP-1 and PYY concentrations during and shortly after exercise and hunger ratings in one study [92] but not in the other [93]. The only study that reported the association between a satiating gut peptide and exercise anorexia during fasting period was that of Broom et al. [89]. There was a brief overlap between the period of exercise anorexia and an increased plasma PYY concentration in this study. A subsequent meal did not elicit increases in PYY. Another gut hormone secreted by the proximal part of GI tract is glucose-dependent insulinotropic peptide (GIP), secreted by the K cells in the duodenum and jejunum [106]. The association of GIP with

satiation has not been established, but it reflects early gastrointestinal filling and plays a role in suppression of gastric emptying [107] and for that reason will be measured in these studies.

Influence of macronutrient composition on the secretion of gut peptides

The secretion of postprandial gut hormones is affected by the macronutrient composition of the meals. The secretion of GLP-1 is predominately stimulated by CHOs and fat [51, 52, 108], while a higher concentration of plasma PYY is seen after fat-, rather than CHO-, or protein-rich meals [108-110]. Both GLP-1 [111] and PYY [112] act as the ileal brake, a distal-to-proximal gastrointestinal feedback mechanism that optimizes nutrient digestion and absorption [113]. Although the association of GIP and satiation has not been established, GIP is secreted in the proximal small intestine in response to ingested fat and CHO [51, 55] and was be measured along with GLP- 1 and PYY in Study 3.

Study 3 of this dissertation tests the hypothesis that exercise anorexia is in part caused by increased exercise-associated concentrations of GLP-1, and PYY. Sensations of hunger and satiation are measured during exercise and postprandial periods under three experimental conditions: Experiment 1: where exercise is performed in fasted state before the first meal and both the early PP (1-3 hours after the first meal) and late PP (4-6 hours after the first meal), Experiment 2: where the duration of exercise before the meals differs by a factor of two (1-hour vs. 2-hour exercise bouts), and Experiment 3: where the CHO content of the meals differs by a factor of two (60% vs 30% CHO content) and exercise is performed before the meals.

The three hypotheses exploring the relationship of exercise anorexia and digestive gut peptide secretion are:

(1) Both exercise anorexia and the satiating gut peptide concentrations will be higher during postprandial periods than during the pre-meal fasting period because of the coincidence of exercise and gut peptide secretion in the former condition; also the association between exercise anorexia and satiating gut peptide concentrations will be stronger during the early than during late PP because of the higher gut peptide concentrations in the early PP;

(2) Longer exercise bouts before the meals will produce greater exercise anorexia and longer elevation of gut peptide secretion than exercise bouts that are half as long; and

(3) Exercise anorexia will be greater during subsequent low-CHO than high-CHO meals because low-CHO meals are reported to have stronger stimulatory effect on postprandial GLP-1 and PYY secretion.

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CHAPTER 3

Postprandial glucose responses to exercise in different prandial states

Abstract

Circulating glucose concentration transiently increases after meals and is cleared by insulin action leading to increased tissue glucose uptake and glucose metabolism. Exercise, on the other hand, increases glucose clearance through insulin-independent mechanism and may lead to hypoglycemia if exercise is performed during early postprandial (PP) period when insulin level is still high. It is not fully understood how exercise affects blood glucose if it is repeated during a day in different prandial states. The design of this study examines the effects of exercise on glycemia as a factor of different prandial states: fasting state before the first meal, early postprandial state 1-4 hours after morning and afternoon meals, and late PP state 4-7 hours after the morning meal. It addresses the question of whether the changes in exercise-associated glycemia are a function of prandial state or also of a known afternoon increase in glucose intolerance that could be independent of prandial state. This study was guided by the hypotheses that moderate-intensity exercise during both early PP periods in this repeat-event design will (1) reduce the glycemic and insulinemic responses to high-carbohydrate (CHO) meals because of coincident actions of insulin and exercise and (2) eliminate afternoon decline in glucose tolerance compared to the same exercise performed during fasting or late PP period and to sedentary state, where only one of the two variables is operating.

Methods. Subjects were healthy postmenopausal women matched by body weight and BMI. Two isocaloric high-CHO meals containing 60% CHO, 15% protein and 25% fat were provided at 1000 h and 1700 h. Two 2-hour moderate-intensity exercise bouts were completed either 1-hour before (EBM), or started 1-hour after (EAM) the two meals. Metabolism was measured by indirect calorimetry at rest and during exercise. The effects of exercise before the high-CHO meals (EBM, n=13) were contrasted to the effects of exercise after the meals (EAM, n=13) and compared to sedentary (SED, n=8) trials with the same type of meals.

Measurements. Changes in concentrations of glucose, insulin, glucagon, free fatty acids (FFAs) and D-3-hydroxybutyrate ketone body were assessed as areas under the curve (AUCs). The AUCs were calculated for the 4-hour PP periods after the onset of each meal (early PP) and for the 3-hours from the onset of each 2-hour bout of exercise.

Results. Exercise reduced blood glucose only when it was performed during late PP period 4 hours after the first meal (second EBM exercise bout), but not in fasted state before the first meal (first EBM exercise bout), or during both early PP periods 1-hour after both meals (EAM exercise bouts). However, in all three groups, PP glycemic response after the afternoon meal was significantly higher than after the morning meal. EAM exercise during both early PPs led to a significant decline in insulin AUCs compared to the PP insulin response in EBM group when exercise was performed during fasting or late PP state. Glucagon responses were significantly higher during late PP than during fasting period in all three groups. Exercise during both early and late PP periods led to an increase in FFA AUCs, and the increase was greatest during late PP. D-3-hydroxybutyrate AUC increased significantly more during the late PP exercise, and the rise extended through the early part of the second PP period compared to changes during fasting exercise (first EBM exercise bout) and early part of the first PP period.

Conclusions. Confluence of high insulin and exercise during early PP state after high-CHO meals did not have a significant and prolonged hypoglycemic effect due to rapid and significant rise in glucagon and decline in insulin AUCs. A significant and protracted decline in blood glucose occurred during 2 hours of exercise and one post-exercise hour in late PP. This was associated with high FFA and ketone body responses at the time insulin had returned to low basal concentration. Thus exercise 4 hours after a large high-CHO meal appears to reduce CHO availability during exercise in late PP period relative to exercise performed 1 hour after such a meal or while fasting. An afternoon decline in glucose tolerance was unaffected by 2 hours of moderate-intensity exercise performed either preceding or following the meal indicating the afternoon rise in PP glucose intolerance is refractory to moderate-intensity exercise when coupled with large high-CHO meals. Therefore, regarding hypothesis (1) it appears that the metabolic consequences of timing of high-CHO meals to late PP period rather than the coincidence of high insulin state and exercise-associated glucose uptake are responsible for protracted declines in blood glucose. With respect to hypothesis (2), increased afternoon PP

glycemia to the large high-CHO meal is a consequence of some circadian variable independent of actions of 2-hour long moderate-intensity exercise.

Introduction

Glycemic responses to food ingestion and exercise have been extensively investigated due to their relevance to glucose availability for exercise performance and for mitigation of some chronic conditions such as the metabolic syndrome or type 2 diabetes. Increased plasma glucose concentration after meal absorption and digestion triggers secretion of insulin from the pancreatic β cells. Insulin stimulates glucose uptake particularly by the muscle and some other tissues, increases carbohydrate utilization, and inhibits hepatic glucose production by glycogenolysis and gluconeogenesis and its release into circulation. In contrast, secretion of the counterregulatory pancreatic hormone glucagon is stimulated by a decline in plasma glucose to a threshold concentration of about 50 mg/dL [1]. Several additional counterregulatory hormonal and autonomic reflexes restore euglycemia through increased hepatic glycogenolysis and gluconeogenesis in hypoglycemic states.

Exercise, on the other hand, activates sympathetic outflow to the pancreatic β cells that, in turn, suppresses insulin secretion through norepinepherine action on α adrenergic receptors [2]. Exercise also can increase muscle glucose uptake through an insulin-independent mechanism [3-5].

Exercise in different prandial states may profoundly influence postprandial (PP) glucose concentration by affecting both insulin-mediated and insulin-independent glucose clearance. The significant but inconsistent effects of the prandial state are evident in so-called rebound hypoglycemia that ensues when high-intensity exercise is performed during early PP state following ingestion of glucose [6-9]. This rapid hypoglycemic effect results from the additive effects of both the insulin-independent muscle glucose uptake during exercise and insulin-induced glucose uptake before sympathetic suppression of insulin secretion starts taking effect. Similarly, a moderate-intensity exercise bout performed immediately after a carbohydrate (CHO)-rich meal was reported to acutely reduce meal-induced hyperglycemia [10, 11] while the same exercise performed before the same type of meal does not [12]. However it also was reported that exercise performed 2 hours after the meal produces a 15% greater PP hyperglycemia (rather than reduced glycemic response) than when it is performed in fasted state

[13]. Therefore, the consequences of meal eating have variable and inadequately understood effects on glucoregulation during exercise.

Glucose lowering effect also can be produced by temporal structuring of exercise. PP and 12-hour overall glucose concentrations can be lowered by repeated short, intermittent bouts of exercise compared to an isocaloric single bout of exercise [14, 15]. Also, three bouts of 15-minute exercise reduced PP hyperglycemia in older obese subjects to a greater extent than a single bout of energy- and intensity-matched 45-min walk [14]. In addition, hourly 5-minute moderate-intensity exercise reduced plasma glucose concentrations over the 12-hour period to a greater extent than a single 1-hour bout of morning exercise in young obese individuals [15]. All of these studies suggest that in addition to the prandial state, patterns of repeat exercise can affect glycemia differently than a single energy-matched bout of exercise.

The effects of prandial state and patterning of exercise on glucoregulation are poorly understood, and the knowledge gap is even greater when these two variables are combined. Since human life entails intermittent patterning of eating and physical activity, it is particularly important to understand how the prandial state during exercise and the temporal structuring of exercise affect glucoregulation. Glucoregulation appears severely disrupted when repeated bouts of exercise are performed in fasted or postabsorptive state than in PP state. Thus, when two bouts of moderate-intensity exercise are separated by 7 hours and carried out in fasted state, plasma glucose concentration declines between the two exercise bouts [16]. After the second exercise bout in postabsorptive state, sustained blood glucose lowering was reported [16], and deficient counterregulation to a hypoglycemic challenge occurred several hours later [17-19].

The final complexity regarding temporal circumstances of glucoregulation entails an apparent circadian influence on PP glycemia and insulin response. Glucose tolerance and insulin sensitivity are lower in the afternoon or evening than in the morning. A diurnal decline in glucose tolerance has been reported in comparisons of a single oral challenge of between 50 and 100 g [20-22] or twice daily as 25 g intravenous infusions in the morning (7-9 am) and in the afternoon (3pm) or evening (7 pm) [20, 23], or when the effects of a continuous intravenous infusion at a constant low rate are examined [24, 25]. In all these tests, glucose tolerance is

reduced in the afternoon over an extended period of time compared to the more rapid plasma glucose clearance in the morning.

In view of the habitual human intermittent patterning of meals and exercise, and because of the contradictory reports on the effects of prandial state during single and repeated exercise bouts and the presence of a circadian influence on glycemia, this study was designed to systematically examine the influence of fasting and of early and late PP states on glycemia and insulin responses during two exercise bouts, one taking place in the morning, and the other in the afternoon. Two isocaloric weight-maintenance meals were provided at fixed times, and two 2-hour moderate-intensity exercise bouts were performed either 1 hour before the meals (EBM) or 1 hour after the completion of the meals (EAM). This provided the opportunity to contrast the effects on glucoregulation of exercise during fasting (first EBM exercise bout), early PP state (two EAM exercise bouts), and late PP (second EBM exercise bout), as well of morning vs. afternoon exercise bout in both EBM and EAM trials.

The two hypotheses of this study posited that (1) a combination of high insulin response and exercise during early PP state will produce greater lowering of plasma glucose than exercise performed during fasting or late PP period when insulin level is low, and (2) the afternoon 2-hour moderate-intensity exercise will eliminate diurnal decline in glucose tolerance.

Methods

Subjects

The eligibility criteria included: healthy and non-smoker postmenopausal women, age 50-65 years; body mass index (BMI) between 20-30 kg/m^2; fasting glucose level < 100 mg/dl; hematocrit > 32%, hemoglobin > 12 mg/dl; and absence of restricted food intake, endocrine and metabolic disorders requiring medication other than hormonally corrected hypothyroidism and musculoskeletal disabilities that would prevent walking.

Thirty-four postmenopausal women, mean age of 57.7±0.71 years and BMI of 24.0±0.52 kg/m^2 met study criteria. After matching by body weight and BMI, subjects were assigned to either

sedentary (SED, n=8) or one of the exercise groups, exercise-before-the-meals (EBM, n=13) or exercise-after-the-meals (EAM, n=13).

General experimental protocol

All subjects signed an informed consent approved by The University of Michigan Medical School Institutional Review Board and underwent two preliminary screening tests at Michigan Clinical Research Unit (MCRU). The health screening test included measurements of weight, height, and body fat by a dual-energy X-ray absorptiometry (General Electric Lunar Prodigy Advance), physical and health-history interview, and a fasting blood draw for checking laboratory chemistries and thyroid function. The fitness screening test assessed individual maximal aerobic effort by indirect calorimetry to assign a relative exercise intensity. It consisted of walking on a treadmill at 3 miles per hour with 2% slope increments every 3 minutes and the subject's respiratory gases routed through a mouthpiece analyzed by a Max II metabolic system (AEI Technologies, Inc., Bastrop, TX). The criterion of maximal effort (VO_2max) was a respiratory quotient of 1.

After matching by body weight and BMI, subjects were assigned to study groups. A meal was provided at 1900 h after subject's admission to MCRU at 1800 h on the day before the study trial. The meal contained one-third of subject's weight-maintenance energy need (30 kcal/kg body-weight). A template menu for a pre-study meal differing in CHO content is presented in Tables 3-2. The macronutrient composition of the pre-trial evening meal was the same as that provided during the trial. A catheter was inserted into an arm vein at 1930 h for blood collection and kept patent with sodium heparin. Blood was collected hourly, and also at 15- and 30-min intervals during meals and exercise sessions on study day (Table 3-1).

Exercise

In exercise trials, treadmill walking was carried out at 45% of maximal aerobic effort. In exercise-before-the-meals (EBM) trials, 2-hour exercise bouts were completed 1-hour before the start of each meal, from 0700- 0900 h to assess the effect of exercise in fasted state and from 1400- 1600 h to assess the effect of exercise during late PP period (Figure 3-1). In exercise-after-the-meals (EAM) trial, the exercise was completed 1-hour after the onset of each meal, from

1100 to 1300 h and from 1800 to 2000 h to assess the effect of exercise during early PP period (Figure 3-1). Exercise intensity was adjusted by modifying the treadmill incline while the walking speed remained constant at 3 miles per hour. Sedentary subjects engaged in no structured physical activity (Figure 3-1).

Indirect calorimetry

Resting metabolism was measured by indirect calorimetry between 0600-0630 h on the study day and on the morning of discharge from the MCRU using Viasys apparatus (Respiratory Care Inc., Yorba Linda, CA). The same method was also used to measure early PP metabolism at 1030-1100 h and 1730-1800 h, immediately after the meals, and exercise metabolism during the first half hour of each hour of exercise bouts. Energy expenditure (EE) and relative CHO and fat utilization during rest and exercise were estimated using the Weir equation [26].

Meals

Two isocaloric high-CHO meals were provided on the study day at 1000 h and 1700 h and contained one half of daily energy intake (25 kcal/kg body weight). The meals contained 60% CHO, 15% protein (PRO), and 25% fat in the form of egg salad, wheat roll with butter, graham crackers, coleslaw salad, carrots, skim milk, orange juice and fruit in the morning (Table 3-3), and ham-bacon and cheese sandwich, Romaine greens salad with diet French dressing, carrots, pretzels, cranberry juice, fruit and vanilla ice cream in the afternoon (Table 3-4). Subjects were encouraged to complete eating within 30 minutes. The food provided and any left uneaten was weighed to calculate the actual energy and macronutrient consumption.

Measurements

Plasma glucose, insulin and glucagon were measured hourly at sleep and at more frequent intervals during exercise and meals. Free fatty acids (FFAs) and D-3-hydroxybutyrate ketone body concentrations were also measured as they can impair glucose tolerance and are elicited both by exercise and declines in plasma glucose and are inhibited by PP insulin. The times for metabolite and hormone measurements are listed in Table 3-1.

Analytical procedures

Blood samples were collected into ice-chilled EDTA-coated tubes containing aprotinin (50 KIU/ml blood, Sigma Chemical, St. Louis, MO) and dipeptidyl peptidase-4 inhibitor (10 μl/ml blood; EMD Millipore Corporation, Billerica, MA). Plasma was kept frozen at -80°C for metabolite and hormone measurements. Plasma glucose (Fisher Diagnostics, Middletown, VA) and FFAs (Wako Diagnostics, Richmond, VA) were measured with enzymatic colorimetric assays. D-3-hydroxybutyrate ketone body was measured with a kinetic enzymatic method (Randox Laboratories-US, Ltd., Kearneysville, WV). Plasma insulin and glucagon were measured with radioimmunoassays (EMD Millipore Corporation, Billerica, MA). The intra-assay coefficients of variation (CV) for the insulin and glucagon assays were respectively 2.3% and 3.6% and inter-assay CVs were 16.2% for both assays.

Calculations and statistics

Data are presented as means and standard errors (SEs). Subject characteristics, energy consumption, and expenditure were evaluated with the analysis of variance (ANOVA) using Statistical Analysis System program (SAS; version 9.3, SAS Institute, Cary, NC). Areas under curve (AUCs) of appetite responses and hormones were calculated by the trapezoid rule. The exercise AUCs were calculated during 2 hours of exercise and 1 hour post-exercise period. The AUCs of exercise periods were 0700-1000 h and 1400-1700 h where exercise was performed before the meals in EBM trial and 1100-1400 h and 1800-2100 h for exercise performed after the meals in EAM trial. The AUCs of PP periods were 1000-1400 h and 1700-2100 h. The overall 24-h (0600 h to 0600 h) concentrations of hormones and metabolites also were calculated. Mixed-model repeated measures ANOVA was used to analyze the effects of exercise at different prandial states on plasma concentrations of metabolites (glucose, FFAs, D-3-hydroxybutyrate) and hormones (insulin and glucagon) during exercise and PP periods. Exercise and PP AUCs were analyzed as between-subject effects, while the times of exercise (morning vs. afternoon) and the interaction of the time and exercise were analyzed as within-subject effects. Bonferroni correction was applied to multiple comparisons.

Results

Subjects' characteristics, including body weight, percentage of body fat, BMI, fitness level, and energy intake and expenditures are summarized in Table 3-5. The groups did not differ in body weight, percent body fat, BMI, or fitness level. However, subjects in EBM group were significantly older than those assigned in SED group ($t=2.72$, $p=.0107$).

Metabolism and fuel utilization (Table 3-5)

Subjects in EAM group expended more calories during the first than the second early PP exercise bout ($t=2.76$, $p=.0108$). In addition, the EAM group utilized more CHO and less fat during their first exercise bout in early PP period than the EBM group exercising in fasted state before the first meal ($t=5.28$, $p<.0001$). On the other hand, exercise EE in EBM group was similar during both exercise bouts, but CHO utilization increased to 59% during late PP exercise from 39% when exercise took place during fasting period ($t=5.9$, $p<.0001$). Both exercise groups had significantly lower daily energy balance than the SED group (EBM<SED: $t=-5.55$, $p<.0001$; EAM<SED: $t=-7.44$, $p<.0001$) because the energy expended through exercise was not replaced with additional food.

Plasma glucose (Figure 3-2)

Exercise performed during the late PP (second EBM exercise bout, 4-hours after the first meal) caused a significant decline in plasma glucose during 2 hours of exercise and 1 post-exercise hour compared to the EAM trial when no exercise was taking place at the same time ($t=2.99$, $p=.0054$). In addition, increased PP glucose AUCs after the afternoon meal relative to the morning meal was not eliminated by either EBM exercise performed before the afternoon meal ($t=3.82$, $p=.0006$) or EAM exercise during the second early PP period ($t=3.4$, $p=.0018$) and was of the same magnitude as in the SED trial ($t=2.23$, $p=.0328$).

Plasma insulin (Figure 3-3)

Insulin AUCs significantly declined during exercise in both early PPs (EAM exercise bouts) relative to the non-exercise period in EBM trials in which exercise was completed before the meals (PP1: $t=3.63$, $p=.001$; PP2: $t=3.77$, $p=.0007$). There also was a trend for the insulin AUC to be lower in EAM than in SED group during the second early PP ($t=2.27$, $p=.0304$). The

overall 24-hour insulin concentration was significantly higher in the EBM than in the EAM trials (t=2.8, p=.0086).

Plasma glucagon (Figure 3-4)

Glucagon AUCs were significantly higher in all three groups during late PP period than during the fasting period before the first meal (SED: t=2.63, p=.0132; EBM: t=4.92, p<.0001; EAM: t=3.76, p=.0007). Also during late PP period, glucagon AUC tended to be higher during EBM exercise than in the SED trial (t=2.38, p=.0237). On the other hand during early PP, glucagon concentration trended higher during EAM exercise than SED trial (t=1.9, p=.0666).

Plasma FFAs (Figure 3-5)

FFA increases were associated with exercise but to a greater extent in EBM than in EAM trials, as in the latter the meal consumption reduced the FFA rise. Thus highest FFA AUC relative to SED trial occurred during the late PP period when subjects in the EBM trial engaged in their second exercise bout (t=4.75, p<.0001). This late PP FFA rise in the EBM trial also tended to be higher than the EAM FFA rise that was initiated during the early PP exercise and extended through the start of the second meal (t=2.43, p=.0211). During the late PP period, the FFA AUCs were greater than during the morning fasting period in both EBM (t=2.31, p=.0278) and EAM (t=2.09, p=.0449) trials. By contrast in the SED trial, morning meal consumption lowered the late PP FFA AUC below the fasting FFA concentration (t=2.31, p=.0277). The rises in FFAs during EAM exercise taking place during early PPs were blunted within 3 hours after the consumption of meals but were greater than the SED FFA concentration only during the second early PP period (t=3.03, p=.0049). The overall 24-hour plasma FFA concentration was significantly higher in EAM than in SED trials (t=2.7, p=.0112). There also was a trend for the 24-hour FFA concentration to be higher in EBM than in SED trial (t=2.32, p=.0268)

Plasma D-3-hydroxybutyrate ketone body (Figure 3-6)

D-3-hydroxybutyrate ketone body AUC in the EBM trial was significantly higher during late-PP exercise than during the morning fasting exercise (t=3.64, p=.001) and extended into the early PP period (t=3.13, p=.0038) at which time it was higher than in SED (t=2.16, p=.0387) and EAM (t=2.42, p=.0214) trials. The rise in D-3-hydroxybutyrate AUC during late-PP exercise in the

EBM trial tended also to be higher than in the EAM (t=2.48, p=.0188) and SED trials (t=2.42, p=.0216) when no exercise was conducted at the time.

Discussion

The two objectives of this study were to determine first whether exercise during the high-insulin state in early PP causes the greatest decline in blood glucose in exercising individuals and whether (2) exercise prior to, or 1 hour after, the afternoon meal can abolish the afternoon PP glucose intolerance. The rationale for the first hypothesis was stemmed from the demonstration in numerous studies that ingestion of different simple sugars [7, 9] or CHO–rich foods [6, 10, 11] 15 to 60 minutes before the exercise produces in most individuals so-called rebound hypoglycemia during exercise with glucose levels dropping rapidly below 3.5mmol/L and then rebounding. In some of these studies, however, insulin concentration gradually declined during exercise and did not reach hypoglycemia [7, 9]. The present study confirmed a rapid drop in blood glucose when the 2-hour moderate-intensity exercise was performed an hour after high-CHO morning meal, but the decline in glucose concentration was non-significant relative to simultaneous glucose variation in sedentary trial and trial where the exercise was performed before the meal. This rebound hypoglycemia lasted less than 45 minutes and was associated with a rapid rise in plasma glucagon and a rapid decline in plasma insulin. Similar to previous studies, not all subjects in the present study showed rebound hypoglycemia during exercise. Only 6 of 13 subjects in the EAM trial experienced blood glucose declines below their pre-meal basal values during the 30-45 minutes of exercise performed 1 hour after the morning meal and in only one was it necessary to provide 25 g of glucose as per MCRU institutional anti-hypoglycemic protocol. The decline in blood glucose was again observed when exercise took place during early PP period after the second meal, but the decline in plasma glucose concentration was only to the pre-meal concentration. During second early PP, the apparent PP hyperglycemia in the EBM trial and the apparent hypoglycemia in the EAM trial relative to the SED trial, did not reach significance most likely due to insufficient statistical power. No exercising subject had to be treated with extra glucose during the second early PP period.

The absence of significant hypoglycemia when exercise and high insulin state coincided in the present study compared to studies where it appeared can most likely be attributed to the type of

pre-exercise nutrient stimulus as well as the intensity and duration of exercise employed. Most of the studies reporting pronounced hypoglycemic response to exercise taking place between 15 and 60 minutes after CHO ingestion used pure glucose and other sugar solutions. The amount of simple sugar typically provided in these studies was 50 to 75 g in the form of a beverage [7, 9-11]. The present study provided 119 to 129 g of CHOs consisting of both sugars and starches in a solid form (Tables 3-3 and 3-4) which are digested more gradually than a simple sugar solution. This difference in the CHO stimulus could have affected the impact of the CHO administration on the magnitude and rate of insulin response. The aforementioned studies also usually applied a 15- to 40-minute long exercise bout at intensities between 60 and 74% of maximal effort [6, 7, 9] compared to 120 minutes of exercise at 45% of maximal effort implemented in this study. Exercise at these higher intensities relies more extensively on muscle glycogen and liver-derived glucose than the 120-min long exercise bout at intensity of 45% VO_2max of the presen study [27]. The studies showing greater hypoglycemic response also predominantly engaged younger subjects, whereas the present study engaged postmenopausal women.

The first of the two important findings of this study is that it identified the late PP period as the prandial state that has a significant and sustained glucose-lowering effect impact on glucoregulation during exercise. Moderate-intensity exercise performed during late PP period, 4 to 6 hours after a large high-CHO meal, led to significant and sustained decline in plasma glucose compared to the trial where exercise was performed during early PP state, 1 to 3 hours after the meal. The finding that exercise during late PP is effective in lowering plasma glucose, but exercise in fasting state or in early PP is not, was also reported in studies with type 2 diabetic men [28, 29]. Similar beneficial glucose-lowering effect of late PP exercise was also reported in the healthy population where 75 min of high-intensity cycling performed 2 hours after breakfast sustained lower glycemia throughout the exercise session and delayed peak glycemia after the next meal in healthy men [30].

Since the sustained hypoglycemia associated with late PP exercise occurred at the time when insulin had declined to the basal level as low as during the fasting. Its cause could not have been the coincidence of increased exercise-induced glucose uptake and increased plasma insulin

concentration. Three lines of evidence suggest that this sustained lowering of plasma concentration during late PP exercise is a consequence of inadequate CHO fuel availability.

First, there was a shift in fuel used during late PP exercise to 59% CHO utilization from 39% CHO when the exercise was performed during fasting period before the first meal although insulin concentration in both situations remained at the basal level. The shift in fuel utilization may have been influenced by the morning high-CHO meal as CHO consumption several hours before exercise was previously described to increase glucose utilization during subsequent exercise [31-34]. The second line of evidence for a decline in CHO availability for exercise in late PP period was the very large increase in FFA and ketone body concentrations during that time period. The largest increases FFA AUC in response to late PP exercise compared to the exercise in other three prandial states indicates inadequate availability of circulating fuel and an increased need for an additional metabolic fuel. A large ketone body rise during late PP period but not during the three other exercise episodes is an index of relative glycogen depletion in the liver [35-37]. This suggests that muscle glycogen re-synthesis after the fasting exercise bout and the morning CHO meal was not completed to allow for liver glycogen re-synthesis in this trial. Post-exercise muscle glycogen repletion has priority over liver glycogen repletion on account of 100-times lower Km of glucose-phosphorylating hexokinase compared to liver glucokinase. The final line of evidence in support of glucose insufficiency for late PP exercise is the contrast between the FFA concentrations in the sedentary trial compared to the FFA response during exercise at that time. FFA concentration during late PP in the sedentary trial was significantly lower than during the fasting period. This indicates that, in contrast to the repeat EBM exercise in late PP that elicited a very large FFA and ketone release, the supply of 119 to 129 g of CHO from the high-CHO meal was sufficient to suppress lipolysis and ketogenesis in the SED trial while this CHO supply was not available in the EBM trial. While the CHOs in the meals were sufficient to block FFA and ketone body production in the sedentary trial, they also were sufficient to arrest the early rise in lipolysis during early PP exercise 1-3 hours after the meal onset. This showed that the immediate availability of absorbed glucose for exercise within 3 hours of the high-CHO meal provided sufficient glucose to blunt FFA and ketone body elevation that was necessary for exercise in the late PP. One immediate application of the demonstration that exercise during late PP period leads to sustained hypoglycemia is that athletes whose

performance critically depends on glucose availability should avoid scheduling a large high-CHO meal 4 hours before the onset of exercise. Another application is that exercise during late PP period may lower hyperglycemia in prediabetics and Type 2 diabetics who eat high-CHO meals. Additional studies designed for direct monitoring of glucose turnover and changes in muscle glycogen concentration are needed to elucidate the mechanism of glucose lowering phenomenon during late PP exercise.

The second important finding of this study is that 2 hours of moderate-intensity exercise completed either 1 hour before, or performed 1 to 3 hours after the high-CHO meal, had no impact on PP hyperglycemic response to the afternoon high-CHO meal as a similar level of afternoon PP hyperglycemia and glucose intolerance also occurred in the sedentary trial. Thus a substantial 450-Kcal exercise energy expenditure either before or immediately after the meal containing about 119 to 129 grams of CHO produced significantly higher afternoon than morning PP hyperglycemia in all three trials with a suggestion that the afternoon hyperglycemia was aggravated by exercise before the afternoon meal and mitigated by exercise within an hour after the afternoon meal. Studies with greater statistical power will be needed to determine whether these variations in the afternoon PP hyperglycemia might be significant.

Absence of a significant decline in afternoon PP hyperglycemia is unexpected and contrary to the universal consensus that aerobic exercise improves glycemia and reduces insulin responses [38-43]. One possible reason for the results is the extended duration and the moderate intensity of exercise bout in this study. Exercise is known to improve glucose tolerance 12-15 hours after moderate- or high-intensity exercise performed at 55-80% VO_2max, for between 20 and 75 minutes [44-50], which were not present in this study. It is not clear why 2 hours of moderate-intensity exercise performed either before or after two high-CHO meals did not reduce glycemia. Increased FFA release during late PP exercise could not be responsible through its inhibitory effect on insulin signaling [51] because the 24-hour FFA concentration was significantly greater in EAM than that in the SED trial, yet the 24-hour insulin responses were significantly higher in EBM than in the EAM trials. Glucagon measurements also did not offer an explanation for the dissociation between insulin and glucose responses when exercise was performed in different

prandial states. In general, glucagon AUCs were showing reactive increases during mid-day, but not fasting, exercise and during late PP when plasma glucose declined.

Another possibility for the afternoon glucose intolerance may possibly be a diurnal change in the capacity of insulin to clear CHOs from circulation. A 50-g oral glucose load produces higher and more protracted glycemia at 1500 h or 2000 h than at 0900 h [20-22] and is associated with a delayed but overall greater PP insulin response [20-22]. The delayed and protracted pattern of insulin response resembles that observed in pre-diabetes [52]. This change in the afternoon insulin response to a uniform glucose challenge may be the primary cause of the afternoon glucose intolerance because it does not appear in type 1 diabetics or after afternoon intravenous injection of insulin in non-diabetic subjects, and the insulin secretagogue tolbutamide has lower stimulatory effect in the afternoon than in the morning [20]. Thus, the capacity of insulin to stimulate glucose uptake is limited by the size of the CHO load. All of these inferences will require resolution in additional studies with suitable experimental designs.

This study has several limitations. First, only postmenopausal women were recruited as subjects, so the results could not represent the general population. Second, number of subjects could have been too small for some observed differences to survive Bonferroni correction for multiple comparisons. Third, morning and afternoon meals were not identical for a test of actual diurnal effect. However, the supplemental study 3A in the appendix demonstrates that small (17%) glycemic differences in the two daily meals of this study had no impact on glycemic and insulin responses in exercise trials. PP glycemia and insulin responses can be affected by GI of the previous meal [53-58] but the GI difference have to be 2 to 3-fold to significantly affect glycemic and insulin responses

In summary, the principal condition leading to significant and sustained lowering of blood glucose was exercise during late PP in contrast to non-significant and transient rebound hypoglycemia during morning exercise in early PP, and no change in blood glucose during fasting exercise before the morning meal. Thus exercise parameters as used in this study did not consistently reduce glycemia except when it was performed in late PP. The probable cause of the hypoglycemia during late-PP exercise was of metabolic nature as insulin concentration at the

time reached the low basal level as in fasting state. The relative contribution of prior exercise and large high-CHO meal in this phenomenon will require additional research with appropriate analytical methods. Despite the expectations that two hours of moderate-intensity exercise used in this study would be effective in lowering the afternoon glucose intolerance this did not occur either when the exercise was completed 1-hour before, or started 1-3 hours after the afternoon high-CHO meal. This failure of exercise to abolish the afternoon glucose intolerance may be caused either by the parameters of exercise used in this study or a diurnal change in the insulin secretory capacity to high-carbohydrate meals, suggestions that require further experimental exploration. Within the limitations of this study that preclude generalizations to the other gender and age groups than used in this study, exercise performed 4 hours after a large high-CHO meal can significantly reduce plasma glucose concentration but exercise either before or after the afternoon meal does not abolish afternoon decline in glucose tolerance. Glucose lowering after late-PP exercise may be beneficial in circumstances where hyperglycemia is a health problem [28, 29], but it also may have negative implications for high-intensity sport performance or other conditions where a decline in blood glucose presents a problem.

Table 3-1: Timing of metabolites and hormones measurements

Day 2	Glucose	Insulin	Glucagon	FFA	Ketone
6:00	√	√	√	√	√
7:00	√	√	√	√	√
7:15	√	√		√	
7:30	√	√		√	√
7:45	√	√		√	
8:00	√	√	√	√	√
8:30	√	√		√	√
9:00	√	√	√	√	√
9:15	√	√		√	
9:30	√	√		√	√
10:00	√	√	√	√	√
10:15	√	√		√	√
10:30	√	√	√	√	√
10:45	√	√		√	√
11:00	√	√	√	√	√
11:15	√	√		√	
11:30	√	√		√	√
11:45	√	√		√	
12:00	√	√	√	√	√
12:30	√	√	√	√	√
13:00	√	√	√	√	√
13:15	√	√		√	√
13:30	√	√	√	√	√
13:45	√	√		√	√
14:00	√	√	√	√	√
14:15	√	√		√	
14:30	√	√	√	√	√
14:45	√	√		√	
15:00	√	√	√	√	√
15:30	√	√	√	√	√
16:00	√	√	√	√	√
16:15	√	√		√	
16:30	√	√		√	√
17:00	√	√	√	√	√
17:15	√	√		√	√
17:30	√	√	√	√	√
17:45	√	√		√	√
18:00	√	√	√	√	√
18:15	√	√		√	
18:30	√	√	√	√	√

18:45	√	√		√	
19:00	√	√		√	√
19:30	√	√	√	√	√
20:00	√	√		√	√
20:15	√	√		√	
20:30	√	√	√	√	√
21:00	√	√		√	√
22:00	√	√	√	√	√
23:00	√	√		√	√
Day 3					
0:00	√	√	√	√	√
1:00	√	√		√	√
2:00	√	√	√	√	√
3:00	√	√		√	√
4:00	√	√	√	√	√
5:00	√	√		√	√
6:00	√	√	√	√	√

Table 3-2: Menu template for pre-study high-carbohydrate (CHO) dinner

Meal composition: 60% CHO, 15% protein (PRO), 25% fat

Food items	Wt (g)	CHO (g)	PRO(g)	FAT(g)	Kcal
Spaghetti w/Meat sauce	163g noodle/113g meat sauce	57	23	12	428
Tossed Greens using Romaine Blend	2 oz; 57g	6.25	1.25		30
Sysco French dressing	1 package; 12 g	2.1	0	5.5	57.9
Orange juice	1 carton; 125g	15	0	0	60
1 serving fresh fruit (options listed below†)	depends†	15	0.8	0.2	65
Total‡		**95.35**	**25.05**	**17.7**	**640.9**
Percentage (%)		**59.5**	**15.6**	**24.9**	
†Fruits options	**Wt (g) (1 serving)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
Fresh Strawberries	8 oz; 226.8g	15.9	1.4	0.8	76.4
Fresh Nectarine	1 med; 5oz;	16	1	0.6	73.4
Asian pear	1 each	16	1	0	68
Peach Halves	1/2 cup; 2 halves	14	1	0	60
Pineapple rings	1/2 cup; 3 rings	14	0	0	56
Pear halves	1/2 cup; 2 halves	13	0	0	52
Pear slices	1/2 cup	14	1	0	60
‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-third of the subject's daily caloric need. Subjects' daily caloric need=30kcal/kg BW					

Table 3-3: Menu template for morning high-carbohydrate (CHO) meal

Meal composition: 60% CHO, 15% protein (PRO), 25% fat, glycemic index=58

Food items	Wt (g)	CHO (g)	PRO(g)	FAT(g)	Kcal
Egg salad plate on Multi grain bun	1 plate; 156 g	26	13	15	291
Wheat roll	0.5 each; 18.4 g	8.6	1.7	0.8	48.4
Margarine country c	0.5 tub; 2.4 g			1.2	10.8
New Coleslaw	85g	11	1	3	75
Carrot sticks	1 serving; 86 g	4	1	0	20
Skim milk	1 cup; 243g	11	8	0	76
Orange juice	0.5 carton, 63g	7.5	0	0	30
1 banana	70g	16	0.7	0	66.8
1 serving fresh fruit (options listed below†)	depends†	15	0.8	0.2	65
Graham crackers	4 squares; 28 g	22	2	3	123
Total‡		**121.1**	**28.2**	**23.2**	**806**
Percentage (%)		**60.1**	**14.0**	**25.9**	
†Fruits options	**Wt (g) (1 serving)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
Fresh Strawberries	8 oz; 226.8g	15.9	1.4	0.8	76.4
Fresh Nectarine	1 med; 5oz;	16	1	0.6	73.4
Asian pear	1 each	16	1	0	68
Peach Halves	1/2 cup; 2 halves	14	1	0	60
Pineapple rings	1/2 cup; 3 rings	14	0	0	56
Pear halves	1/2 cup; 2 halves	13	0	0	52
Pear slices	1/2 cup	14	1	0	60

‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-half of the subject's daily caloric need. Subjects' daily caloric need=25kcal/kg BW

Table 3-4: Menu template for afternoon high-carbohydrate (CHO) meal

Meal composition: 60% CHO, 15% protein (PRO), 25% fat, glycemic index=68

Food items	Wt (g)	CHO (g)	PRO(g)	FAT(g)	Kcal
Bacon, Cheese, & Ham sandwich					
▪Wheat Toast	2 slices; 73.4 g	28	6	2	154
▪Silvered Ham	50g	0	8	1.4	44.6
▪Bacon	1 slice; 4.7g	0	1.5	2.7	30.3
▪Cheddar cheese	1 slice; 0.8 oz	0	6	8	96
▪Tomatoes	2.5 slices; 55g	4	0.8	0	19.2
▪Lettuce, green leaf, raw	14g	0	0	0	0
▪Diet mayonnaise	1 pkg.; 12 g	0	0	0	0
▪Ketchup or mustard can be provided	1 pkg.;10g	0	0	0	0
Broccoli, Cauliflower, Carrots (cooked, salt can be added)	0.5 cup;86 g	7	1	0	32
1.6 oz Tossed Greens using Romaine Blend	1.6 oz; 45.5g	5	1	0	24
Diet French Dressing	1 package; 12 g	2	0	0.5	12.5
1 serving fresh fruit (options listed below†)	depends†	22	1.2	0.3	95.5
Cranberry Juice cocktail	0.9 carton,110g	17	0	0	68
Rold Gold Pretzels	1 oz; 28.3g	23	2	1	109
Vanilla Ice Cream	57g	13	1.8	6	113.2
Total‡		**121**	**29.3**	**21.9**	**798.3**
Percentage (%)		**60.6**	**14.7**	**24.7**	
†Fruits options	**Wt (g) (1 serving)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
Fresh Strawberries	8 oz; 226.8g	15.9	1.4	0.8	76.4
Fresh Nectarine	1 med; 5oz;	16	1	0.6	73.4
Asian pear	1 each	16	1	0	68
Peach Halves	1/2 cup; 2 halves	14	1	0	60
Pineapple rings	1/2 cup; 3 rings	14	0	0	56
Pear halves	1/2 cup; 2 halves	13	0	0	52
Pear slices	1/2 cup	14	1	0	60

‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-half of the subject's daily caloric need. Subjects' daily caloric need=25kcal/kg BW

Table 3-5: Subjects' characteristics in sedentary (SED), exercise-before-the-meals (EBM), and exercise-after-the-meals (EAM)

Groups	SED (n=8)	EBM (n=13)	EAM (n=13)
Age (years)	55.0±1.07	59.7±0.93	65.6±2.75
Weight (Kg)	66.1±2.22	68.8±2.69	63.7±2.10
Percentage of Body Fat (%)	35.1±2.18	38.3±2.37	35.5±2.47
BMI (Kg/m^2)	23.6±0.91	24.9±0.79	23.4±0.97
Fitness level (VO_2/min×Kg)	25.6±3.66	23.8±1.81	24.7±1.73
EI in meal 1 (Kcal)	769.5±32.82	836.7±35.74	784.9±36.15
EI in meal 2 (Kcal)	803.8±33.69	839.7±38.85	767.3±53.32
1st Exercise EE (Kcal)	NA	423.6±30.90	501.0±48.58*
Carbohydrate utilization (%) during 1st exercise	NA	39%±3.6%a,#	64%±3.7%b
Fat utilization (%) during 1st exercise	NA	61%±3.6%a,#	36%±3.7%b
2nd Exercise EE (Kcal)	NA	420.8±25.87	471.6±40.84
Carbohydrate utilization (%) during 2nd t exercise	NA	59%±2.4%	68%±3.5%
Fat utilization (%) during 2nd exercise	NA	41%±2.4%	32%±3.5%
EB: EI - exercise EE (Kcal)	1573.3±65.19a	832.0±79.30b	462.2±103.29b

EI: energy intake; EE: energy expenditure; EB: energy balance

a,b Within the same variable, groups with different superscripts are significantly different ($p<.05$)

* Within EAM group, energy expenditure during 1st exercise was significantly higher than during the 2nd exercise ($p<.05$)

Within EBM group, less carbohydrate and more fat were utilized during 1st exercise than during the 2nd exercise ($p<.05$)

Figure 3-1: Repeat-event experimental paradigm

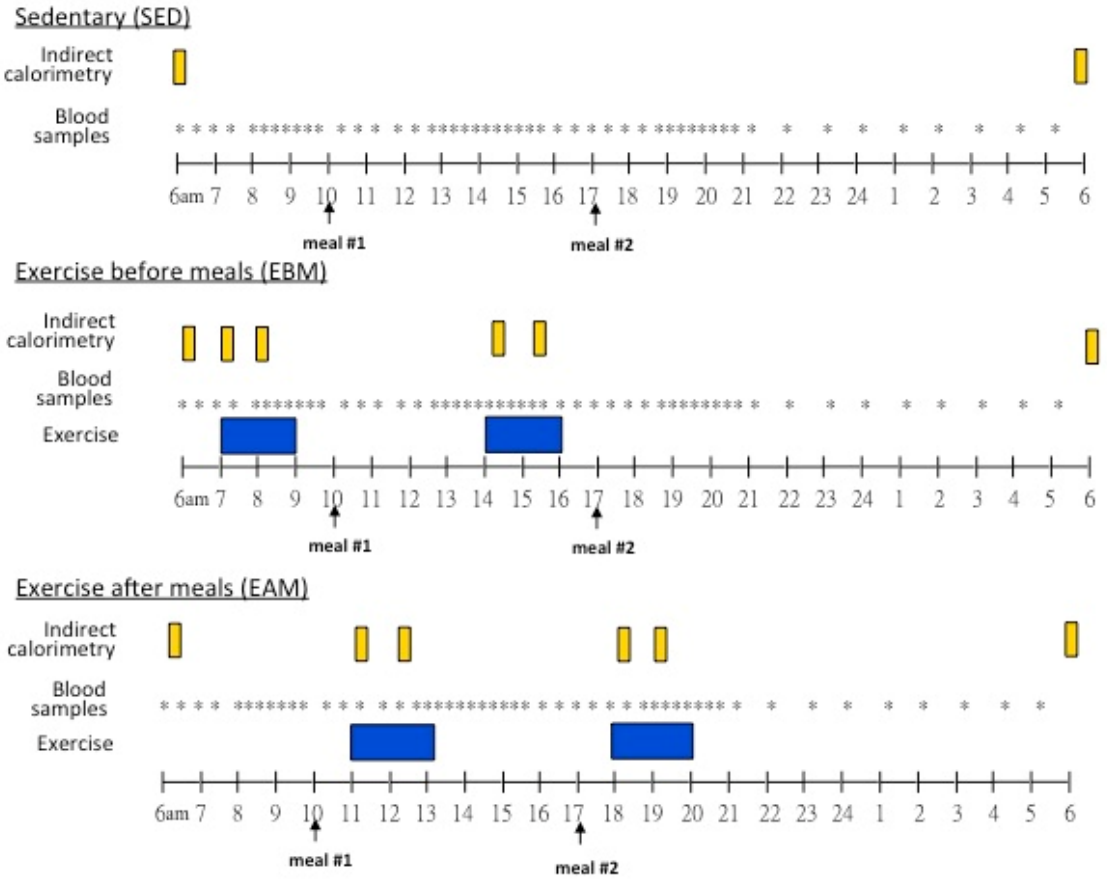
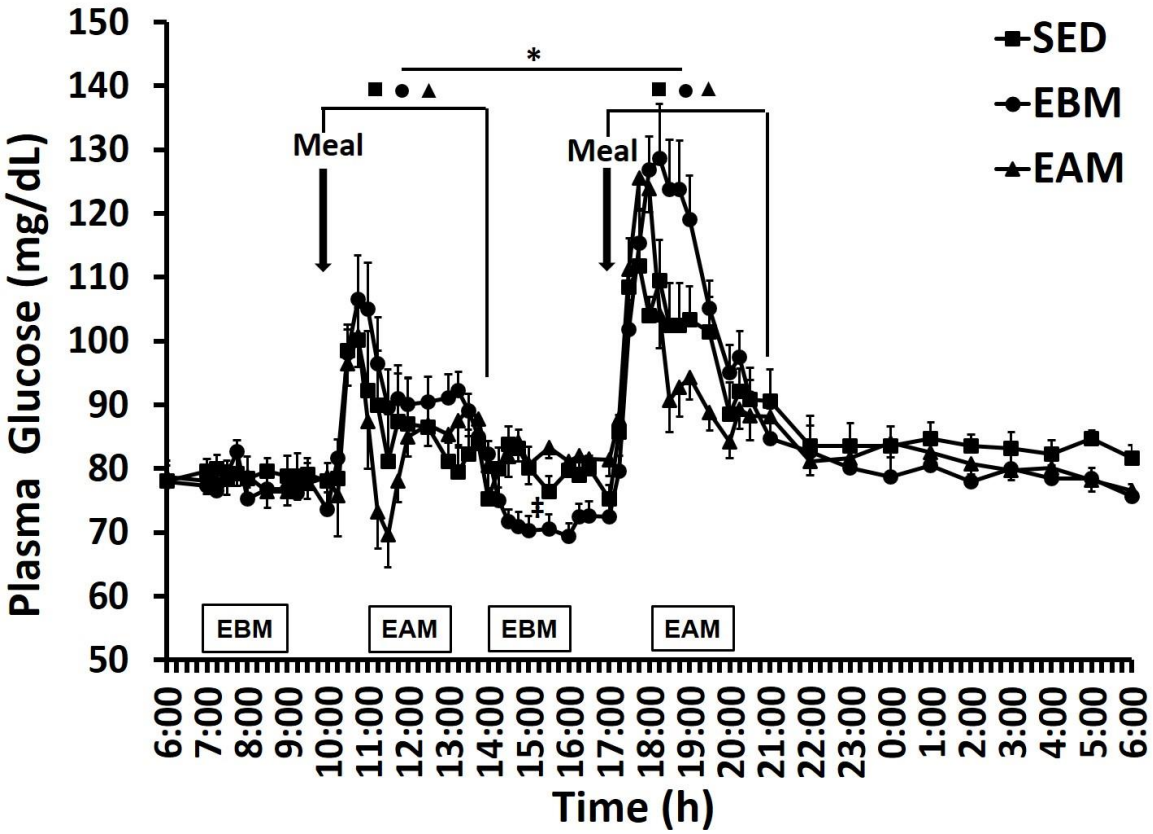
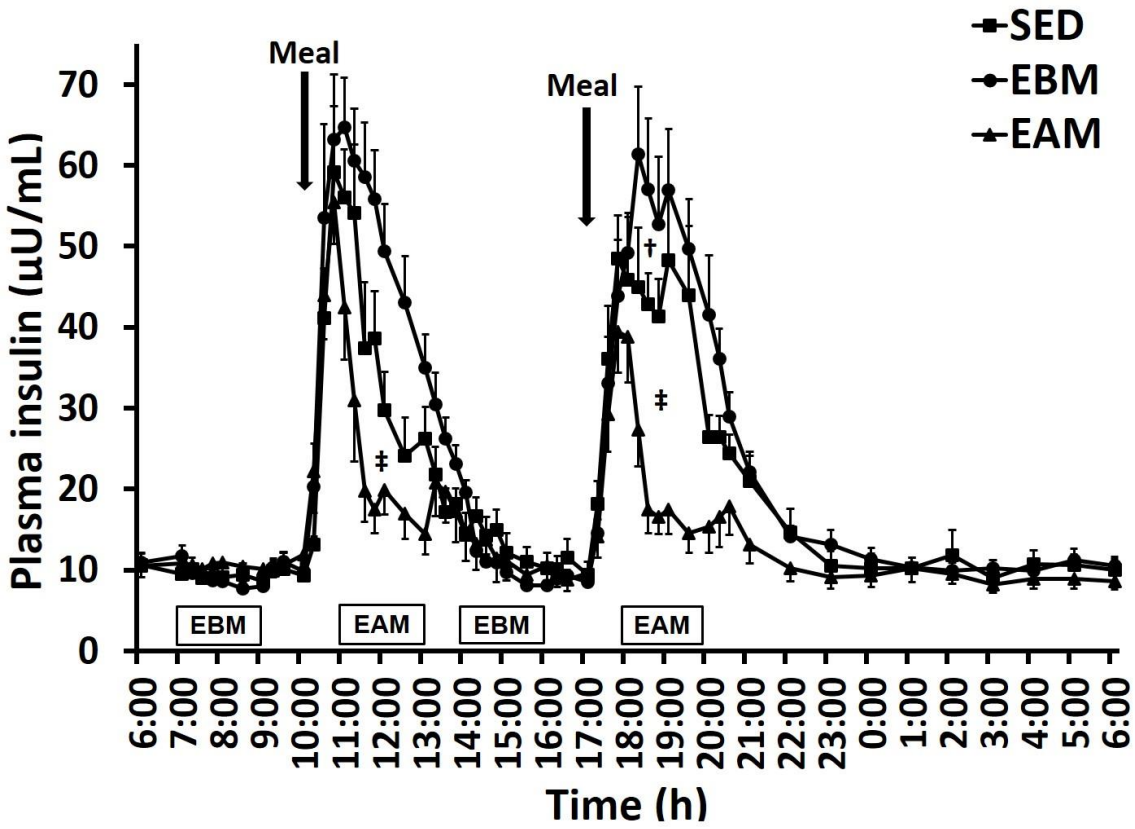


Figure 3-2: Plasma glucose responses to sedentary (SED), exercise before meals (EBM) and exercise after meals (EAM)



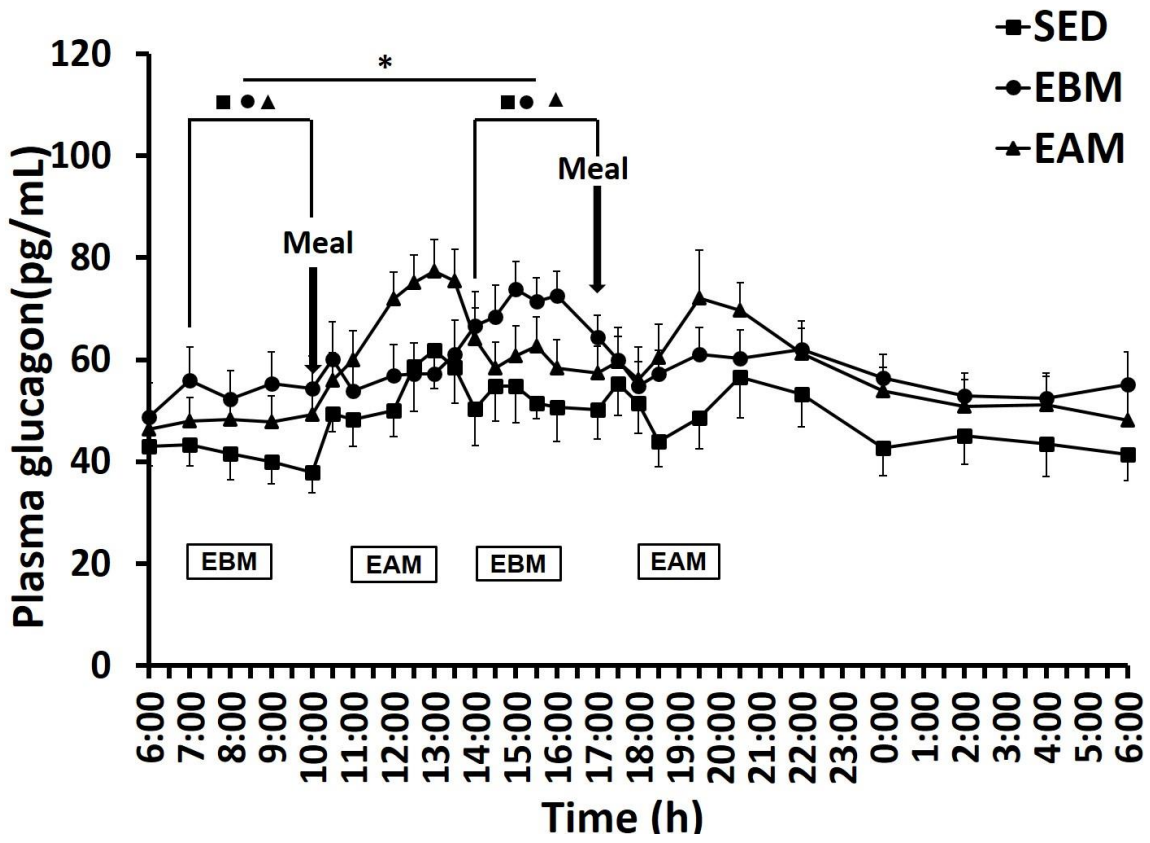
"*" indicates the statistical significance between two selected time periods.

Figure 3-3: Plasma insulin responses to sedentary (SED), exercise before meals (EBM) and exercise after meals (EAM)



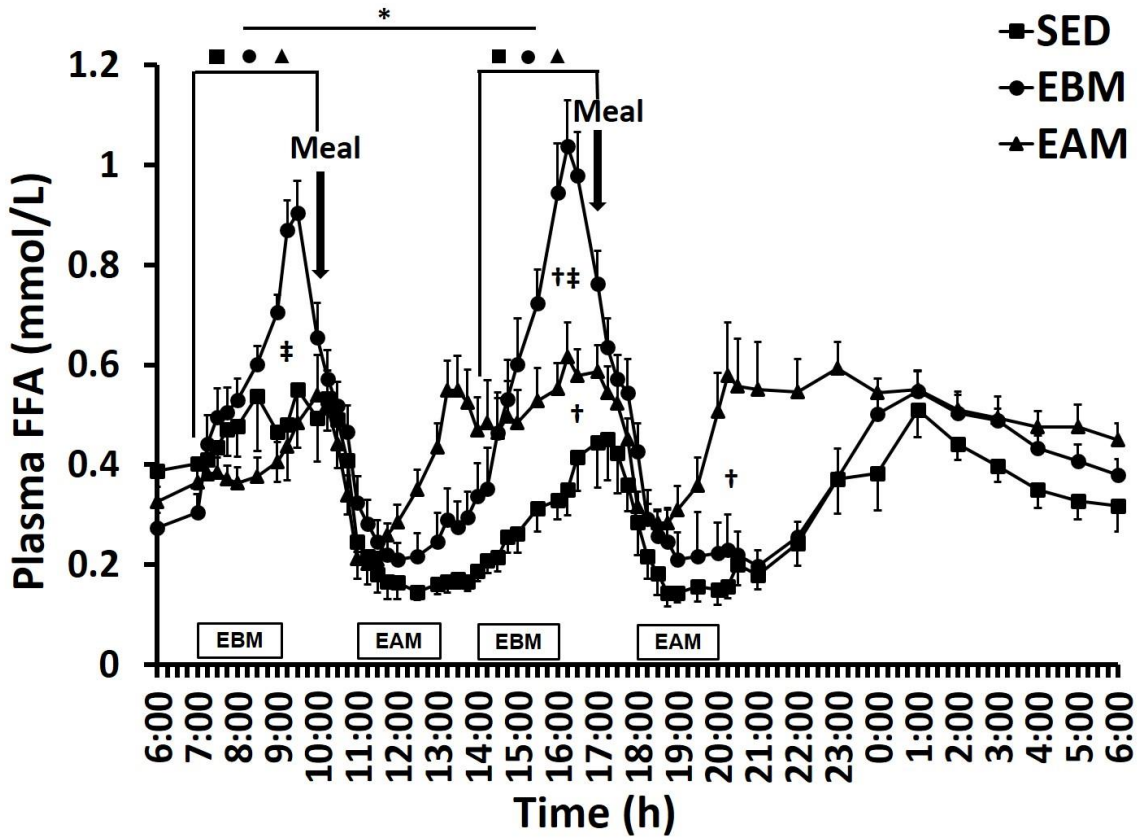
"‡" indicates the statistical significance between EBM and EAM trials in the selected areas under the curve.

Figure 3-4: Plasma glucagon responses to sedentary (SED), exercise before meals (EBM) and exercise after meals (EAM)



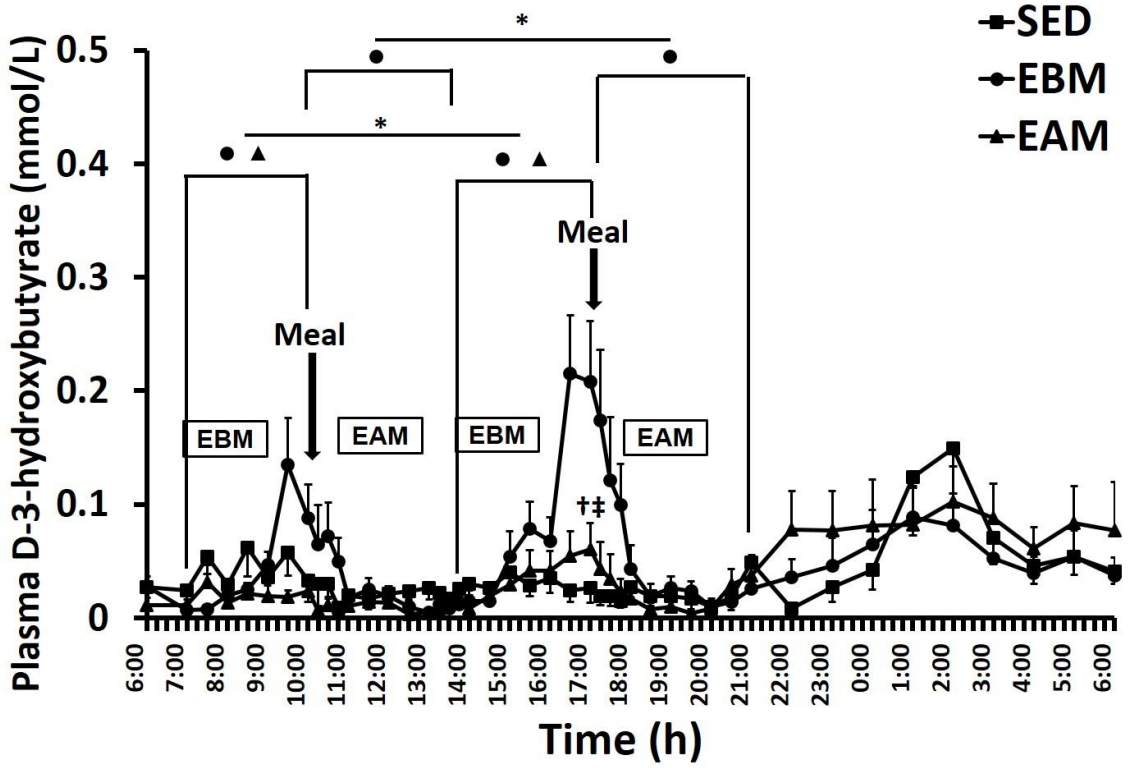
"*" indicates the statistical significance between two selected time periods.

Figure 3-5: Plasma free fatty acids (FFAs) to sedentary (SED), exercise before meals (EBM) and exercise after meals (EAM)



"*" indicates the statistical significance between two selected time periods. "†" indicates the significant difference compared to sedentary (SED) trial in the selected areas under the curve. "‡" indicates the significant difference in the selected areas under the curve between EBM and EAM trials.

Figure 3-6: Plasma D-3-hydroxybutyrate ketone body responses to sedentary (SED), exercise before meals (EBM) and exercise after meals (EAM)



"*" indicates the statistical significance between two selected time periods.

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CHAPTER 4

Postprandial glucose and insulin responses to dietary macronutrient content and exercise

Abstract

Afternoon postprandial (PP) glycemia to a high-carbohydrate (CHO) meal is greater in the afternoon and evening than in the morning and is associated with a similarly high insulin response as in the morning. The afternoon PP hyperglycemia and daily hyperinsulinemia may predispose to development of insulin resistance and prediabetes. It is unclear what restrains the afternoon insulin response commensurate to the magnitude of hyperglycemia and whether the effect is related to any changes in insulin-stimulating gut hormones (incretins) that are responsive to the nutrient content of the meals. The hypothesis 1 in this study posits that the large dietary CHO load is the cause of the afternoon glucose intolerance and of a large insulin response and reduction in the CHO content of the meal will abolish the afternoon PP glucose intolerance and restore insulin response to be commensurate to the magnitude of glycemia. In the experiment 1, this hypothesis is tested by comparing glycemic and insulin responses to a morning and afternoon customary high-CHO (60%) meals and meals containing half as much CHO (30%) . In addition, it is not clear whether exercise could abolish diurnal decline in glucose tolerance in conjunction with or independently of the CHO content of the meals and restore insulin response to be commensurate to the magnitude of glycemia. The hypothesis 2 posits that exercise performed before each meal will abolish the afternoon glucose intolerance and restore insulin response to be commensurate to the magnitude of glycemia with either high-CHO meals or meals containing half as much CHO. In the experiment 2, this hypothesis is tested by comparing glycemic and insulin responses to a morning and afternoon customary high-CHO (60%) and meals containing half as much CHO (30%) after the completion of 2 hours of moderate-intensity exercise 1 hour before each meal.

Methods Thirty-two healthy postmenopausal subjects were matched by body weight and BMI. In the experiment 1, 8 subjects received 30%-CHO meals (LCS) and another 8 received 60%-CHO meals (HCS) at 1000 h and 1700 h. In the experiment 2, another 16 subjects performed 2-

hour treadmill exercise at 45% of VO_2max between 0700 and 0900 h and 1400-1600 h and were assigned to 30%-CHO (LCX) or 60%-CHO (HCX) meals, eight subjects each. Areas under the curve (AUCs) for insulin, glucagon, glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1, glucose, free fatty acids, and D-3-hydroxybutyrate ketone body were measured during 4-hour PP periods after each meal and analyzed by a mixed-model analysis of variance.

Results In the experiment 1, afternoon PP glucose AUC was significantly higher than the morning AUC in the HCS, but not in the LCS trial. Afternoon PP insulin AUC declined 39% after the low-CHO, but not after the high-CHO, meal in conjunction with PP glycemia that also was lower than in HCS trial. CHO content of the meals had no effect on the glycemia or the insulin response to the morning meal. The 48% afternoon decline in GIP AUC after low-CHO, but not after high-CHO, meal paralleled the decline in insulin AUC. Similar results were obtained in the exercising experiment (experiment 2) in that afternoon PP insulin response declined by 31% in parallel with a 45% decline in GIP in the LCX trial with no such change in the HCX trial, while the afternoon PP glycemia remained higher than the morning glycemia in both LCX and HCX trials.

Conclusions Two daily high-CHO meals result in significantly higher PP glycemia after the afternoon compared to the morning meal but equally high insulin and GIP responses after both meals. A reduction in CHO content of the meals by half reduces afternoon glycemia to approximate the morning glycemia, reduces the magnitude of the afternoon PP insulin response by 39% and the afternoon GIP AUC by 48%. Two-hour pre-meal moderate-intensity exercise does not reduce afternoon PP glycemia on either diet. It also does not alter the afternoon PP decline in insulin AUC (31%) or GIP AUC (45%). Insulin lowering effect by low-CHO meals may be functionally related to a parallel change in GIP response. Reducing dietary CHO content from 60% to 30% reduces afternoon insulin over-secretion and hyperglycemia and could possibly lower the risk of developing insulin resistance.

Introduction

Progressive and sustained increase in the overweight and obesity among US adults during the recent decades [1] has been paralleled by a similar increase in insulin resistance, prediabetes, and type 2 diabetes (T2D) [2]. In 2012, twenty-one million individuals in United States were diagnosed with T2D and another 8.1 million people are believed to be undiagnosed [3]. In 2013, about 382 million people world-wide had diabetes, and this number is projected to increase to 592 million by 2035 [2]. The deleterious effects of T2D on the cardiovascular disease and life span [4] are well known, as is the financial burden associated with the treatment of its symptoms [5]. The connection between the overweight, obesity and development of diabetes is well established as insulin resistance in peripheral tissues and compensatory insulin over-secretion lead progressively to loss of beta cell secretory capacity [6]. Insulin resistance and decline in its metabolic actions is the keystone of the cluster of pathologies in the metabolic syndrome and tends to progress to T2D when the overweight reaches obesity level [7]. Besides the increase in daily energy intake (168-335 kcal) from 1971-2000 [8] and approximately threefold reduction in the physical effort required in the current US jobs compared to the energy-demanding agrarian lifestyle of some contemporary communities [9-11], two additional behavioral changes and a feature of human physiology may be contributing to the rise in the incidence of insulin resistance and T2D.

The two behavioral trends during the past three decades that had accompanied the rapid rise in obesity and T2D in developed countries have been a shift toward eating the largest meal later in the day [12] and a shift toward greater carbohydrate (CHO) content at the expense of fat content of the diet [13]. Increased selection of CHO in the US diet was a response to the reports of the association of high blood cholesterol concentration with cardiovascular morbidity in populations consuming a high proportion of animal fat [14], the feasibility of the reversal of both with low-fat diets [15], and the dietary guidelines issued since 1980 by the US Department of Agriculture and Health and Human Services in favor of lowering of dietary fat and of increasing starch and fiber intake [16]. The possibility that CHO restriction can reduce glucose and insulin responses in general has been supported by studies where long-term low-CHO (LC) diets were found to benefit diabetes control and short-term weight loss [17-22]. Johnsson et al. (2009) reported that 3-months of Paleolithic-type LC diet lowered HbA1c more than the nutritional approach for

treatment and prevention of diabetes [18]. Better glycemic control, often with no change in body fat, in T2D was reported with CHO-restriction and ketogenic diets [23].

The feature of human physiology where the insulin response to the same CHO challenge differs in the afternoon from the morning response has been known for at least four decades [24-26]. The phenomenon is seen most clearly when the oral glucose challenge is administered at different times of day. A 50-g oral glucose load produces higher and more protracted glycemia at 1500 h or 2000 h than at 0900 h [24-26] and is associated with a delayed but overall greater PP insulin response [24, 26]. The rate of glucose clearance is slower after intravenous glucose administration at 1500 h compared to at 0900 h [27]. When a large meal containing 60% of daily energy is consumed in the evening, the insulin response is 16 to 20% greater than in the morning regardless of the meal glycemic index (GI) [28]. Therefore, the afternoon insulin response to a uniform glucose load differs in its overall magnitude, the rate at which the peak concentration is reached, and in the duration of the elevation in its concentration compared to the morning response. This delayed and protracted pattern of insulin response resembles that observed in pre-diabetes [29] and appears to be the cause rather than the consequence of the afternoon glucose intolerance. In support of the latter conclusion are the observation that afternoon glucose intolerance does not appear after afternoon intravenous injection of insulin while intravenous administration of insulin secretagogue tolbutamide has lower stimulatory effect in the afternoon than in the morning [24].

The cause of the diminished afternoon insulin response to CHO remains uncertain but has been attributed in some studies to increased afternoon concentrations of free fatty acids (FFAs) [24, 26]. Another, largely unexplored, possibility is that it is modulated by the afternoon decline in the incretin response to CHOs. Incretin gut hormones, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), are established insulin secretagogues and inhibitory to glucagon secretion [30, 31]. The incretin hypothesis is suggested by the 30-40% lower magnitude of insulin response seen after intravenous than that after oral glucose load [32]. Exercise is another variable known to acutely facilitate insulin-independent glucose tolerance [33-35] and to stimulate insulin sensitivity 3 to 48 h after its termination [36-38]. Exercise can increase glucose tolerance and insulin sensitivity when performed twice in a day in a fasted state

[39-41]. Exercise can therefore be expected to decrease afternoon PP insulin responses by increasing glucose tolerance and insulin sensitivity. Exercise could also affect insulin secretory response by altering the PP responses of gut hormones, GIP and GLP-1 and their insulinotropic actions [31, 42]. In support of incretin hormone responsiveness to exercise, a reduced PP GIP response to glucose after a single bout of exercise has been reported [43].

Two hypotheses were formulated based on the data and inferences generated by others [25-27]. Hypothesis 1 posits that the large dietary CHO load is the cause of the afternoon glucose intolerance and of a large insulin response mediated, in part, by the insulinotropic incretin hormones GIP and GLP-1. The experiment 1 tested the hypothesis 1 that a reduction in the CHO content of the meals by a factor of two will reduce PP glycemia and insulin response and abolish the afternoon PP hyperglycemia. This hypothesis was tested by measuring glycemic, insulin, glucagon, and incretin gut hormone responses to a morning and afternoon meals containing either 30% or 60% CHO and provided at 1000 and 1700 h. Hypothesis 2 posits that that moderate-intensity exercise performed before each meal will reduce glycemia and insulin responses to high-CHO diets and abolish afternoon hyperglycemia on either high-CHO or low-CHO diet. Hypothesis 2 was tested in the experiment 2 by measuring the same variables as in experiment 1 to a morning and afternoon meals that were preceded by two bouts of moderate-intensity exercise in trials with low-CHO (LCX) or high-CHO (HCX) meals.

The expected outcomes in the experiment 1 regarding the hypothesis 1 were that: insulin and glucose responses would be lower to a low-CHO diet than to a high-CHO diet, afternoon hyperglycemia would be abolished, and reduction in insulin response on low-CHO diet would be associated with a parallel reduction in the PP incretin hormone responses. The expected outcomes in the experiment 2 were that exercise before the meals would lower PP glucose and insulin responses to a greater extent in trials with high-CHO meals than in trials with low-CHO meals, abolish afternoon hyperglycemia in trials with both diets, and lead to corresponding reductions in PP insulin and incretin hormone responses.

Methods

Subjects

In both experiments, subject eligibility criteria were the same and included: healthy postmenopausal women, age 50 to 65 years; body mass index (BMI) 20 to 30 kg/m^2; fasting glucose level < 100 mg/dL; hematocrit > 32%, hemoglobin > 12 mg/dL; non-smoker; and absence of restricted food intake and endocrine and metabolic disorders requiring medication other than hormonally corrected hypothyroidism. All subjects signed an informed consent approved by The University of Michigan Medical School Institutional Review Board. Women underwent a preliminary health and a fitness screen at the Michigan Clinical Research Unit (MCRU). The health screen included health history, measurements of weight, height, and body fat by a dual-energy X-ray absorptiometry (General Electric Lunar Prodigy Advance), and a fasting blood draw for laboratory chemistries and thyroid function. A fitness screen assessed individual maximal aerobic effort. It consisted of a treadmill test at 3 miles per hour with 2% slope increments every 3 minutes with the subject breathing through a mouthpiece using a Max II metabolic cart (AEI Technologies, Inc., Bastrop, TX). The criterion of maximal effort (VO_2max) was a respiratory quotient of 1.

General Experimental Protocol

Subjects were assigned to groups after matching by body weight and BMI. In experiment 1, eight subjects were assigned to low-CHO meals (LCS) and another 8 to high-CHO meals (HCS). In experiment 2, another sixteen subjects, eight each, exercising before the meals were assigned to either low-CHO meals (LCX) or high-CHO meals (HCX). After admission to MCRU at 1800 h on the day before the study trial, a meal of one-third of subject's weight-maintenance energy need (30 kcal/kg body-weight) was provided at 1900 h with the composition appropriate to dietary treatment was provided. The template of pre-trial high-CHO and low-CHO evening meal was shown in Tables 3-2 and 4-2, respectively. A catheter was inserted into an arm vein around 1930 h for hourly blood collection with additional samples taken at 15- and 30-min intervals during meals and exercise on the study day. It was kept patent with sodium heparin. In all experiments, plasma insulin, glucagon (the major insulin-counterregulating hormone), insulinotropic incretin hormones GIP and GLP-1, and glycemic responses were examined as a function of meal CHO content and/or exercise. The FFAs and D-3-hydroxybutyrate ketone body

concentrations also were measured as they are elicited by reduced glucose availability after low-CHO meals and can impair insulin sensitivity [44, 45]. Most endocrine and metabolic variables were measured at hourly intervals between 0600 h of the day of the experiment (day 2) through 0600 h of the day after the study (day 3). Measurement frequency during exercise and PP periods (0-4 hours after meals) for some of the variables was up to fourfold greater (Table 4-1).

Meals

In both experiments during the study day, two iso-caloric meals were provided at 1000 and 1700 h, equally containing a half of daily energy allotment of 25 kcal/kg body-weight. The high-CHO meals contained 60% CHO, 15% protein (PRO), and 25% fat provided in the form of egg salad, wheat roll with butter, graham crackers, coleslaw salad, carrots, skim milk, orange juice and fruits in the morning (Table 3-3) and ham-bacon-and-cheese sandwich, Romaine-greens salad with diet French dressing, carrots, pretzels, cranberry juice, fruit and vanilla ice cream in the afternoon (Table 3-4). The GI, calculated with Nutrition Data System for Research, was 58 for the morning high-CHO meal and 68 for the afternoon high-CHO meal. The low-CHO meals contained 30% CHO, 25% PRO, and 45% fat provided in the form of a chef salad platter including romaine lettuce, slivered turkey-and-ham strips, cheddar and Swiss cheese, cherry tomatoes, cucumber and croutons, wheat roll and butter, macaroni and cheese, sausage, yogurt with shredded almonds in the morning (Table 4-3), and vegetarian burger, chicken Caesar salad with shredded almonds, minestrone soup, fruit juice and fruits in the afternoon (Table 4-4). The GI was 53 for the morning low-CHO meal and 51 for the afternoon low-CHO meal. Subjects were encouraged to eat the meals within 30 minutes. The food provided and any left uneaten was weighed to calculate the actual energy and macronutrient consumption.

Exercise

During the study days, treadmill walking in experiment 2 was carried out at 45% of maximal aerobic effort. The 2-hour exercise bouts were completed 1 hour before each meal and carried out between 0700 and 0900 h and between 1400 in 1600 h. Exercise intensity in both experiments was adjusted by modifying the treadmill incline, while the walking speed remained constant at 3 miles per hour. In experiment 1, sedentary subjects engaged in no structured physical activity.

Indirect calorimetry

Resting metabolism was measured between 0600-0630 h on the study day and on the discharging day by indirect calorimetry using Viasys apparatus (Respiratory Care Inc., Yorba Linda, CA). Exercise metabolism was measured by indirect calorimetry during the first half hour of each hour of morning and afternoon exercise bouts as described above. Energy expenditure (EE) and relative CHO and fat utilization during rest, and exercise were estimated using the Weir equation [46].

Analytical procedures

Blood samples were collected into ice-chilled EDTA-coated tubes containing aprotinin (50 KIU/mL blood, Sigma Chemical, St. Louis, MO) and dipeptidyl peptidase-4 inhibitor (10 μL/mL blood; EMD Millipore Corporation, Billerica, MA). Plasma was kept frozen at -80°C for hormone and metabolite measurements. Plasma glucose (Fisher Diagnostics, Middletown, VA) and FFAs (Wako Diagnostics, Richmond, VA) were measured with enzymatic colorimetric assays. Ketone body D-3-hydroxybutyrate was measured with a kinetic enzymatic method (Randox Laboratories-US, Ltd., Kearneysville, WV). Plasma insulin and glucagon were measured with radioimmunoassays (EMD Millipore Corporation, Billerica, MA), and GIP and GLP-1 with a milliplex chemiluminescent assay kit (HGT-68K, EMD Millipore Corporation, Billerica, MA). All metabolites and hormones were measured in both experiments. The intra-assay coefficients of variation (CV) for the insulin and glucagon assays were respectively 2.3% and 3.6% and inter-assay CVs were 16.2% for both assays. For GIP and GLP-1 milliplex assay, intra-assay CV was <11% and inter-assay CV was <19%.

Statistical analyses

Data are presented as the mean and the standard error of the mean (SEM). Subject characteristics, energy consumption, and expenditure were evaluated with the analysis of variance (ANOVA) using Statistical Analysis System program (SAS; version 9.3, SAS Institute, Cary, NC). In both experiments, hormone and metabolite areas under the curves (AUCs) were calculated by the trapezoid rule to assess PP responses over 4-hour period after the two meals, between 1000 and 1400 h and between 1700 and 2100 h. In the experiment 2, hormone and metabolite responses were calculated as AUCs during 2-hour exercise and 1-hour postexercise

periods (between 0700 and 1000 h and 1400-and 1700 h exercise AUCs). Mixed-model repeated-measures ANOVA was used to analyze the effects of diurnal meal timing and macronutrient composition of meals. PP AUCs between low-CHO and high-CHO groups were analyzed as between-subject effects, while the times of meals (morning vs. afternoon), the interaction of the time and macronutrient composition (low-CHO vs. higih-CHO) were analyzed as within-subject effects.

Results

Experiment 1: Effect of the carbohydrate content of the meals on glucoregulation

The characteristics of the 16 subjects matched to a low-CHO sedentary (LCS) or high-CHO sedentary (HCS) group, eight each, are shown in Table 4-5. No group difference was found in age, weight, or BMI.

Endocrine responses

Plasma insulin (Figure 4-1)

The LCS trial resulted in 39% reduction in the afternoon PP insulin response relative to the corresponding morning insulin response (F=22.37, p=.0003), while no such change in the afternoon relative to the morning PP insulin response was seen in the HCS trial. Afternoon PP insulin AUC to low-CHO meal was 36% lower than to high-CHO meal (F=5.93, p=.0289 The effect of meal timing (morning vs. afternoon) on PP insulin response was significant (F=6.87, p=.0201) as was the interaction between the time of the meal and diet composition (F=16.54, p=.0012), although there was no overall diet effect on PP insulin responses.

Plasma glucagon (Figure 4-2)

PP glucagon responses in LCS group were significantly higher than in HCS group both in the morning (F=7.12, p=0.0184) and in the afternoon (F=1.09, p=0.428). Meal timing had no significant effect.

Plasma GIP (Figure 4-3)

LCS diet was associated with 48% lower afternoon PP GIP AUC than the HCS diet (F=8.87, p=.01). Morning PP GIP AUC appeared lower in the LCS than the HCS trial, but the difference did not reach significance.

Plasma GLP-1 (Figure 4-4)

The PP GLP-1 responses were unaffected by either meal timing or the macronutrient composition.

Metabolites

Plasma glucose (Figure 4-5)

The PP glucose AUC in the HCS trial was significantly higher in the afternoon than in the morning (F=5.51, p=.0341), but no such difference was seen in the LCS trial. The significant PP meal-timing effect was attributable to the afternoon hyperglycemia in the HCS group (F=5.51, p=.0342). Diets had no overall effect on PP glucose responses.

Plasma FFAs (Figure 4-6)

The FFA AUC was significantly lower 4 hours after the first meal compared to the FFA responses during fasting period before the first meal in HCS trial (F=6.13, p=.0267), but not in the LCS trial.

Plasma D-3-hydroxybutyrate (Figure 4-7)

The PP D-3-hydroxybutyrate AUCs were unaffected by either meal timing or the diets.

Experiment 2: Effect of exercise before the meals differing in carbohydrate content on glucoregulation

The characteristics of the 16 subjects matched to a low-CHO exercising (LCX) or high-CHO exercising (HCX) group of 8 women, each, are shown in Table 4-6. No group difference was found in age, weight, or BMI. However, the HCX group utilized significantly more CHO and less fat than the LCX groups (F=4.69, p=.0481) in the morning resting period on the study day. In addition, the HCX group utilized more CHO than fat the next day morning after the study than

during the corresponding period on study day (F=6.21, p=.0258). The HCX group utilized significantly more CHO and less fat during exercise in two circumstances: during the second compared to the first exercise session (F=27.03, p=.0001) and during the second exercise session compared to LCX group (F=11.65, p=.0042).

Endocrine responses

Plasma insulin (Figure 4-8)

Two hours of moderate-intensity exercise before the low-CHO meals resulted in a 31% reduction in the afternoon PP insulin response relative to the corresponding morning insulin response (F=17.72, p=.0009), while no such diet-associated change was seen in the morning meal of either composition or between the morning and afternoon high-CHO meals. The afternoon PP insulin AUC to the low-CHO meal was 35% lower than to the high-CHO meal (F=5.3, p=.0371). The effect of meal timing (morning vs. afternoon) on PP insulin response was significant (F=14.39, p=.002) as was the interaction between meal timing and the diet (F=4.67, p=.0486), but overall macronutrient composition effects were not significant.

Plasma glucagon (Figure 4-9)

Exercise before the morning low-CHO meal elicited a higher PP glucagon response than to the afternoon low-CHO meal (F=13.9, p=.0023), but overall macronutrient composition effect was not significant. In both exercising groups, plasma glucagon responses during afternoon exercise were significantly higher than during the morning exercise (LCX: F=38.7, p<.0001; HCX: F=25.3, p=.0002).

Plasma GIP (Figure 4-10)

Exercise before the afternoon low-CHO meal resulted in 45% lower PP GIP AUC than did exercise before the high-CHO meal (F=12.66, p=.0031). The afternoon PP GIP AUC after exercise and high-CHO diet was significantly higher (25%) than after the morning exercise and the meal (F=11.75, p=.0041). Afternoon exercise on either diet elicited greater GIP responses than morning exercise in fasted state (LCX: F=21.81, p=.0004; HCX: F=25.69, p=.0002). There was no overall significant effect of macronutrient composition on PP GIP responses.

Plasma GLP-1 (Figure 4-11)

Macronutrient composition had no overall effect on PP GLP-1 AUCs in exercise trials. However, GLP-1 responses were significantly higher during afternoon than morning exercise period in both LCX ($F=11.75$, $p=.0065$) and HCX ($F=9.03$, $p=.0132$) trials.

Metabolites

Plasma glucose (Figure 4-12)

Afternoon PP glycemia was significantly greater than in the morning to meals of either macronutrient composition (LCX: $F=5.44$, $p=.0351$; HCX: $F=18.67$, $p=.0007$). No overall dietary effect on glycemia reached significance. However, PP glycemia was significantly higher during exercise both in the morning ($F=5.35$, $p=.0365$) and in the afternoon ($F=5.54$, $p=.0337$).

Plasma FFAs (Figure 4-13)

In exercise trials, afternoon PP FFA AUC was significantly higher in the LCX than in the HCX group ($F=8.44$, $p=.0115$). No overall diet or meal timing effect was observed in PP FFAs responses. On the other hand, plasma FFA responses during afternoon exercise were significantly higher than during morning exercise in both exercising groups (LCX: $F=10.91$, $p=.0052$; HCX: $F=4.64$, $p<.05$).

Plasma D-3-hydroxybutyrate (Figure 4-14)

The PP D-3-hydroxybutyrate response in the LCX group was greater in the afternoon than in the morning ($F=5.83$, $p=.03$). These responses also were significantly higher during the afternoon exercise period than during morning exercise in both the LCX ($F=5.05$, $p=.0412$) and the HCX ($F=5.2$, $p=.0388$) trials. No significant overall effect of diet was found during PP or exercise periods.

Discussion

The main objective of this study was to determine whether the large CHO load characteristic of habitual US meals was responsible for the afternoon decline in glucose tolerance (hypothesis 1 and experiment 1), whether this diurnal phenomenon was influenced by incretin hormone effects on the afternoon insulin secretion (hypothesis 1), and whether the exercise could mitigate the

effects of the large CHO load on glycemia and abolish the afternoon glucose intolerance (hypothesis 2 and experiment 2).

The two experiments uncovered four unexpected and striking results. The first important finding was that the afternoon insulin response can be reduced by 39% within a sedentary day by exposure to a low-CHO meal in contrast to no such change when an iso-caloric high-CHO meal were eaten or when meals differing by two-fold in their CHO content were eaten in the morning. The finding only partially supported the hypothesis 1 that in the sedentary experiment 1, low-CHO meals attenuated the difference between afternoon and morning glycemia and resulted in large decline in the afternoon PP insulin response, but did not support the part of hypothesis 1 expecting an overall reduction in PP glycemia. Also, in support of hypothesis 1, the significantly lower afternoon GIP AUC in the low-CHO compared to high-CHO trials paralleled the changes seen in insulin responses thus indicating that the two events may be functionally related.

The second important finding was that in experiment 2 a substantial energy expenditure during 2-hour moderate-intensity exercise performed before the meals differing in CHO composition produced no additional decline in glycemia or in insulin response compared to the non-exercise trials. This finding did not support hypothesis 2 that was based on the expectation for a greater reduction in glycemic and insulin responses to exercise before high-CHO than low-CHO meals. There was no attenuation of the afternoon glucose intolerance to either diet despite a 31% decline in the afternoon PP insulin of similar magnitude as seen in experiment 1 sedentary condition. These results were unexpected and contrary to the universal consensus that aerobic exercise improves glycemia and reduced insulin responses [33-38].

The third striking finding is a parallel afternoon declines in PP insulin (39% in sedentary and 31% in exercise trials) and GIP (48% in sedentary and 45% in exercise trials) but not in GLP-1, when diet contains about 60 g of CHO but not when it delivers 120 g. And the final unexpected finding was that reduction of the CHO content of the two meals by a factor of two, from 60% that is customary in contemporary American diet to 30%, did not reduce or abolish the afternoon PP hyperglycemia while the low-CHO diet paradoxically reduced the afternoon PP insulin response. The results of the two experiments therefore require discussion of the four unexpected

findings, first the decline in the afternoon insulin and GIP responses to low-CHO meals and no such declines to morning low-CHO meals or to either morning or afternoon high-CHO meals; second the failure of substantial moderate-intensity pre-meal exercise to reduce glycemia and insulin responses and abolish the afternoon glucose intolerance; third, the possible explanation for parallel declines in the afternoon PP insulin and GIP, but not GLP-1, in both sedentary trials in experiment 1 and exercising trials in experiment 2; and finally the failure of dietary CHO restriction to lower PP glycemia or abolish the afternoon glucose intolerance.

The expected decline in the afternoon PP insulin and GIP responses to low-CHO meal compared to morning PP insulin response has been known for over four decades. Carroll et al. (1973) reported higher glycemia and lower first-hour insulin response during oral or intravenous glucose administration at 7 am and 7 pm. They also found reduced effectiveness of intravenous tolbutamine during first 40 minutes post-injection to elicit insulin secretion in the evening than in the morning [24]. Jarrett et al. (1972) observed lower insulin responses to oral glucose tolerance tests in the evening than in the morning with the reverse diurnal effects on glycemia [25]. Service at al. (1983) described progressive decline in CHO meal tolerance in the course of the day and associated the decline with impaired late-day insulin secretion as estimated by C-peptide concentrations [47]. A decline in insulin action in the course of a day also was demonstrated by Tatò et al. (1991) through increased insulin requirement for the maintenance of euglycemia in Type 1 diabetics from 8.5 IUs in the morning to about 11 IUs in the evening [48]. Wichelow et al. (1974) described faster decline in blood glucose to intravenous glucose tolerance test in the morning than in the afternoon, while the reverse diurnal pattern in the insulin response [27]. The impaired afternoon glucose tolerance was attributed to impaired insulin release.

The present two experiments confirm this phenomenon but sheds additional information beyond these early studies. While in the present two experiments, like in the studies reported four decades ago, glucose clearance was faster in the morning than in the afternoon, the quantity of CHO in the morning meal (about 120 g in the high-CHO meal vs 60 g in the low-CHO meal) had no impact on the magnitude of the PP insulin response. By contrast, in the afternoon, the size of the CHO load led to a proportional scaling of the insulin response. This observation suggests that there is a diurnal change in the stoichiometric relationship between the magnitude of the CHO

stimulus and the insulin response. Meal size, and by implication CHO load in a meal, was shown by Service et al. (1983) to correlate with the magnitude of PP glycemia and insulin and afternoon hyperglycemia accompanied with reduced PP insulin response, C-peptide concentration and impaired insulin secretion. In the present study, the large high-CHO meals elicited quantitatively equal PP insulin responses in the morning and in the afternoon, although their temporal pattern differed. The morning insulin response reached a high peak of about 60 μU and started declining within an hour of the onset of the meal. By contrast, highest afternoon PP insulin concentration was 60 μU and it took 2 hours before it started to decline. These observations are consistent with the interpretation that the afternoon insulin secretory response is directly or indirectly more dependent on the magnitude of glucose stimulus than in the morning.

The striking similarity in the timing of afternoon PP declines in insulin and GIP prompt the consideration that the two responses are functionally related. This points to the possibility that GIP, but not GLP-1, contributes to the diurnal difference in the insulin response to dietary CHO. Incretin gut hormones GIP and GLP-1 are well known insulin secretagogues and inhibitory to glucagon secretion [30, 31]. Secretion of GIP from the K cells in the duodenum and proximal small intestine is sensitive to glucose and facilitates pancreatic secretion of insulin [49] but circadian changes in its secretory response to nutrients are less understood. There was an apparent but non-significant decline in the morning PP GIP response to low-CHO meal in the present study that may have been a precursor to the significant afternoon decline and possibly influenced insulin secretory capacity. The present results suggest that only GIP secreted in the duodenum rather than the GLP-1 released from the distal part of small intestine are likely to be involved in the afternoon mediation of the insulin secretory responses to glucose, an inference that requires further direct experimental testing.

It is also necessary to consider whether the decline in the afternoon PP insulin responses may be a consequence of the secretion and actions of counterregulatory hormones glucagon, cortisol and growth hormone (GH) rather than the gut peptide GIP. Glucagon secretion in experiment 1 was uniformly higher after low-CHO than after high-CHO meals, but the afternoon PP concentration was not higher than in the morning and thus was unlikely to influence afternoon PP insulin response. Food ingestion during the day is inhibitory to GH secretion which exhibits a largest

secretory peak at night [50] eliminating the likelihood of the role of this hormone in the afternoon PP insulin counterregulation. Likewise, cortisol exhibits a strong diurnal decline in its concentration despite a mid-day peak that coincides with the mid-day meal [51, 52]. However, since peak cortisol concentrations are observed in the early morning, and its circadian nadir in the evening, afternoon cortisol secretion cannot account for the afternoon decline in the PP insulin response.

Possibly the least expected findings of these two studies are absence of any hypoglycemic and insulin lowering effects of exercise and of the reduction of CHO content of the meals by half. Exercise is widely known to acutely facilitate insulin-independent glucose tolerance [33-35] and to stimulate insulin sensitivity 3 to 48 h after its termination [36-38]. Exercise can increase glucose tolerance and insulin sensitivity when performed twice in a day in a fasted state [39-41]. Hypothesis 2 therefore predicted a decline in overall glycemia and disappearance of afternoon PP glucose intolerance by 2 hours of moderate-intensity exercise generating about 450 Kcal of energy expenditure 1 hour before the two meals. Exercise also could potentially affect insulin secretory response by altering the PP responses of incretin gut hormones, GIP and GLP-1 and their insulinotropic actions [31, 42]. As the results showed, exercise did not alter the pattern of glycemic, insulinemic or GIP responses, and GLP-1 PP responses were unaffected by either diet or exercise. Exercise trials actually maintained afternoon PP hyperglycemia relative to the morning PP glycemic responses on both diets and removed the beneficial effect of low-CHO diet on the afternoon hyperglycemia seen in the sedentary trial.

Although there is no obvious explanation for the absence of a hypoglycemic and hypoinsulinemic effect of exercise in this study, three possible explanations could be proffered. One is that the differences observed between the morning and afternoon insulin responses to meals of different CHO composition were selectively influenced by postingestive effects of the two diets and therefore unaffected by the energy cost of preceding exercise. The second possibility is that the prolonged exercise of moderate intensity exerts less of an influence over subsequent PP insulin secretion than does more intense and shorter exercise typically deployed in studies showing glycemic and insulin changes [33-38]. The third possibility is that the difference between the present and previous results resides in the gender and age of the subjects

as the present study was performed with postmenopausal women and the previous ones typically with young men.

The final unexpected finding of an unanticipated absence of a PP hypoglycemic and hypoinsulinemic response to low-CHO meal could possibly be a function of the large, about 800-Kcal meals used in present study. Afternoon hyperglycemia was shown to be increased with increased size of the meals [47] especially when the meals have high-CHO content and glycemic load. PP glycemia increased by about 10%, insulin response by about 47%, and insulin resistance estimated by HOMA by about 230% when the GI of 61% CHO meals was 84 compared to 34. The effect was amplified when the meals provided 60% as compared to 20% of daily energy [28]. Since we offered close to weight-maintenance amount of energy in only two rather than customary three meals, it is possible that even 60 g of carbohydrates in the afternoon low-CHO meal taxed the afternoon insulin-secretory capacity.

The data prompt new hypotheses that would require a different experimental design to determine whether there is either a greater afternoon reliance of GIP secretion on CHO absorption, a greater afternoon reliance of insulin secretion on incretin hormones, or another cause of greater reduction of afternoon glucose tolerance.

This study has several limitations. We engaged only healthy normal-weight postmenopausal women as subjects, so the results cannot be generalized to the different gender or age and body weight groups. The caloric intake at 25 kcal/kg of body weight during the study day was slightly lower than the estimated weight-maintenance energy balance of 30 kcal/kg of body weight. Our ability to make general inferences about the relative role of individual dietary macronutrients on PP insulin and glycemic responses is also limited by our use of only two diets that systematically altered CHO content without controlling the quantities of protein and fat. The diurnal effect on glycemia and insulinemic responses need to be further confirmed as the meal provided at different time of the day in present study were only controlled by its macronutrient composition, different food items of different GI values could possibly alter glycemic and insulinemic responses. However, the GI difference between the morning and the afternoon meal was not large enough to potentially show the influence of the meal GI on glycemic and insulin responses

as discussed in Appendix A. The small sample size did not provide sufficient power to show significant effects. Finally, while using the repeat-meal study design approximated habitual human feeding pattern, it precluded a distinction between effects of circadian versus repeated dietary exposure effects.

The present study demonstrate the ability of low-CHO meals to within a day substantially lower afternoon insulin response and to a lesser extent reduce high afternoon glucose concentration. By showing that this phenomenon is largely refractory to the glucose- and insulin-lowering effects of prolonged moderate-intensity exercise, the afternoon insulin-lowering effect of a repeat low-CHO meal is likely mostly responsive to postingestive influence of the GIP incretin gut peptide the secretion of which and stimulatory activities in the afternoon may be dependent on the quantity of ingested glucose. The efficacy of low-CHO diets to lower afternoon insulin response and decrease afternoon PP glycemia suggests that current preference and advocacy for high-CHO diets [16] may increase the risk of promoting insulin over-secretion and thus possibly compromising long-term pancreatic capacity to secrete insulin. This hormonal and glycemic effects of this simple dietary change provides an explanation for the efficacy of low-CHO diets in improving glycemic control and possibly reducing the risks of developing T2D reported in long-term studies [17-19]. Data of present study are also consistent with a recent studies showing that long-term intake of low-CHO and CHO restrictive diets or meals low in grain and milk products in insulin-resistant individuals reduces the progression to T2D [53].

Despite its limitations, this study is important in that it reveals three new phenomena: (1) a rapid 39% reduction in the afternoon insulin response and some improvement in glucose tolerance by simply reducing the CHO load of the meal from 60% to 30%; (2) apparent ineffectiveness of extensive moderate-intensity exercise to influence this dietary effect, and (3) an apparent involvement in the incretin gut hormone GIP in the dietary change of the afternoon PP insulin response. The present study also provides evidence that the convention of studying the effects of single, usually morning, exposures to specific diets on endocrine control of metabolism provides incomplete and potentially misleading information about physiological consequences of their repeat exposures within a day. Findings from this study may be of clinical significance in situations where reduction in PP hyperinsulinemia in general, and afternoon insulin over-

secretion in particular, is necessary, as hyperinsulinemia is viewed as a core defect associated with insulin resistance, pre-diabetes and T2D [54].

Table 4-1: Timing of metabolites, hormones, and appetite assessments (VAS) measurements

Day 2	Insulin	Glucagon	GIP	GLP-1	PYY	Glucose	FFA	Ketone
6:00	√	√	√	√	√	√	√	√
7:00	√	√	√	√	√	√	√	√
7:15	√					√	√	
7:30	√					√	√	√
7:45	√					√	√	
8:00	√	√	√	√	√	√	√	√
8:30	√					√	√	√
9:00	√	√	√	√	√	√	√	√
9:15	√					√	√	
9:30	√					√	√	√
10:00	√	√	√	√	√	√	√	√
10:15	√					√	√	√
10:30	√	√	√	√	√	√	√	√
10:45	√					√	√	√
11:00	√	√	√	√	√	√	√	√
11:15	√					√	√	
11:30	√					√	√	√
11:45	√					√	√	
12:00	√	√	√	√	√	√	√	√
12:30	√	√				√	√	√
13:00	√	√	√	√	√	√	√	√
13:15	√					√	√	√
13:30	√	√				√	√	√
13:45	√					√	√	√
14:00	√	√	√	√	√	√	√	√
14:15	√					√	√	
14:30	√	√				√	√	√
14:45	√					√	√	
15:00	√	√	√	√	√	√	√	√
15:30	√	√				√	√	√
16:00	√	√	√	√	√	√	√	√
16:15	√					√	√	
16:30	√					√	√	√
17:00	√	√	√	√	√	√	√	√
17:15	√					√	√	√
17:30	√	√				√	√	√
17:45	√					√	√	√
18:00	√	√	√	√	√	√	√	√
18:15	√					√	√	
18:30	√	√				√	√	√

18:45	√					√	√	
19:00	√		√	√	√	√	√	√
19:30	√	√				√	√	√
20:00	√		√	√	√	√	√	√
20:15	√					√	√	
20:30	√	√				√	√	√
21:00	√		√	√	√	√	√	√
22:00	√	√	√	√	√	√	√	√
23:00	√					√	√	√
Day 3								
0:00	√	√	√	√	√	√	√	√
1:00	√					√	√	√
2:00	√	√				√	√	√
3:00	√					√	√	√
4:00	√	√				√	√	√
5:00	√					√	√	√
6:00	√	√				√	√	√

Table 4-2: Menu template for pre-study low-carbohydrate (CHO) dinner

Meal composition: 30% carbohydrate (CHO), 25% protein (PRO), 45% fat

Food items	Wt (g)	CHO (g)	PRO (g)	FAT(g)	Kcal
Turkey roll					
▪Silvered turkey	2 oz	0	17	0.5	72.5
▪Multigrain roll	1 each;54 g	25	5	2.5	142.5
▪Menu Magic Mayo	1/2 pkg.; 6 g	0.5	0	2.3	22.7
cheese omelet	1 each; 98 g	3	12	13	177
Tossed Greens using Romaine Blend	0.8 oz; 22.8g	2.5	0.5	0	12
Kraft Ranch dressing	1 package; 12.4 g	0	0	7	63
shredded almonds (on the top of tossed greens)	12 almonds	3	3	7.5	91.5
1 serving fresh fruit (options listed below†)	depends†	15	0.8	0.2	65
Total‡		**49**	**38.3**	**33**	**646.2**
Percentage (%)		**30.3**	**23.7**	**46.0**	
†Fruits options	**Wt (g) (1 serving)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
Fresh Strawberries	8 oz; 226.8g	15.9	1.4	0.8	76.4
Fresh Nectarine	1 med; 5oz;	16	1	0.6	73.4
Asian pear	1 each	16	1	0	68
Peach Halves	1/2 cup; 2 halves	14	1	0	60
Pineapple rings	1/2 cup; 3 rings	14	0	0	56
Pear halves	1/2 cup; 2 halves	13	0	0	52
Pear slices	1/2 cup	14	1	0	60
‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-third of the subject's daily caloric need. Subjects' daily caloric need=30kcal/kg BW					

Table 4-3: Menu template for morning low-carbohydrate (CHO) meal

Meal composition: 30% carbohydrate (CHO), 25% protein (PRO), 45% fat

Food items	Wt (g)	CHO (g)	PRO(g)	FAT(g)	Kcal
Macaroni and cheese	50g	6	4	5.5	89.5
Sausage	2.5 links	0	7.5	7.5	97.5
Silvered ham	2 oz; 57 g	0	9	1.5	49.5
Wheat roll	0.8 each; 29.4 g	13.8	2.7	1.2	76.8
Margarine country c	1 tub; 4.8 g			2.4	21.6
CHEF SALAD					
▪romaine blend	100 gm	3.5	1	0	18
▪slivered turkey strips	29 gm	0	4.25	0.1	13
▪slivered ham strips	29 gm	0	4.6	0.7	20
▪cheddar cheese	20 gm	0	5	6.7	78
▪Swiss cheese	15 gm	0	3.7	4.8	59
▪cherry tomatoes	two	1.5	0	0	6
▪cherry tomatoes	two	1.5	0	0	6
▪cucumber slices	12 gm	1			5
▪croutons	7 gm pkg	5	1	1	30
▪croutons	7 gm pkg	5	1	1	30
Straw/Ban Lite & Fit Yogurt	4 oz cont.	7	3	0	40
shredded almonds (on the top of Yogurt)	12 almonds	3	3	7.5	91.5
1 serving fresh fruit (options listed below†)	depends†	15	0.8	0.2	65
Total‡		62.3	50.55	40.1	796.4
Percentage (%)		31.3	25.4	45.3	
†Fruits options	**Wt (g) (1 serving)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
Fresh Strawberries	8 oz; 226.8g	15.9	1.4	0.8	76.4
Fresh Nectarine	1 med; 5oz;	16	1	0.6	73.4
Asian pear	1 each	16	1	0	68
Peach Halves	1/2 cup; 2 halves	14	1	0	60
Pineapple rings	1/2 cup; 3 rings	14	0	0	56
Pear halves	1/2 cup; 2 halves	13	0	0	52
Pear slices	1/2 cup	14	1	0	60

‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-half of the subject's daily caloric need. Subjects' daily caloric need=25kcal/kg BW

Table 4-4: Menu template for afternoon low-carbohydrate (CHO) meal

Meal composition: 30% carbohydrate (CHO), 25% protein (PRO), 45% fat

Food items	Wt (g)	CHO (g)	PRO(g)	FAT(g)	Kcal
Flame Grilled Garden burger	3.4 oz; 99 g	10	19	5	161
Minestrone Soup US	6oz; 150 g	12	3.6	6	116.4
Chicken Caesar Salad					
▪Romaine lettuce	90.7 gm	2.2	1.5	0.2	13
▪sliced chicken	1 breast cooked	0	24.3	1.4	112
▪parmesan cheese	9 gm	0.3	3.4	2.5	38
▪croutons	1 pkt	5	1	1	29
Caesar dressing	1.5 oz pkg.; 43 g	2	1	22	210
shredded almonds (on the top of Caesar salad)	4 almonds	1	1	2.5	30.5
Cran-grape Juice	1 carton	20			80
0.5 serving fresh fruit (options listed below†)	depends†	7.5	0.4	0	31.6
Total‡		**60**	**55.2**	**40.6**	**821.5**
Percentage (%)		**29.2**	**26.9**	**44.5**	
†Fruits options	**Wt (g) (.5 serving)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
Fresh Strawberries	4 oz; 113.4g	8	0.7	0.4	38.4
Fresh Nectarine	0.5 med; 2.5oz;	8	0.5	0.3	36.7
Asian pear	0.5 each	8	0.5	0	34
Peach Halves	1/4 cup; 1 halve	7	0.5	0	30
Pineapple rings	1/4 cup; 1.5 rings	7	0	0	28
Pear halves	1/4 cup; 1 halve	6.5	0	0	26
Pear slices	1/4 cup	7	0.5	0	30

‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-half of the subject's daily caloric need. Subjects' daily caloric need=25kcal/kg BW

Table 4-5: Subject characteristics and energy intake in low-carbohydrate (LCS) and high-carbohydrate (HCS) sedentary trials

Groups	LCS (n=8)	HCS (n=8)
Age (years)	56.9±1.54	55.0±1.07
Weight (Kg)	69.9±3.41	66.1±2.22
Percentage of Body Fat (%)	38.0±1.65	35.1±2.18
BMI (Kg/m^2)	25.4±0.75	23.6±0.91
Fitness level (VO_2/min×Kg)	24.7±2.49	25.6±3.66
EI in meal 1 (Kcal)	751.8±59.69	769.5±32.82
EI in meal 2 (Kcal)	648.6±112.98	803.8±33.69

EI=Energy intake

Table 4-6: Subject characteristics and energy balance in exercise before low-carbohydrate meals (LCX) and exercise before high-carbohydrate meals (HCX) trials

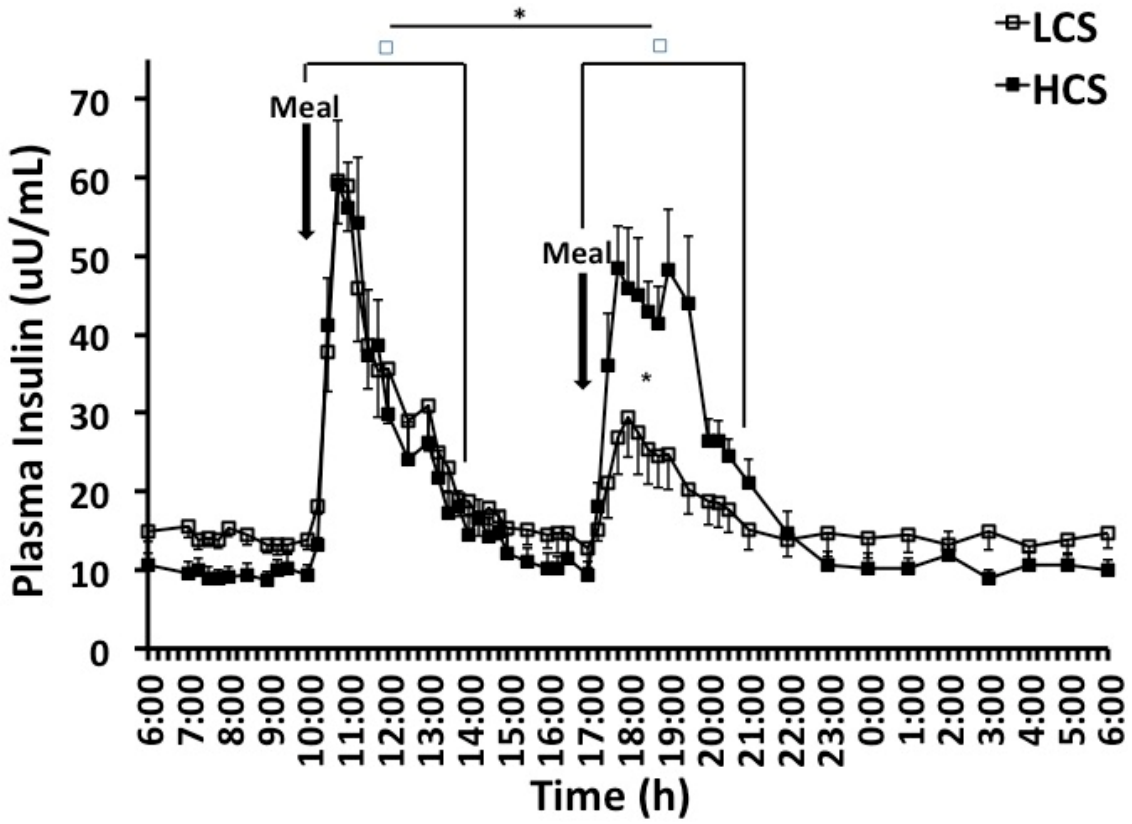
Groups	LCX (n=8)	HCX (n=8)
Age (years)	59.3±1.46	59.9±1.03
Weight (Kg)	71.8±2.76	65.9±3.19
Percentage of Body Fat (%)	39.0±3.30	36.9±2.65
BMI (Kg/m^2)	25.7±1.16	24.1±0.90
Fitness level (VO_2/min×Kg)	26.2±3.60	22.6±1.79
EI in meal 1 (Kcal)	782.1±58.24	816.4±43.14
EI in meal 2 (Kcal)	873.2±38.00	821.7±41.72
1st Exercise EE (Kcal)	485.2±63.28	413.7±24.55
CHO utilization (%) during 1st exercise	41%±3.8%	43%±5.4%*
Fat utilization (%) during 1st exercise	59%±3.9%	57%±5.4%*
2nd Exercise EE (Kcal)	472.5±55.81	408.6±24.54
CHO utilization (%) during 2nd exercise	42%±2.1%[a]	61%±3.3%[b]
Fat utilization (%) during 2nd exercise	58%±2.1%[a]	39%±3.3%[b]
EB: EI - exercise EE (Kcal)	697.6±143.91	815.9±88.11

EB=energy balance; EE=energy expenditure; EI=energy intake

[a,b] Within the same variable, groups with different superscripts are significantly different ($p<.05$)

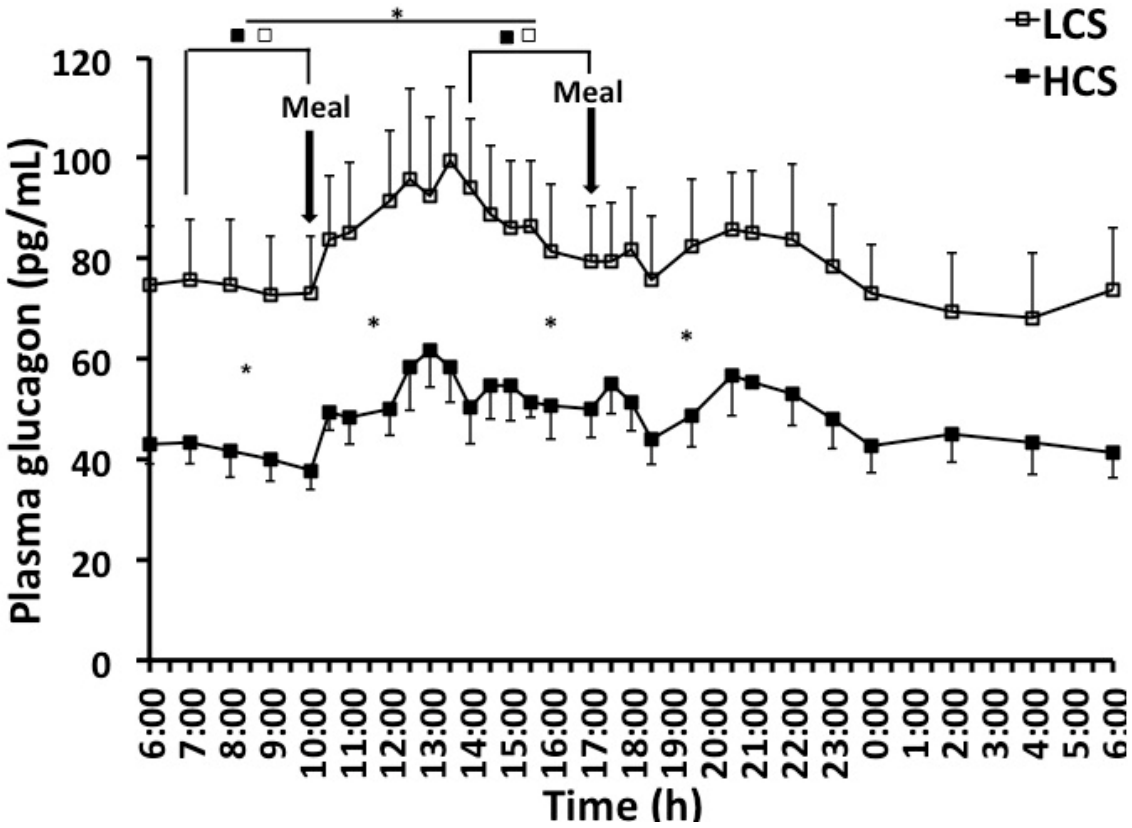
* Within HCX group, less carbohydrate and more fat were utilized during 1st exercise than during the 2nd exercise ($p<.05$)

Figure 4-1: Plasma insulin responses in low-carbohydrate sedentary (LCS) and high-carbohydrate sedentary (HCS) trials



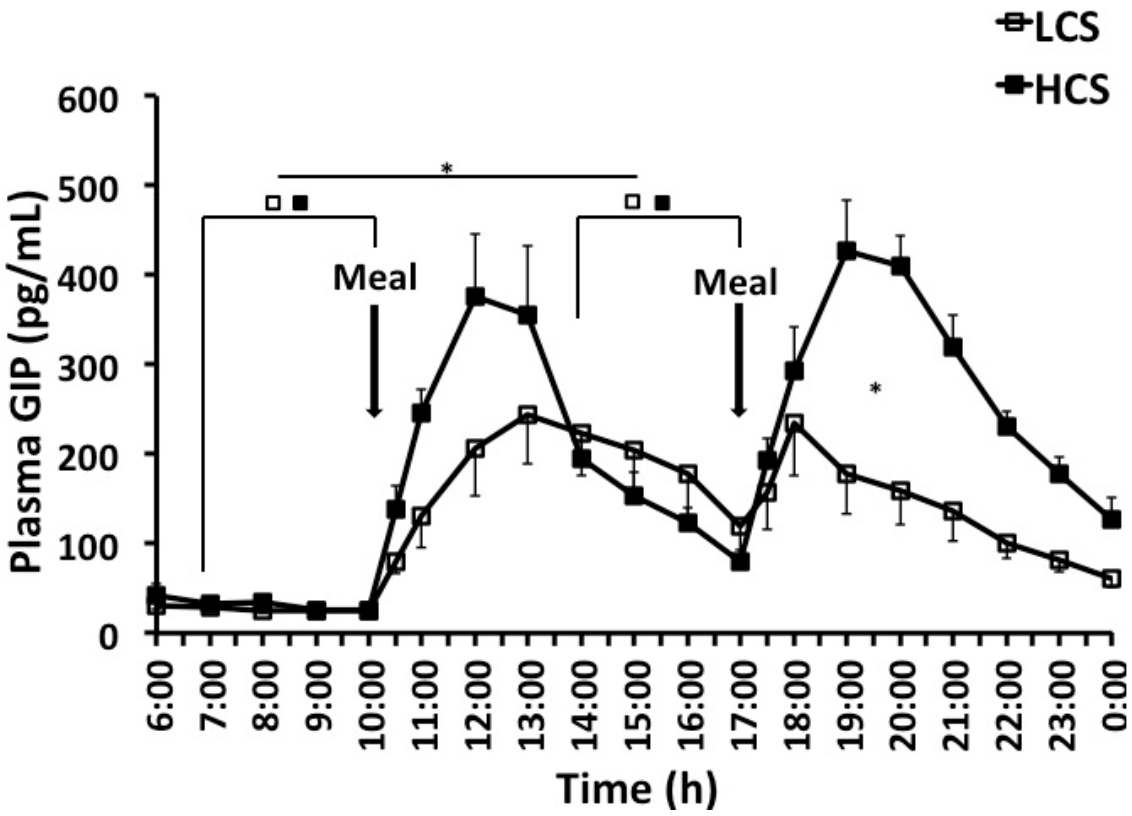
"*" indicates the significance between two selected time periods/areas under the curve.

Figure 4-2: Plasma glucagon responses in low-carbohydrate sedentary (LCS) and high-carbohydrate sedentary (HCS) trials



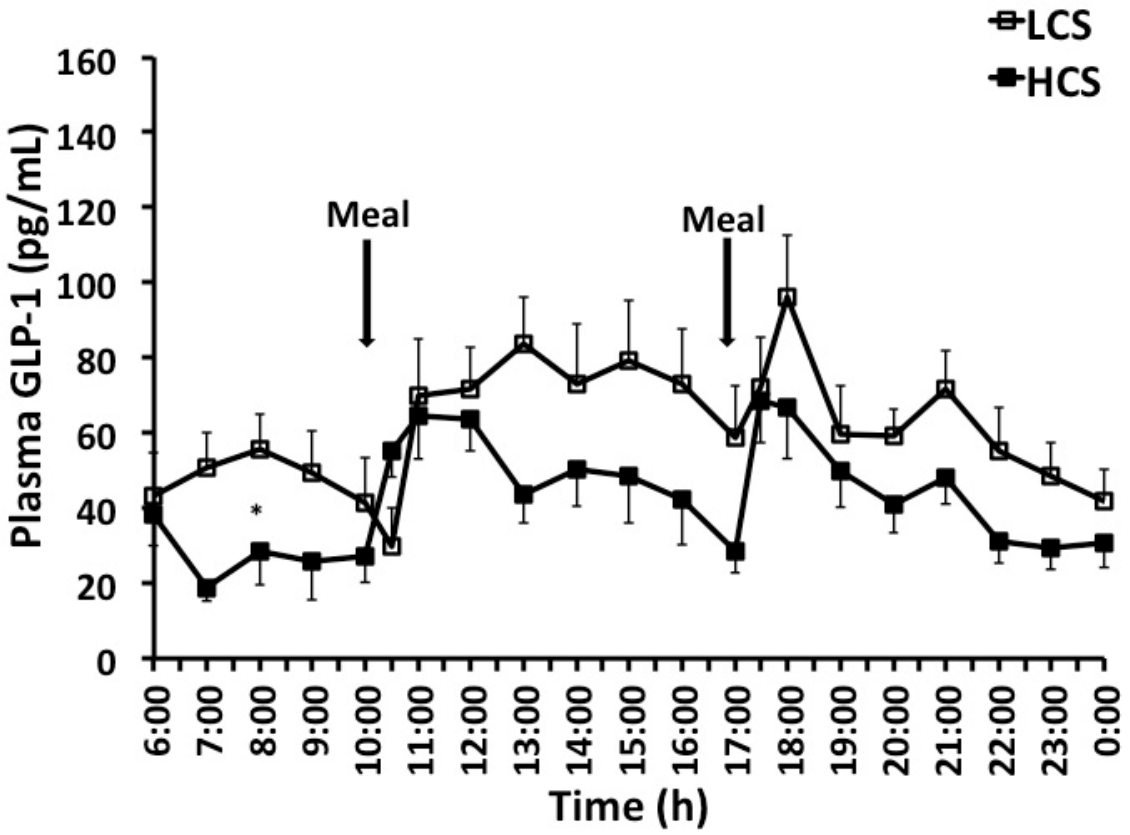
"*" indicates the significance between specified areas under the curve.

Figure 4-3: Plasma GIP responses in low-carbohydrate sedentary (LCS) and high-carbohydrate sedentary (HCS) trials



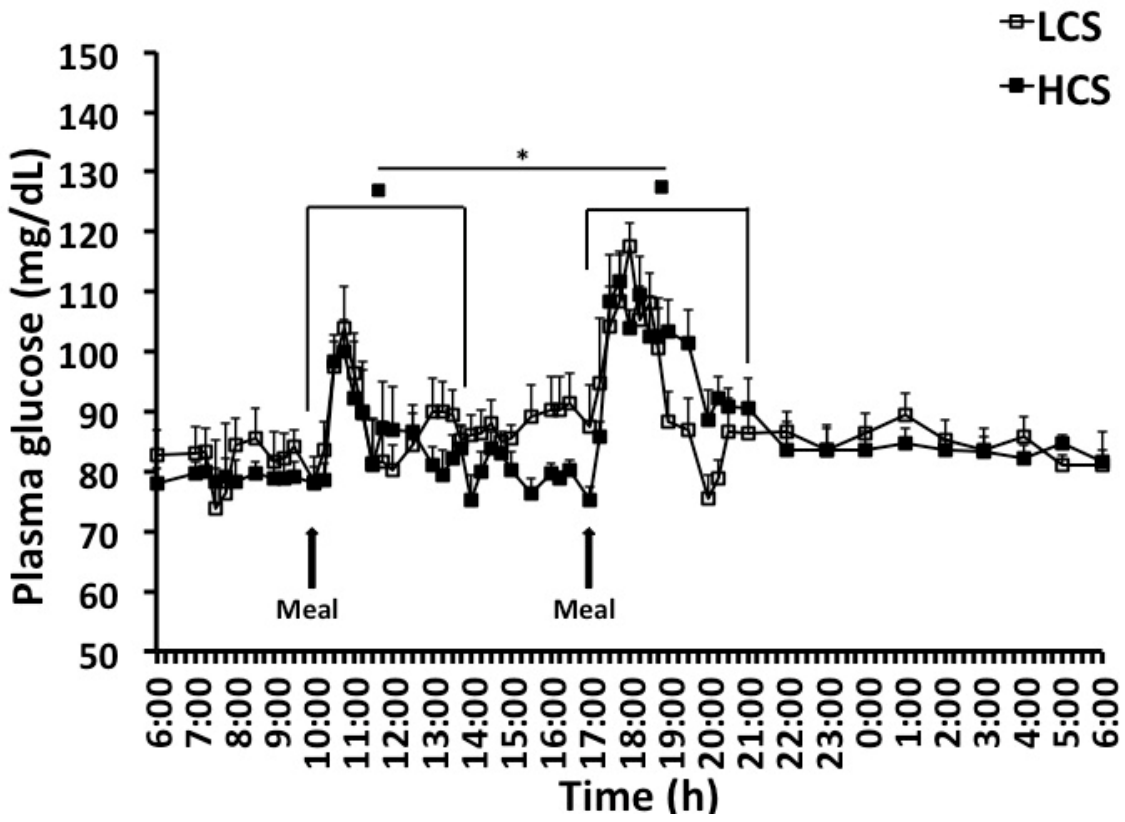
"*" indicates the statistical significance between two selected time periods/areas under the curve.

Figure 4-4: Plasma GLP-1 responses in low-carbohydrate sedentary (LCS) and high-carbohydrate sedentary (HCS) trials



"*" indicates the statistical significance between LCS and HCS sedentary groups in the specified areas under the curve.

Figure 4-5: Plasma glucose responses in low-carbohydrate sedentary (LCS) and high-carbohydrate sedentary (HCS) trials



"*" indicates the statistical significance between two selected time periods.

Figure 4-6: Plasma free fatty acids (FFAs) responses in low-carbohydrate sedentary (LCS) and high-carbohydrate sedentary (HCS) trials

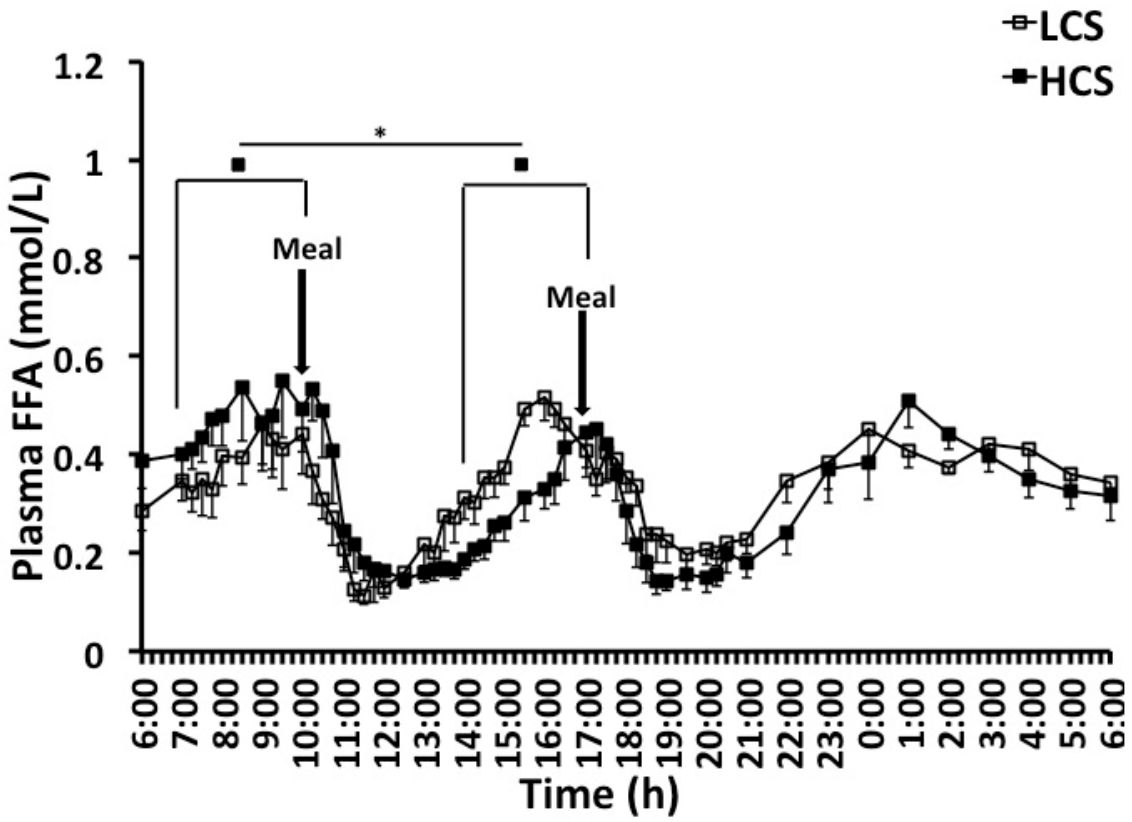


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Figure 4-7: Plasma D-3-hydroxybutyrate ketone responses in low-carbohydrate sedentary (LCS) and high-carbohydrate sedentary (HCS) trials

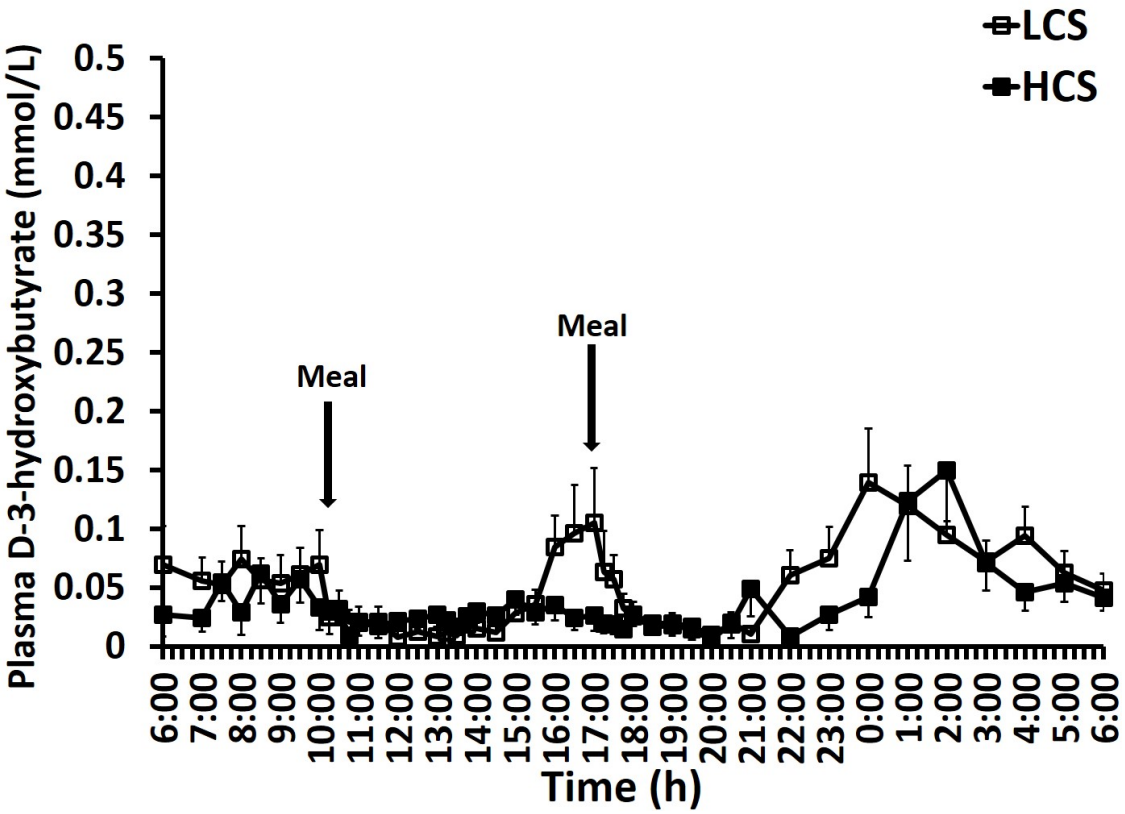
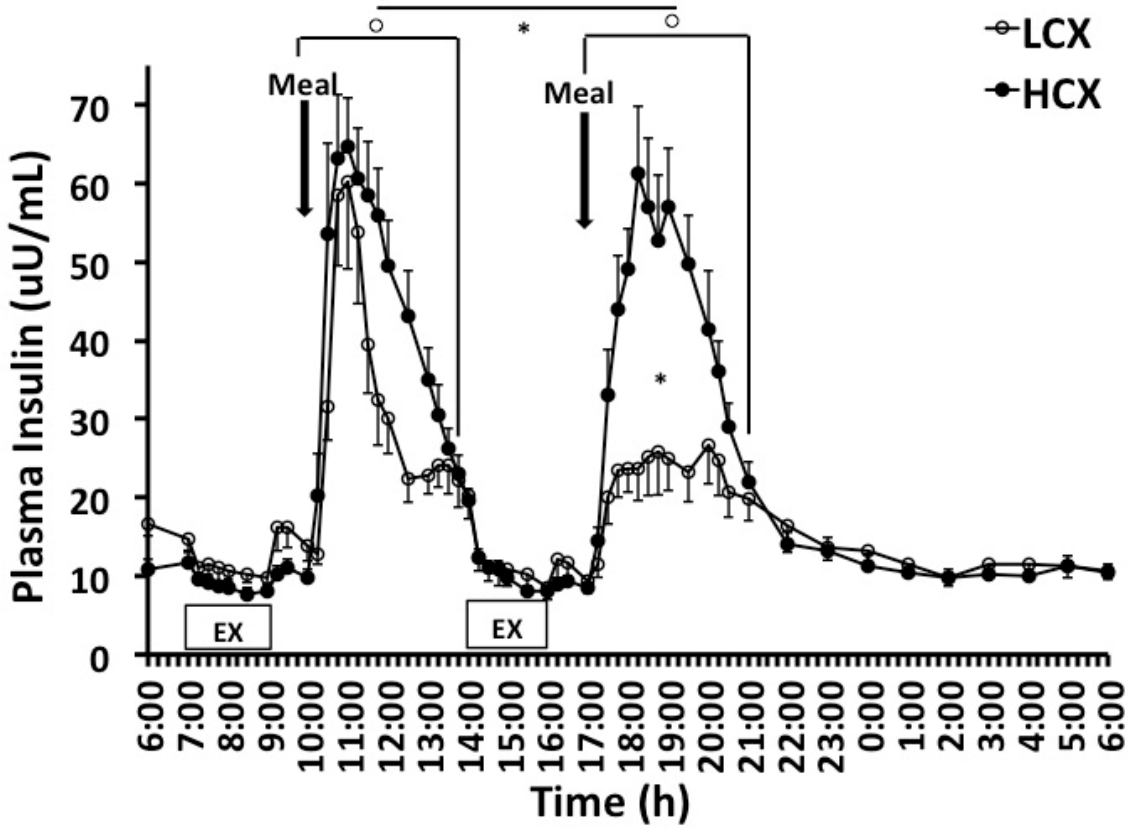


Figure 4-8: Plasma insulin responses in exercise before low-carbohydrate (LCX) meals and exercise before high-carbohydrate meals (HCX) trials



"*" indicates the statistical significance between two selected time periods/areas under the curve.

Figure 4-9: Plasma glucagon responses in exercise before low-carbohydrate (LCX) meals and exercise before high-carbohydrate meals (HCX) trials

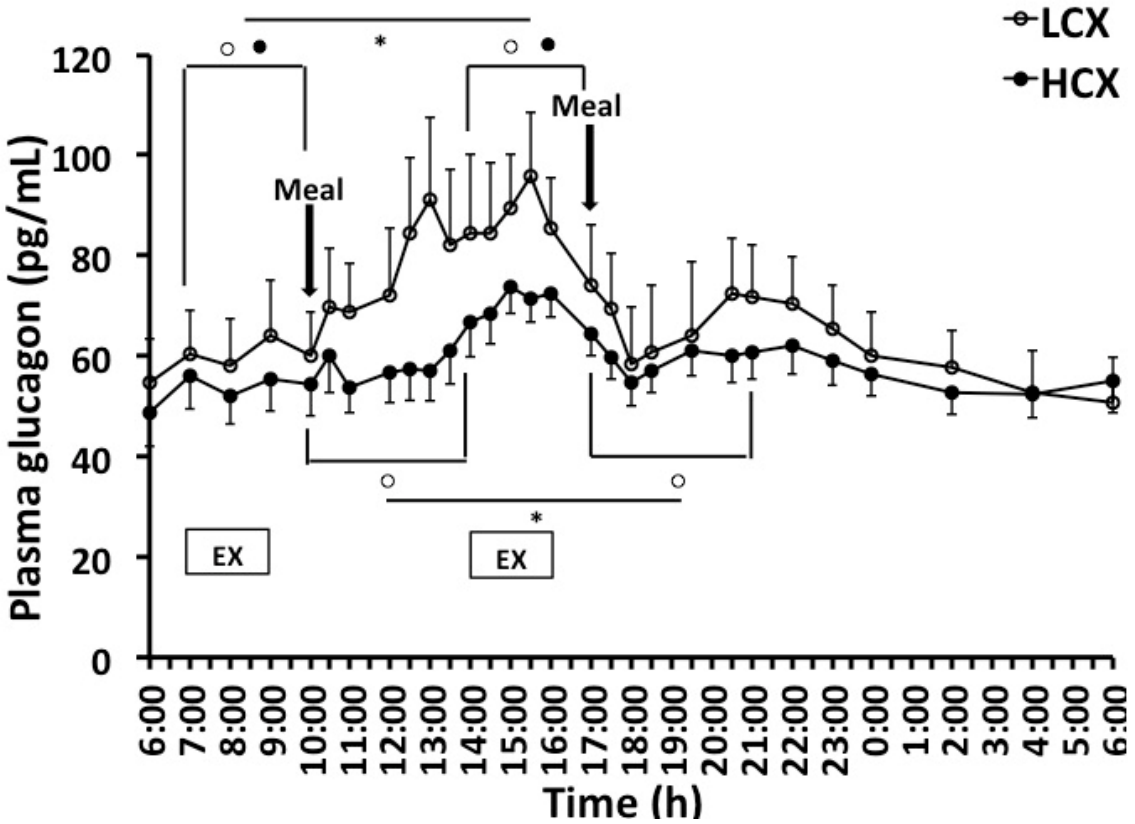


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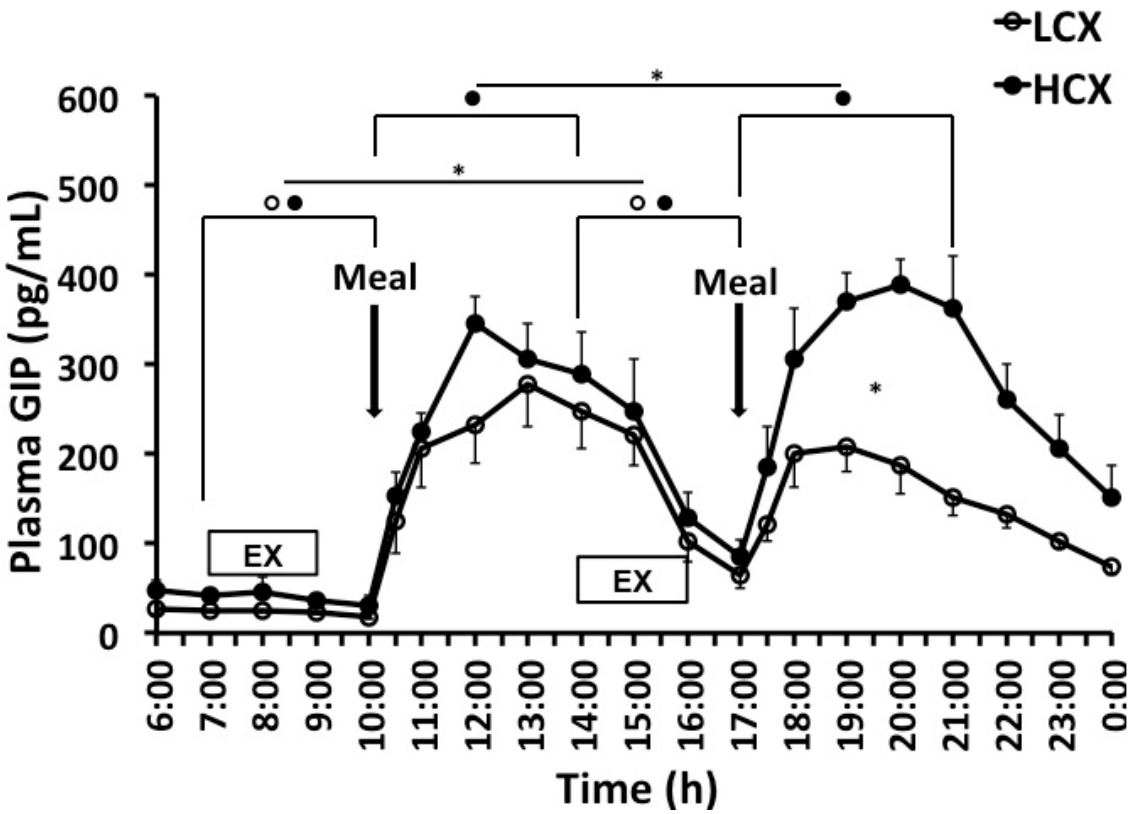
Figure 4-10: Plasma GIP responses in exercise before low-carbohydrate (LCX) meals and exercise before high-carbohydrate meals (HCX) trials



"*" indicates the statistical significance between two selected time periods/areas under the curve.

Figure 4-11: Plasma GLP-1 responses in exercise before low-carbohydrate (LCX) meals and exercise before high-carbohydrate meals (HCX) trials

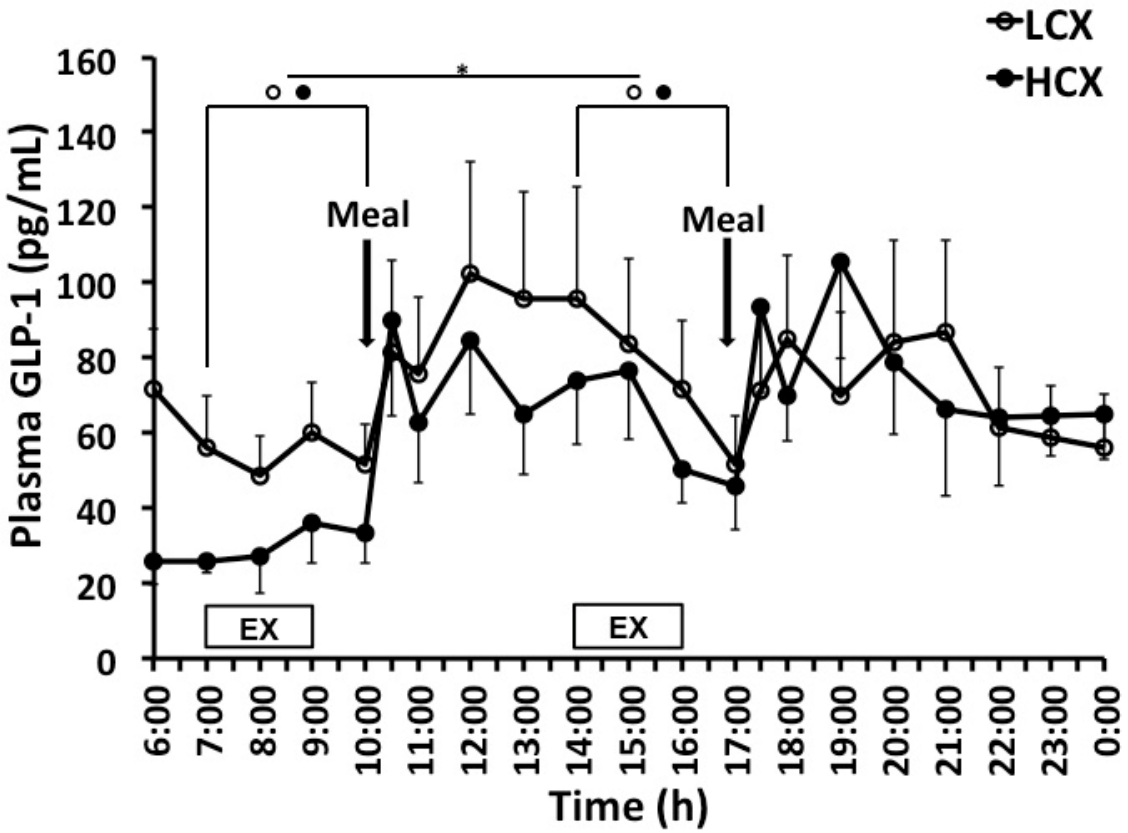


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Figure 4-12: Plasma glucose responses in exercise before low-carbohydrate (LCX) meals and exercise before high-carbohydrate meals (HCX) trials

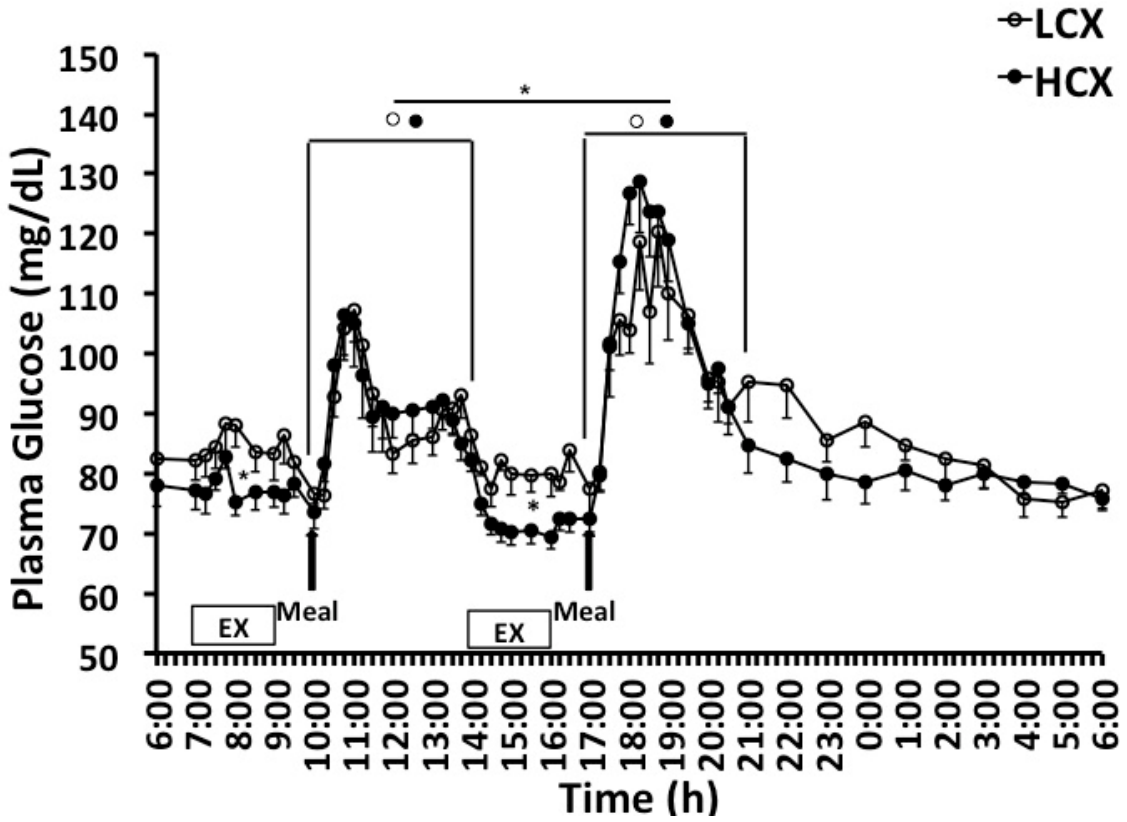

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Figure 4-13: Plasma free fatty acids (FFAs) responses in exercise before low-carbohydrate (LCX) meals and exercise before high-carbohydrate meals (HCX) trials

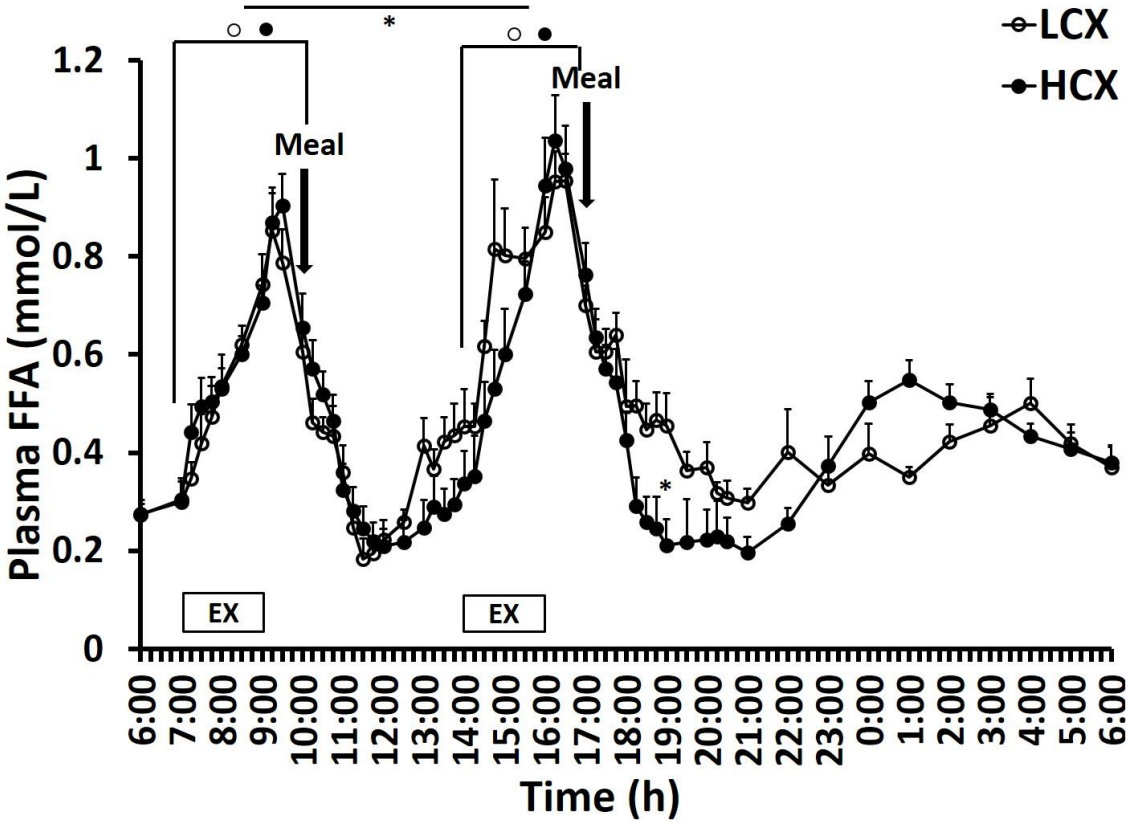

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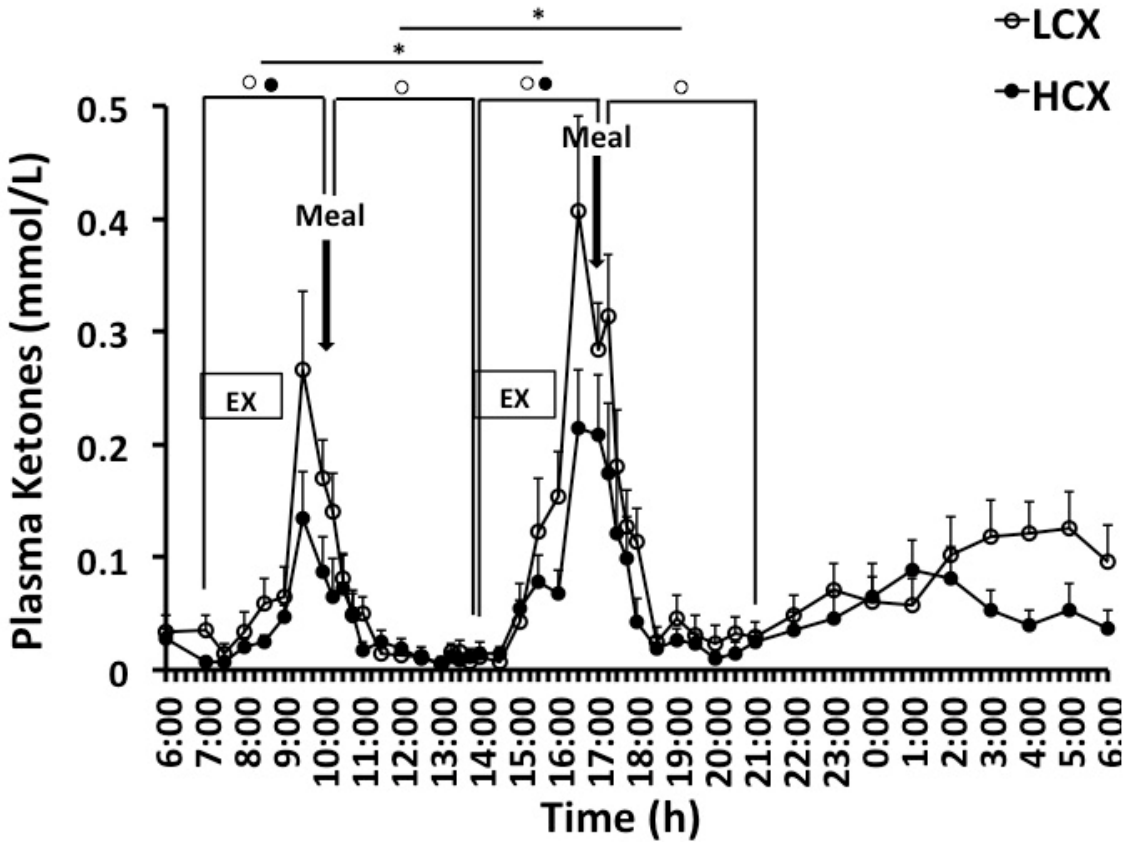
Figure 4-14: Plasma D-3-hydroxybutyrate ketone body responses in exercise before low-carbohydrate (LCX) meals and exercise before high-carbohydrate meals (HCX) trials



"*" indicates the statistical significance between two selected time periods.

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CHAPTER 5

Exercise anorexia: Influence of gut peptides and macronutrient composition

Abstract

Several studies have attributed exercise anorexia to increased concentrations of satiating hormones glucagon-like peptide 1 (GLP-1), and peptide tyrosine tyrosine (PYY). The hypothesis that exercise anorexia is in part caused by exercise-associated changes in the concentration of GLP-1 and PYY was tested in a repeat-event design under three study conditions: (1) timing of exercise during fasting condition as well as during both early and late postprandial (PP) exercise (experiment 1), (2) varied duration and volume of exercise before the meals (experiment 2), and (3) effects of exercise before the meals that differed in the carbohydrate content by a factor of two (experiment 3). We hypothesized that (1) exercise during the PP period, but not during fasted state, would suppress hunger and enhance sensation of fullness because of the coincident increased secretion of GLP-1 and PYY; in addition, that the suppression of hunger would be greater during the early rather than late PP periods because of greater GLP-1 and PYY concentrations (experiment 1); (2) prolonged exercise before the meals would suppress hunger more than exercise of shorter duration (experiment 2), and (3) exercise would elicit greater satiation to subsequent low-carbohydrate (LC) than to a high-carbohydrate (HC) meal because a LC meal stimulates greater release of GLP-1 and PYY than a HC meal (experiment 3).

Methods. Healthy postmenopausal women were matched for weight and BMI. Two iso-caloric meals given at 1000 h and 1700 h. HC meals contained 60% carbohydrate (CHO), 15% protein (PRO), and 25% fat, and LC meals contained 30% CHO, 25% PRO and 45% fat. Two bouts of moderate-intensity exercise were performed in exercise trials; timing (before- or after-meals) or duration (1- and/or 2-hour long) of exercise bouts depends on experimental settings. Blood samples were collected for hormone and metabolite measurements and ratings of hunger and fullness were assessed with a visual analog scale. Resting and exercise metabolism were measured by indirect calorimetry.

Measurements. Changes in concentrations of gut hormones, GIP, GLP-1, and PYY, and appetite (hunger and fullness) ratings were assessed as areas under the curve (AUCs). The AUC periods were 4-hour after each meal (early PP period) and 3-hour after each bout of exercise. The second bout of exercise occurred 4-hour after the first meal coincided with the 3-hour late PP period in both exercising and sedentary trials.

Experiments. In experiment 1 compared the effects of 2-hour long exercise before (EBM) or after (EAM) two HC meals to sedentary trials. In experiment 2, the effects of two bouts of 1-hour exercise (X1) before LC meals were contrasted to two bouts of 2-hour exercise (X2) and the sedentary trial (X0). In experiment 3, the effects CHO content of meals (LC vs. HC) were examined in sedentary (LCS vs. HCS) and exercise (LCX vs. HCX) conditions.

Results. In experiment 1, fullness ratings and the declining portion of GLP-1, PYY, and GIP AUCs were significantly higher when EBM exercise took place during the late PP than during the fasting period. Higher fullness was also registered during late PP period in EAM trial when no exercise was performed during that time. GIP and GLP-1 AUCs also were significantly higher during late PP than the fasting period in all three groups while PYY AUCs changes in the same way in the non-exercising SED and EAM groups. In experiment 2, neither fullness ratings nor gut peptide AUCs were higher in X2, than in X1 exercising trials. Both the fullness ratings and PYY AUC were significantly higher when exercise in X1 and X2 trials took place during the late PP than during the fasting period. GIP and GLP-1 AUCs also were significantly higher during late PP than the fasting period in all three groups. In experiment 3, hunger ratings in LCS group were significantly lower during late PP than during the fasting period. During the fasted state, LCX group with LC meal previous night reported lower hunger ratings than HCX group with HC meal previous night. Fullness ratings and GLP-1 AUC were also higher during late PP compared to the fasting period in both LCX and HCX groups, while PYY AUC changes in the same way in both sedentary (LCS and HCS) trials and in HCX.

A trend toward higher sensation of fullness during late PP than during the fasting period were seen in exercising as well as sedentary trials with LC meals while all three gut peptides were unaffected by macronutrient composition of meals and were consistently higher during late PP than during the fasting state.

Conclusions. No consistent association was seen between the exercise-associated increased ratings of fullness and concentrations of GIP, GLP-1, and PYY. However, both sets of variables

were higher during late PP than during the fasted state independently of the presence or absence of exercise. Two-fold difference in exercise duration and in CHO content of the meals did not alter this outcome. The hypothesized causal relationship of exercise anorexia and exercise-associated changes in GLP-1 and PYY was not supported.

Introduction

Exercise is frequently used as a means of weight control or weight loss. The features of exercise that may aid in these changes is the transient suppression of hunger and increased sense of fullness during and shortly after the exercise as well as the absence of post-exercise compensatory food intake for energy expended during exercise [1-7]. The cause of exercise anorexia is not known, and as it not consistently elicited, the dietary and exercise conditions necessary for its elicitation also are not well understood. The possibility that it depends on exercise intensity is supported by some studies where it appears when a threshold exercise intensity of about 65-70% of maximal effort is exceeded [3, 5, 7]. In these studies, appetite suppression often lasts for less than an hour after exercise cessation, and the magnitude of hunger suppression is proportional to duration of exercise above this threshold intensity [3]. However, this working hypothesis is not supported by other studies in which exercise anorexia can be elicited at lower intensity of 46% of VO_2max [2] but does not extend to the post-exercise period [8]. Two recently published studies using isocaloric bouts of moderate and high-intensity exercise showed differential effect of exercise intensity on appetite responses in normal- weight vs. overweight/obese men [9, 10]. Deighton et al. [9] reported that normal weight men showed a greater suppression of hunger if they exercised at high-intensity than at moderate-intensity exercise. However, overweight/obese individuals showed no differential appetite responses between moderate and high-intensity exercise [10]. Despite its inconsistent manifestation, the phenomenon of exercise anorexia is of interest because it, along with absence of compensatory increase in post-exercise food intake, may contribute to weight loss and weight-loss maintenance [2, 3].

Increased release of gut hormones have also been hypothesized to cause exercise anorexia. Gastric hormone ghrelin [11, 12] is viewed as an appetite-stimulating hormone as its concentration changes in parallel with hunger sensation [13-15]. In support of its role in exercise anorexia, parallel declines in hunger and acylated ghrelin, but not total ghrelin, concentrations were reported during and after exercise [16-18]. On the other hand, gut hormones cholecystokinin (CCK), glucagon-like pepetide-1 (GLP-1) and peptide tyrosine tyrosine (PYY) have all been implicated in producing increased sensations of fullness and satiation [4, 16-20]. Cholecystokinin (CCK) is secreted by I cells in the duodenum and jejunum in response to fat and

protein [21]. The role of CCK in conscious sensation of satiation has been extensively studied and validated [22-25]. However, due to the difficulty of its measurement because the antibodies against it cross-react with gastrin, it was not included in this study. GLP-1 and PYY, secreted by L cells in the distal intestine and colon in response to food ingestion [26, 27] have been implicated in conscious sensations of fullness or satiation [28-33]. The secretion of GLP-1 is predominantly stimulated by carbohydrate and fat [34-36], while a higher concentration of plasma PYY is seen after fat-, rather than carbohydrate-, or protein-rich meals [36-38]. Both GLP-1 [39] and PYY [40] act as the ileal brake, a distal-to-proximal gastrointestinal (GI) negative feedback mechanism over additional food intake that optimizes nutrient digestion and absorption [41].

The hypothesis that GLP-1 and PYY may cause exercise anorexia is supported by several studies, in which the concentrations of both hormones were reported to increase significantly during, and shortly after a half-, or 1-hour bout of moderate-intensity exercise performed 1 hour after eating [4, 19, 20]. However, the changes in GLP-1 and PYY concentrations were not invariably associated with changes in appetite responses or subsequent energy intake. Ueda et al. (2009) reported reciprocal relationship between increases in GLP-1 and PYY concentrations during and shortly after exercise and hunger ratings in one study [19] but not in the other [20]. The only study that reported the association between a satiating gut peptide and exercise anorexia during fasting period was that of Broom et al. [18] where there was a brief overlap between the period of exercise anorexia and an increased plasma PYY concentration And where a subsequent meal did not elicit increases in the hormone. We therefore concluded that the effect of the prandial state at the time of exercise may provide the answer whether exercise anorexia is consistently elicited when the satiating gut peptides are released. The study design

To test the hypothesis that exercise anorexia is in part caused by increased exercise-associated concentrations of GIP, GLP-1, and PYY, the experiment was designed to allow the measurement of the concentrations of GLP-1 and PYY during exercise that was performed in three prandial states, (a) fast before the morning meal (first EBM exercise bout), (b) early postprandial period (early PP period, 1 hour after the meal, EAM), and (c) late PP period (afternoon EBM exercise, 4 hours after the meal). The appetite ratings and gut peptides during exercise were contrasted to the

corresponding measurements in the sedentary trials (experiment 1).Glucose-dependent insulinotropic peptide (GIP) was included in the measurements as a marker of early gastric filling although its association with satiation has not been established. GIP is secreted by K cells in the proximal small intestine [42, 43] in response to ingested fat and carbohydrate [34, 44] and it suppresses gastric emptying [45]. Additional test of the gut peptide hypothesis of exercise anorexia entailed a comparison between exercise bout durations that differed by a factor of two with the expectation that there would be parallel dose-dependent changes in exercise anorexia and gut peptides (experiment 2). Finally, the association between exercise anorexia and gut peptide secretion was also examined against the background of meals of different macronutrient composition as these peptides display differential responsiveness to dietary components. The expectation was that there would be diet-specific changes in PP gut peptide secretion and parallel changes in exercise anorexia (experiment 3).

We hypothesized that:
(1a) Both exercise anorexia and satiating gut peptide concentrations will be higher during PP than during pre-meal fasting period because of the coincidence of exercise and gut peptide secretion in the former condition and the absence of gut peptide secretion in fasted state (experiment 1);
(1b) The association between exercise anorexia and satiating gut peptide concentrations will be stronger during early than during the late PP period because of the higher post-meal concentrations of gut peptides in the early compared to the late PP period (experiment1);
(2) Longer exercise bouts before the meals will produce greater exercise anorexia and longer elevation of gut peptide secretion than exercise bouts that are half as long (experiment 2), and
(3) Exercise anorexia will be greater during subsequent low-carbohydrate (LC) than high-carbohydrate (HC) meals because LC meals are reported to have stronger stimulatory effect on PP GLP-1 and PYY secretion (experiment 3).

Methods

Subjects

In all three studies, the eligibility criteria were the same and included: healthy postmenopausal and non-smoker status, age 50-65 years; body mass index (BMI) between 20-30 kg/m^2; fasting

glucose level < 100 mg/dl; hematocrit > 32%, hemoglobin > 12 mg/dl; and absence of restricted food intake, endocrine and metabolic disorders requiring medication other than hormonally corrected hypothyroidism and musculoskeletal disabilities that would prevent walking. All subjects signed an informed consent approved by The University of Michigan Medical School Institutional Review Board and underwent two preliminary screening tests at Michigan Clinical Research Unit (MCRU). The health screening test included measurements of weight, height, and body fat by a dual-energy X-ray absorptiometry (General Electric Lunar Prodigy Advance), physical and health-history interview, and a fasting blood draw for checking laboratory chemistries and thyroid function. The fitness screening test assessed individual maximal aerobic effort by indirect calorimetry to assign a relative exercise intensity. It consisted of walking on a treadmill at 3 miles per hour with 2% slope increments every 3 minutes and the subject's respiratory gases routed through a mouthpiece analyzed by a Max II metabolic system (AEI Technologies, Inc., Bastrop, TX). The criterion of maximal effort (VO_2max) was a respiratory quotient of 1. In all three studies, the indirect calorimetry, appetite assessment, and analytical and statistical procedures were the same, as described in later sections.

General experimental protocol

After matching by body weight and BMI, subjects, eight each, were assigned to either sedentary (SED in experiment 1, X0 in experiment 2, LCS & HCS in experiment 3a) or one of the exercise groups (EBM in experiment 1a, EAM in experiment 1b, X1 & X2 in experiment 2, LCX & HCX in experiment 3b). A meal was provided at 1900 h after subject's admission to MCRU at 1800 h on the day before the study trial. The meal contained one-third of subject's weight-maintenance energy need (30 kcal/kg body-weight). The macronutrient composition of the meal was the same as that provided during the trial (Tables 3-2, 4-2). A catheter was inserted into an arm vein at 1930 h for blood collection and kept patent with sodium heparin. Blood was collected hourly, and also at 15- and 30-min intervals during meals and exercise sessions.

Exercise

During the study days, treadmill walking in all three studies was carried out at 45% of maximal aerobic effort. Where exercise consisted of 2-hour bouts before the meals (EBM group in experiment 1a, X2 group in experiment 2, and LCX and HCX groups in experiment 3b), 2-hour

exercise bouts were carried out from 0700 to 0900 h and 1400 to 1600 h. Where exercise consisted of two 1-hour bouts (X1 group in experiment 2), it took place from 0700 to 0800 h, and 1400 to 1500 h. Where exercise was performed 1 hour after the meals (EAM group in experiment 1b), it took place from 1100 to 1300 h and from 1800 to 2000 h. Exercise intensity in all three studies was adjusted by modifying the treadmill incline while the walking speed remained constant at 3 miles per hour. In experiments 1 (SED), 2 (X0), and 3a (LCS & HCS) sedentary subjects had no structured physical activity.

Indirect calorimetry

Resting metabolism was measured between 0600-0630 h on the study day and on the discharging day by indirect calorimetry using Viasys apparatus (Respiratory Care Inc., Yorba Linda, CA). Exercise metabolism was measured by indirect calorimetry during the first half hour of each hour of morning and afternoon exercise bouts as described above. Energy expenditure (EE) and relative carbohydrate and fat utilization during rest and exercise were estimated using the Weir equation [46].

Meals

In all studies the two meals were provided at fixed times of 1000 and 1700 h and contained one half of daily energy intake (25 kcal/kg body weight). In experiment 1 and one part of experiment 3, the two isocaloric meals had a high-carbohydrate content (HC) (60% carbohydrate, 15% protein, and 25% fat) provided in the form of egg salad, wheat roll with butter, graham crackers, coleslaw salad, carrots, skim milk, orange juice and fruits in the morning (Table 3-3), and ham-bacon and cheese sandwich, Romaine greens salad with diet French dressing, carrots, pretzels, cranberry juice, fruit and vanilla ice cream in the afternoon (Table 3-4). In experiment 2, and the other part of experiment 3, the two meals had a carbohydrate content that was 50% lower than that of HC diet. The low-carbohydrate meals (LC) contained 30% carbohydrate, 25% protein, and 45% fat provided in the form of a chef salad platter including romaine lettuce, slivered turkey and ham strips, cheddar and Swiss cheese, cherry tomatoes, cucumber and croutons, wheat roll and butter, macaroni and cheese, sausage, yogurt with shredded almonds in the morning (Table 4-3), and vegetarian burger, chicken Caesar salad with shredded almonds, minestrone soup, fruit juice and fruits in the afternoon (Table 4-4).

In all experiments, subjects were encouraged to complete eating the meal within 30 minutes. The food provided and any left uneaten was weighed to calculate the actual energy and macronutrient consumption.

Appetite assessment

On the study day, subjects rated their appetite on a validated [47] 100 mm visual analog scale (VAS) every hour from 0600 to 2100 h and also at 30 minutes right after they completed the meals (~1030 h and ~1730 h). The times of appetite assessment throughout the study trial are listed in Table 5-1.

The VAS questions: How hungry do you feel right now?, How full do you feel right now?, How strongly do you desire to eat right now?, and How much could you eat right now? required a mark on a scale bracketed with "Not at all" at one end and "Extremely" at the opposite end of the scale. The distances marked were converted to percentages of the full scale.

Analytical procedures

Blood samples were collected into ice-chilled EDTA-coated tubes containing aprotinin (50 KIU/ml blood, Sigma Chemical, St. Louis, MO) and dipeptidyl peptidase-4 inhibitor (10 μl/ml blood; EMD Millipore Corporation, Billerica, MA). Plasma was kept frozen at -80°C for gut hormone measurements. The times of gut hormone measurements throughout the study trial are listed in Table 5-1.

Plasma GIP, GLP-1, and PYY were measured with a milliplex chemiluminescent assay kit (HGT-68K, EMD Millipore Corporation, Billerica, MA). For GIP, GLP-1, PYY milliplex assay, intra-assay CV was <11% and inter-assay CV was <19%.

Calculations and statistics

Data are presented as means and standard errors (SEs). Subject characteristics, energy consumption, and expenditure were evaluated with the analysis of variance (ANOVA) using Statistical Analysis System program (SAS; version 9.3, SAS Institute, Cary, NC). Areas under curves (AUCs) of appetite responses and hormones were calculated by the trapezoid rule. The exercise AUC included the time during exercise and 1 hour post-exercise, except for X1 group in experiment 2 where 2 hours post-exercise were included. The fasting exercise AUC were

calculated from 0700-1000 h and the late PP exercise AUC were calculated from1400-1700 h in experiments 1a (EBM), 2 (X1 and X2), and 3b (HCX and LCX). The two early PP exercise AUCs were calculated from 1100-1400 h and 1800-2100 h for exercise performed after the meals trial (EAM) in experiment 1b. The postprandial AUC periods were calculated from 0-4 hours after each meal, 1000-1400 h and 1700-2100 h, in all three experiments. Mixed-model repeated measures ANOVA was used to analyze the effects of exercise timing (experiment 1), duration of exercise (experiment 2), and exercise with different macronutrient composition of meals (experiment 3) as between-subject effects. The times of exercise (morning vs. afternoon), the interaction of the time and exercise (experiments 1-3), and the interaction of time and macronutrient composition (experiment 3) were analyzed as within-subject effects. Bonferroni correction was applied to multiple comparisons in experiment 2.

Results

Experiment 1: Timing of exercise and high-carbohydrate meals

1a: Exercise before the meals

Subjects

Sixteen women, mean age of 57.4±0.95 years; body mass index (BMI) of 23.8±0.62 kg/m^2 met the study criteria.

1b: Exercise after the meals

Subjects

Sixteen women, mean age of 55.3±0.98 years; BMI of 23.1±0.62 kg/m^2 met the study criteria. The results of subjects in SED group of experiment 1a were also used for the control condition in experiment 1b.

Subjects' characteristics, including body weight, percentage of body fat, BMI, fitness level, and energy intake and expenditures for experiments 1a and 1b are summarized in Tables 5-2a and 5-2b, respectively. The groups did not differ in weight, percent body fat, BMI, or fitness level. However, subjects in EBM group were significantly older than those in SED group (t=3.29, p=.0054). Subjects in EAM group expended more calories during the first than the second exercise session (t=2.96, p=.0104). In addition, the EAM group utilized more carbohydrate and less fat than EBM group during their own first exercise session (t=11.4, p=.0045). On the other

hand, exercise energy expenditure in EBM group was similar during both exercise sessions, but less carbohydrate and more fat calories were utilized when exercise took place during fasting period than during late PP period (t=4.57, p=.0004). Both exercise groups had significantly lower daily energy balance than the SED group (EBM vs. SED: t=-6.91, p<.0001 and EAM vs. SED: t=-9.1, p<.0001) because the energy expended through exercise was not replaced with additional food.

Appetite responses

Hunger (Figures 5-1a and 5-1b)

Hunger ratings were unaffected by EBM exercise. Hunger ratings were higher during the first (1000 to 1400 h) than the second (1700 to 2100 h) PP period (t=2.44, p=.0284) in the EAM exercise trials.

Fullness (Figures 5-2a and 5-2b)

Fullness was rated higher during late PP period (1400-1700 h) than the pre-meal fasting period (0700-1000h) in the EBM trial during exercise (Figure 5-2a, t=6.31, p<.0001) and in the EAM trial 1 hour after the completion of exercise (Figure 5-2b, t=2.33, p=.0352). In addition, during late PP period, fullness was significantly higher in the EBM exercise than in the SED trial (t=3.03, p=.0091).

Gut peptide responses

Exercise did not affect the magnitude of GIP (Figure 5-3b), GLP-1 (Figure 5-4b), or PYY (Figure 5-5b) responses during the early PPs.

Plasma GIP (Figures 5-3a and 5-3b)

During late PP period, GIP AUCs were higher than during the fasting period before the first meal in SED trial (t=4.07, p=.0011), during EBM exercise (t=5.6, p<.0001) and during EAM trial, 1 hour after the completion of exercise (t=5.2, p=.0001).

Plasma GLP-1 (Figure 5-4a & 5-4b)

During late PP period, GLP-1 AUC was significantly higher than during the fasting period before the first meal (Figure 5-4b) in both the exercising EBM trial (t=3.83, p=.0033) and in non-exercising SED (t=2.49, p=.0319) and EAM (t=2.32, p=.0407) trials.

PYY (Figure 5-5a & 5-5b)

During late PP period, PYY AUC was significantly higher than during the fasting period in the absence of exercise in SED (Figure 5-5a, t=5.07, p=0.0002) and EAM groups (Figure 5-5b, t=2.86, p=0.0126), but not during exercise by EBM group.

Experiment 2: Exercise anorexia in response to different exercise duration

Subjects

Twenty-four women, mean age of 57.2±0.89 years; BMI of 25.4±0.55 kg/m^2 met the study criteria. Eight subjects per group were matched by body weight and BMI and assigned to 36-hour long sedentary (X0) or one of exercise groups (1-hour exercise bout, X1, or 2-hour exercise bout, X2). Subjects in X0 and X2 were the same subjects in LCS (Experiment 3a) and LCX (Experiment 3b), respectively.

Subjects' characteristics, including body weight, percentage of body fat, BMI, and fitness level, and energy intake and expenditures are summarized in Table 5-3. The groups did not differ in age, weight, percent body fat, BMI, or fitness level. However, both X1 (t=2.37, p=.0275) and X2 (t=2.09, p=.0487) groups consumed more calories in the second meal than the sedentary X0 group. The total energy intake (t=2.24, p=.0363) and energy intake per kg of body weight (t=2.39, p=.0264) were higher in X1 relative to X0 group, which did not remain significance after Bonferroni correction. Energy expenditure during two hours of exercise in X2 was significantly higher than during 1-hour of exercise in X1 group (F=13.81, p=.0023), which made energy balance in X2 50.2% and 46.3% lower, respectively, relative to X0 (t=3.47, p=.0023) and X1 (t=3.12, p=.0052) group. Exercise energy expenditure was not replaced with extra food.

Appetite responses

Hunger (Figure 5-6)

There was a trend for significantly lower hunger ratings in the X2 compared to X1 group during the exercise in fasting condition before the first meal (t=2.28, p=.0335). Exercise of either duration had no effect on hunger ratings during PP periods.

Fullness (Figure 5-7)

Similar and significantly higher fullness ratings were reported during exercise in late PP period than in fasted state by subjects in both 1h-long and 2h-long exercising trials (X1: t=2.5, p=.0206; X2: t=2.44, p=.0235).

Gut peptide responses

All three gut peptide AUCs were significantly higher during the late PP period than during the fasting period before the first meal in the SED condition (GIP: t=4.48, p=.0002; GLP-1: t=2.29, p=.0413; PYY: t=3.95, p=.0008).

Plasma GIP (Figure 5-8)

Plasma GIP AUC was also significantly higher during the late PP than during the fasting period in both exercising groups (X1: t=6.02, p<.0001; X2: t=3.86, p=.0009, Figure 5-8). There was also a trend for the GIP AUC to be higher in 1-hour than in 2-hour exercising groups (t=2.2, p=.0389) during the late PP period.

Plasma GLP-1 (Figure 5-9)

Plasma GLP-1 AUC was also significantly higher during the late PP than during the fasting period in both exercising groups (X1: t=2.64, p=.0215; X2: t=3.44, p=.0049, Figure 5-9).

Plasma PYY (Figure 5-10)

During the late PP period, the PYY AUC was significantly greater than during fasting in the sedentary X0 (t=3.95, p=.0008) and in the 1-hour exercising X1 (t=3.64, p=.0016) groups.

Experiment 3: Effect of meals of different macronutrient composition in sedentary condition and following the exercise bouts

3a: Effects of dietary macronutrients in sedentary condition

Subjects

Sixteen women, mean age of 55.9±0.94 years; BMI of 24.5±0.62 met the eligibility criteria. Eight subjects per group were matched by body weight and BMI and assigned to 36-hour long sedentary trials under two dietary conditions: low-carbohydrate sedentary (LCS) or high-carbohydrate sedentary (HCS). Subjects in LCS and HCS were also the subjects in X0 trial of experiment 2 and in SED trial of experiment 1, respectively.

3b: Effects of dietary macronutrients with exercise

Subjects

Sixteen women, mean age of 59.6±0.87 years; BMI of 24.9±0.74 met the eligibility criteria. Eight subjects per group were matched by body weight and BMI and assigned to 36-hour long exercise before meals trials under two dietary conditions: low-carbohydrate exercise (LCX) or high-carbohydrate exercise (HCX). Subjects in LCX and HCX trials were the subjects in X2 trial of experiment 2 and in EBM trial of experiment 1a, respectively.

Subjects' characteristics, including body weight, percentage of body fat, BMI, and fitness level, and energy intake and expenditures are summarized in Table 5-4a and 5-4b. There were no group differences in any variables, however, HCX group utilized more carbohydrate and less fat than the LCX group ($F=4.69$, $p=.0481$) during early morning resting period on the study day. HCX group also utilized more carbohydrate and less fat next morning after the trial had been completed than during the corresponding period on study day ($F=6.21$, $p=.0258$). The HC groups utilized significantly more carbohydrate than fat than the LC groups in three situations: during the second postprandial period (1800- 2100 h) with ($F=49.71$, $p<.0001$) or without ($F=13.58$, $p=.005$) exercise; during the late PP compared to the fasting exercise session ($F=27.03$, $p=.0001$); and during the late PP exercise session compared to LCX group ($F=11.65$, $p=.0042$).

Appetite responses

Hunger (Figures 5-11a & 5-11b)

In sedentary condition, hunger ratings in LCS groups were significantly lower during late PP than during the fasting period (Figure 5-11a, F=4.72, p=.0475). Hunger ratings were significantly lower in LCX than in HCX group (F=6.33, p=0.0247) during exercise in fasted state (Figure 5-11b).

Fullness (Figures 5-12a & 5-12b)

In sedentary condition, diets had no differential effect on fullness ratings (Figure 5-12a). During exercise in late PP period, fullness ratings were significantly higher than during the fasted state in exercising groups with both diets (LCX: F=6.61, p=.0222; HCX: F=13.95, p=.0022, Figure 5-12b).

Gut peptide responses

In the sedentary trials, GIP (Figure 5-13a, LCS: F=23.74, p=.0002; HCS: F=10.99, p=.0051) and PYY (Figure 5-15a, LCS: F=10.32, p=.0063; HCS: F=23.35, p=.0003), but not GLP-1 (Figure 5-14a), had similarly higher late PP AUCs than during the fasting period before the first meal under both dietary conditions. In the exercise trials, GIP (5-13b, LCX: F=21.81, p=.0004; HCX: F=25.69, p=.0002) and GLP-1 (Figure 5-14b, LCX: F=11.75, p=.0065; HCX: F=9.03, p=.0132), but not the PYY (Figure 5-15b), had similarly higher late PP AUCs than during the fasting period before the first meal under both dietary conditions.

Plasma GIP (Figures 5-13a & 5-13b)

LC meals significantly reduced GIP AUCs compared to HC meals during the second PP period (1800-2100 h) in both sedentary (F=8.87, p=.01, Figure 5-13a) and exercise conditions (F=12.66, p=.0031, Figure 5-13b). In addition, the GIP AUC in HCX group was significantly higher (F=11.75, p=.0041) during the second PP (1700-2100 h) than the first PP (1000-1400 h) period.

Plasma GLP-1 (Figures 5-14a & 5-14b)

During fasting period before the first meal, GLP-1 AUC was significantly higher in LCS than in HCS groups (F=13.58, p=.0078, Figure 5-15a).

Plasma PYY (Figures 5-15a & 5-15b)

PYY AUC was significantly higher during the late PP than during the fasting period in the HCX exercising group ($F=11.28$, $p=.0051$).

Discussion

This experiment examined the proposition developed in several studies [4, 18-20] that exercise anorexia, a temporary and transient suppression of hunger and an increase in satiation, was associated with the release of PP gut hormones. We tested this hypothesis by looking for a consistent association between changes in the appetite and in satiating gut hormone secretion when exercise was performed under three prandial conditions that differentially affect satiating gut peptide secretion: fasting condition when gut peptides are usually not elicited, early PP peak of satiating gut peptide secretion, and late PP when gut peptide secretion is waning. Three hypotheses were formulated to test the gut peptide-exercise anorexia link. The hypothesis 1 posited that if gut peptides were responsible for exercise anorexia, their increased secretion would consistently coincide with exercise. The expectation was therefore that exercise anorexia would be most strongly reported when exercise took place during early PP period when the secretion of GLP-1, PYY and GIP is at peak, less strongly expressed during late PP period when the gut peptide secretion is waning, and not at all during fasting period when gut peptides are typically not secreted. Conversely, exercise anorexia would not be expected during pre-meal fast when gut peptides do not exhibit increased release.

None of these expectations materialized. No exercise anorexia or increased fullness was reported when EAM exercise took place during early PP period in experiment 1b. Instead, increased fullness was recorded when EBM exercise took place during late PP period when gut peptide secretion was waning in experiment 1a. Higher fullness was also recorded during this late PP period 1 hour after EAM group stopped exercising than during the pre-meal fasting period. In experiment 3b, higher fullness was reported when exercise took place during the late PP than during the fasting period regardless of meal composition with a single exception of PYY with exercise and LC meals. In all three experiments, GLP-1, PYY, and GIP AUCs were almost invariably higher during the late PP than during the pre-meal fast under both exercising and

sedentary conditions with the exception of GLP-1 which did not show this change in sedentary trials on both diets.

The converse expectation of hypothesis 1 is that increased satiation should not reported in the absence of increased gut peptide secretion was also not met. In experiment 3b, where subjects were fed LC diet both during the trials and in the evening prior to the trial, hunger suppression was reported during pre-meal exercise in fasted state.

Therefore the proposition of hypothesis 1 that exercise anorexia would be consistently associated with (and presumably caused by) increased secretion of gut peptides was not met because increased satiation was (1) reported during exercise in fasted state in the absence of increased gut peptide secretion (experiment 3b), (2) was absent during peak gut peptide secretion, and (3) was reported in both sedentary condition and during exercise in late PP phase when gut peptide secretion was waning but was increased to a similar extent in both sedentary and exercise condition .

The association between exercise anorexia and satiating gut peptide secretion is not consistently reported in studies by others. For instance, Ueda et al. (2009) showed in two studies a consistent increase in PP concentrations of GLP1 and PYY during exercise carried for 30-60 minutes at 50% VO_2max during early PP period, 1 hour after a 560-kcal breakfast. In only one of these studies was there a temporal association between increased postprandial GLP-1 and PYY secretion and increased fullness ratings [19]. In the other study that included both obese and lean young men, there was no appetite change in spite of increased GLP-1 and PYY concentrations during exercise and 1 hour post-exercise [20]. In experiment 1b EAM exercise was of similar intensity (45% vs. 50% VO_2max) but longer duration (2 hours vs. 30-60 min) and also was performed 1 hour after a similar-size meal (750-kcal vs. 560-kcal), there was no change in appetite ratings despite the increase in PP gut secretion [20]. A possible reason for failing to observe exercise anorexia 1 hour after the meal in experiment 1b and the positive association reported by Ueda in study [19] may be due to possibly two factors that differed between the two studies, the gender of the subjects (postmenopausal women vs. young males) and macronutrient

composition of the pre-exercise meal, a variable discussed later as it relates to all of the studies.

In studies by other investigators where exercise was performed during late PP period [2, 48], increased satiation and decreased hunger ratings were reported during and shortly after exercise performed 2.5 to 4 hours after the meal. In particular, Deighton et al. [48] also reported a negative association of hunger and PYY concentrations when exercise took place during late PP period. These results support experiment 1a only in the timing of the fullness ratings during exercise but they differ in that, increased satiating gut peptide secretion was seen in both sedentary and exercising condition in Experiment 1a and thus inference of specific association between exercise anorexia and gut peptide elevation can be justified. These observations suggest that exercise is more likely to produce hunger suppression and increased satiation when it takes place during late rather than early PP periods but that cannot not attributed to PP gut peptides, the concentrations of which are higher during that prandial phase than during the pre-meal fast in both the sedentary state and during exercise. It is possible that during early PP period the nutritional stimulation of peak PP gut peptide secretion overcomes any exercise effect on the appetite, particularly when the meal size is relatively large (750-800kcal) and the exercise intensity is moderate as was the case in present study.

In experiments 2 and 3b, a trend for exercise anorexia in fasted state was found when a 2-hour exercise was performed in fasted state after having eaten a LC, but not a HC, meal the night before. However, this exercise anorexia was not associated with an increase in either GLP-1 or PYY concentration. Such a disassociation between hunger suppression and an increase in satiating gut hormones was also reported by Cheng et al. [49] who found exercise anorexia in young lean males during 50-min of exercise at 60% VO_2max in fasted state without a coincident change in PYY concentration [49]. In contrast to experiments 2 and 3b and Cheng et al. [49] results, Broom et al. [18] reported a brief and transient temporal association between exercise anorexia and increases in PYY when exercise was performed in fasted state. However, the evidence for the involvement of PYY in exercise anorexia in fasted state is weakened by a minimal duration of the time overlap between anorexia and increased PYY and the observation that anorexia preceded the brief increase in PYY concentration.

The second hypothesis tested the proposition that exercise anorexia and GLP-1 and PYY secretion would be proportionately greater if the duration of exercise increased by a factor of two. This hypothesis also was not supported. In experiment 2, exercise did not increase gut peptide secretion either during the late PP or fasting periods. On the other hand, the hunger ratings tended to be lower during fasting exercise period in 2-hour exercise group (X2) compared to 1-hour exercise group (X1) on a LC diet. The fullness ratings were higher during late PP period in both exercising groups compared to exercise during the fasting period. This stands in contrast to the report of King et al. [3] who described greater decline in hunger ratings when the duration of exercise at 75% of VO_2max was doubled (52 min vs. 26 min). Conversely, a recent study published by Martins et al. [10] reported no difference in the appetite ratings between long (18 min) or short- (9 min) duration of high-intensity intermittent cycling exercise (76-85% VO_2max). The conditions between the present experiment and previous studies differed in several respects. In King's study [3], a bout of high-intensity exercise (75% VO_2max) was performed 2 hours after the meal by young lean male subjects. On the other hand, subjects included both young female and male subjects with mean BMI of 32 kg/m^2 in the Martins's study [10] and the high intensity intermittent exercise at 76-85% VO_2max was performed 1 hour after the meal. These two studies differ substantially from the present experiment in several respects. In the experiment 2, exercise was carried out with normal-weight postmenopausal women during fasting or late PP states as opposed with males [3] or obese men and women [10] and at 45% VO_2max as opposed to higher intensities in the two studies.

The third test of the gut peptide-exercise anorexia hypothesis was the proposition that reducing the carbohydrate content of the diet by half would enhance secretion of GLP-1 and PYY and elicit a parallel suppression of hunger or increased sensation of PP fullness when exercise took place during fast or during early and late PPs. This hypothesis also was not supported. There was no overall dietary effect on the secretion of gut peptides with the exception of GIP 1 which displayed a significant decline during the second PP on LC diet. This effect was described in Study 2, chapter 4. This significant difference in the overall afternoon GIP PP AUC was not associated with a corresponding difference in the afternoon PP ratings of either hunger or satiation. This is not unexpected as no association between this gut peptide secreted from the proximal part of the small intestine with changes in appetite have been reported so far. In the

absence of selective dietary effects on PP GLP-1 and PYY AUCs, the main comparisons were made relating exercise anorexia with secretion of these two hormones under three prandial states in trials with high- and low-carbohydrate diets. In experiment 3, there was a trend toward greater decrease in the sensation of hunger during late PP than during the fasting period in sedentary trials with LC meals (Figure 5-11a) and an increase in the sensation of fullness in exercising trials with either diet (Figure 5-12b). In addition, hunger was rated lower when exercise was performed in fasted state in LC trial where the meal of same composition was fed in the evening before the trial (Figure 5-11b)

Similarly, in experiment 2, lower hunger ratings was reported during two hours of exercise in fasted state when the previous evening meal was of low-carbohydrate content but not of high-carbohydrate content. However, these trends toward appetite suppression or increased sensation of fullness with LC meals were not temporally associated with changes in GLP-1, PYY, and GIP concentrations. All three gut peptides with a single exception each, were unaffected by macronutrient composition of the meals and were generally, higher during the late PP than during the fasting state. However, higher gut peptide levels during late PP than during the fasting state were not temporally consistently associated with exercise. Thus, higher late PP concentrations than in the fasting state were seen in GIP and GLP-1 during exercise on either diet, in GIP and PYY during sedentary condition on either diet, and in PYY during exercise in HC trial. Therefore, while both the manifestations of higher gut peptides and exercise anorexia were most prevalent in late PP phase, their inconsistent temporal association precludes any inference of causality. Greater incidence of anorexia reports in trials with LC meals suggest an effect of macronutrient composition in the meals on appetite suppression that is not specific to exercise, and not selectively attributable to increased secretion of satiating gut hormones. High satiation and lowered hunger ratings to LC meals have been previously reported [50-54] with or without prior exercise although their association with gut peptide secretion was not measured. The outcomes of the three experiments need to be discussed in the context of their experimental design, the size of the meals, the intensity of exercise selected, and the gender of the subjects. The repeat-exercise design was used to study within a single experiment two prandial states (EBM fasting exercise bout EBM exercise during the late PP state) or a comparison of two early PP states (EAM trials). The discussion so far has emphasized appetitive and gut peptide

associations with a specific prandial state without consideration of whether the cumulative effects of two 2-hour bouts of exercise may affect the results through a probable liver and muscle glycogen depletion or fatigue. Some of these issues were reported and discussed in the chapter III. The repeat-event design served three different purposes discussed in this and two pervious chapters and therefore the inability to control for all variables was unavoidable given the complexity of the experimental paradigm. The size of each meal used in this study was large (750-800 Kcal) and contained 50% of daily calories. The two-meal design was chosen to allow for examination of the effects of exercise before and after the meals within a 12-hour day. A stoichiometric association between the size of the meal and the magnitude of gut peptide response has been shown in many studies. Thus GLP-1 area under the curve was greater after a large 520-kcal than a small 260 kcal meal of same macronutrient composition [55]. Similarly, a large carbohydrate-rich meal stimulated higher GLP-1 release than a smaller carbohydrate-rich meal (460-kcal) [56]. Also, a 500-kcal HC meal elicited significantly greater postprandial GIP area under the curve than a 100-Kcal meal [2]. The studies that reported exercise anorexia in association with postprandial increase in GLP-1 and PYY secretion generally provided smaller pre-exercise meals ranging between 500 [4] and 560 kcal [19]. Therefore, one of the reasons that exercise anorexia was not consistently found in the present experiments could be due to the combination of large meals that elicit a strong postprandial gut peptide response in combination with the relatively low exercise intensity of 45% VO2 max. It is possible that elicitation of exercise anorexia depends on the relative magnitude of these two variables. Thus, in previously mentioned studies [4, 19, 20], anorexia during exercise was obtained with smaller-size meals (560 kcal) eliciting smaller gut peptide response 1 hour after eating and with exercise that was of somewhat higher intensity (50% of VO_2max) compared to the larger meals (750-800 Kcal), and lower exercise intensity (45% of VO_2max) in present study.

Another consideration that may have influenced the results is the relatively low, 45% VO2 max exercise intensity used in present experiments. This exercise intensity was chosen to enable successful completion of 4-hour of exercise in untrained postmenopausal women. The argument that exercise intensity may account for our failure to consistently elicit exercise anorexia is supported by some studies [1, 3, 5] where hunger suppression was consistently elicited at exercise intensities ~70% but not at 30-35% of VO_2max. However, exercise at the intensity of

45-50% VO_2max did elicit hunger suppression in girls [57] and postmenopausal women [2] suggesting either that this exercise intensity is close to the threshold of elicitation of the anorexia phenomenon in general, or attributable to the gender difference of the study subjects. Thus, exercise anorexia was elicited at these lower intensities of exercise in young [6, 57], and less consistently in older, women [2] but not in young men [1, 3, 5]. It also could reflect age difference with variable results in postmenopausal women [2] and positive results in young women [6, 57].

Collectively, the present experiments and reviewed studies by other investigators do not support our main hypothesis that exercise anorexia is consistently and presumably causally associated with increased secretion of satiating gut peptides during exercise. Meals elicited peak concentrations of GIP, GLP-1, and PYY during early PP period with progressive declines during late PP period. Under the conditions of the present experimental design, exercise during early PP did not elicit anorexia despite peak secretory responses of the GLP-1 and PYY. Instead these two hormones that have been implicated in satiation [28-33] as well the GIP secreted from the proximal small intestine and not implicated in satiation. Instead, appetite suppression was most consistently found in the present experiments during late PP period when the concentrations of all three gut peptides were significantly higher than during the fasting period before the first meal, but the relationship was temporally not consistent or specific to exercise. Anorexia tended to be associated with LC meals when it was reported both during the fasting period before the first meal and during the late PP period, again not consistently associated with exercise. The conclusion that increased gut peptide secretion cannot account for exercise anorexia is primarily predicated on the frequent temporal dissociation between the two variables and the lack of specific association of both anorexia and gut peptide secretion with exercise. But this conclusion is tempered by the concern that the intensity of exercise applied in our study may have been close to the threshold for consistent elicitation of exercise anorexia as suggested by other studies [3, 5, 6]. The repeat-event design also imposed a statistical limitation in that with the Bonferroni correction some experiments were underpowered and provided only trends rather than significant differences. As such, the trends obtained in these experiments would justify the repetition of the promising but not statistically different results lacked sufficient power to elicit exercise anorexia. However this limitation does not negate our consistent observation that the significant

association of increased fullness with exercise during late PP period in experiment 1a and the trends for hunger suppression or increased sensation of fullness with LC diet in both sedentary and exercise groups in experiments 2 and 3 were in no instance consistently associated with increased secretion of GLP-1 and PYY. Other limitations of the experimental design are that the results obtained with postmenopausal women may not be generalized to the other gender or different age groups and therefore may account for some of differences in experimental results obtained in studies with young men [1, 3, 5], young women [6], and girls [57].

In conclusion, the secretion of GLP-1, PYY, and GIP was consistently increased by meal intake and mostly unaffected by exercise. Exercise anorexia was not associated with peak secretion of these gut peptides during early PP either during exercise or in sedentary condition. Anorexia was most frequently reported during exercise in late PP, but sometimes also at that time by participants in sedentary trials, when gut peptide concentrations were waning but were consistently still significantly higher than during the pre-meal fast. Conversely, exercise in the absence of meals did not consistently elicit increased gut hormone response but tended to decrease hunger or increase ratings of fullness when the meal provided in the evening before the trial had low-carbohydrate content. LC, but not the HC meal, also tended to increase fullness during late PP both during exercise and in sedentary state. The duration of exercise did not affect either of the appetite or gut peptide concentrations in different prandial states. The present experiments do not support a possible functional association between gut hormones GLP-1 and PYY and exercise anorexia s by others [4, 18-20]. While excluding the gut peptides GLP-1 and PYY as possible triggers of exercise anorexia, these experiments did not reveal its probable cause. Elucidation of exercise anorexia may benefit from studies on the possible roles of hormones such as ghrelin, CCK, and catecholamines, or from a more systematic examination of dietary contribution to that phenomenon.

Table 5-1: Timing of hormones and appetite assessment (VAS) measurements

Day 2	GIP	GLP-1	PYY	VAS
6:00	√	√	√	√
7:00	√	√	√	√
8:00	√	√	√	√
9:00	√	√	√	√
10:00	√	√	√	√
10:30	√	√	√	√
11:00	√	√	√	√
12:00	√	√	√	√
13:00	√	√	√	√
14:00	√	√	√	√
15:00	√	√	√	√
16:00	√	√	√	√
17:00	√	√	√	√
18:00	√	√	√	√
19:00	√	√	√	√
20:00	√	√	√	√
21:00	√	√	√	√
22:00	√	√	√	
Day 3				
0:00	√	√	√	
6:00				√

Table 5-2a: Subjects' characteristics and energy balance in sedentary (SED) and exercise before meals (EBM) trials

Groups	SED (n=8)	EBM (n=8)
Age (years)	55.0±1.07[a]	59.9±1.03[b]
Weight (Kg)	66.1±2.22	65.9±3.19
Percentage of Body Fat (%)	35.1±2.18	36.9±2.65
BMI (Kg/m^2)	23.6±0.91	24.1±0.90
Fitness level (VO_2/min×Kg)	25.6±3.66	22.6±1.79
EI in meal 1 (Kcal)	769.5±32.82	816.4±43.14
EI in meal 2 (Kcal)	803.8±33.69	821.7±41.72
1st Exercise EE (Kcal)	NA	413.7±24.55
Carbohydrate utilization (%) during 1st exercise	NA	43%±5.4%[*]
Fat utilization (%) during 1st exercise	NA	57%±5.4%[*]
2nd Exercise EE (Kcal)	NA	408.6±24.54
Carbohydrate utilization (%) during 2nd exercise	NA	61%±3.3%
Fat utilization (%) during 2nd exercise	NA	39%±3.3%
EB: EI - exercise EE (Kcal)	1573.3±65.19[a]	815.9±88.11[b]

EI=energy intake; EE=energy expenditure; EB=energy balance

[a,b] Within the same variable, groups with different superscripts are significantly different ($p<.05$)

[*] Within EBM group, less carbohydrate and more fat were utilized during 1st exercise than during the 2nd exercise ($p<.05$)

Table 5-2b: Subjects' characteristics and energy balance in sedentary (SED) and exercise after meals (EAM) trials

Groups	SED (n=8)	EAM (n=8)
Age (years)	55.0±1.07	55.6±1.72
Weight (Kg)	66.1±2.22	63.7±2.10
Percentage of Body Fat (%)	35.1±2.18	33.8±2.65
BMI (Kg/m^2)	23.6±0.91	22.7±0.89
Fitness level (VO_2/min×Kg)	25.6±3.66	22.9±1.74
EI in meal 1 (Kcal)	769.5±32.82	750.6±32.35
EI in meal 2 (Kcal)	803.8±33.69	714.9±68.62
1st Exercise EE (Kcal)	NA	523.2±66.34*
Carbohydrate utilization (%) during 1st exercise	NA	65%±4.3%
Fat utilization (%) during 1st exercise	NA	35%±4.3%
2nd Exercise EE (Kcal)	NA	480.1±54.00
Carbohydrate utilization (%) during 2nd exercise	NA	67%±5.3%
Fat utilization (%) during 2nd exercise	NA	33%±5.3%
EB: EI - exercise EE (Kcal)	1573.3±65.19[a]	462.2±103.29[b]

EI=energy intake; EE=energy expenditure; EB=energy balance

[a,b] Within the same variable, groups with different superscripts are significantly different ($p<.05$)

[*] Within EAM group, energy expenditure during the 1st exercise was significantly higher than during the 2nd exercise ($p<.05$)

Table 5-3: Subjects' characteristics and energy balance in sedentary (X0), 1-hour pre-meal exercise (X1), and 2-hour pre-meal exercise (X2) trials

Groups	X0 (n=8)	X1 (n=8)	X2 (n=8)
Age (years)	56.9±1.54	55.4±1.48	59.3±1.46
Weight (Kg)	69.9±3.41	70.3±2.97	71.8±2.76
Percentage of Body Fat (%)	38.0±1.65	38.9±2.25	39.0±3.30
BMI (Kg/m^2)	25.4±0.75	25.2±1.01	25.7±1.16
Fitness level (VO_2/min×Kg)	24.7±2.49	20.7±1.39	26.2±3.60
EI in meal 1 (Kcal)	751.8±59.69	913.8±55.08	782.1±58.24
EI in meal 2 (Kcal)	648.6±112.98	902.8±55.25	873.2±38.00
1st Exercise EE (Kcal)	NA	239.5±22.16[a]	485.2±63.28[b]
Carbohydrate utilization (%) during 1st exercise	NA	54%±6.2% [a]	41%±3.8% [b]
Fat utilization (%) during 1st exercise	NA	46%±6.2% [a]	59%±3.8% [b]
2nd Exercise EE (Kcal)	NA	247.0±22.48[a]	472.5±55.81[b]
Carbohydrate utilization (%) during 2nd exercise	NA	57%±1.9% [a]	42%±2.1% [b]
Fat utilization (%) during 2nd exercise	NA	43%±1.9% [a]	58%±2.1% [b]
EB: EI - exercise EE (Kcal)	1400.4±167.50[a]	1330.1±113.4[a]	697.6±143.91[b]

EI=energy intake; EE=energy expenditure; EB=energy balance

[a,b] Within the same variable, groups with different superscripts are significantly different ($p<.05$)

Table 5-4a: Subjects' characteristics and energy balance in sedentary tirals with low-carbohydrate (LCS) and with high-carbohydrate meals (HCS)

Groups	LCS (n=8)	HCS (n=8)
Age (years)	56.9±1.54	55.0±1.07
Weight (Kg)	69.9±3.41	66.1±2.22
Percentage of Body Fat (%)	38.0±1.65	35.1±2.18
BMI (Kg/m^2)	25.4±0.75	23.6±0.91
Fitness level (VO_2/min×Kg)	24.7±2.49	25.6±3.66
EI in meal 1 (Kcal)	751.8±59.69	769.5±32.82
EI in meal 2 (Kcal)	648.6±112.98	803.8±33.69
EB: EI - exercise EE (Kcal)	1400.4±167.50	1573.3±65.19

EI=energy intake; EB=energy balance

Table 5-4b: Subjects' characteristics and energy balance in exercise before low-carbohydrate (LCX) and exercise before high-carbohydrate meals (HCX) trials

Groups	LCX (n=8)	HCX (n=8)
Age (years)	59.3±1.46	59.9±1.03
Weight (Kg)	71.8±2.76	65.9±3.19
Percentage of Body Fat (%)	39.0±3.30	36.9±2.65
BMI (Kg/m^2)	25.7±1.16	24.1±0.90
Fitness level (VO_2/min×Kg)	26.2±3.60	22.6±1.79
EI in meal 1 (Kcal)	782.1±58.24	816.4±43.14
EI in meal 2 (Kcal)	873.2±38.00	821.7±41.72
1st Exercise EE (Kcal)	485.2±63.28	413.7±24.55
Carbohydrate utilization (%) during 1st exercise	41%±3.8%	43%±5.4%*
Fat utilization (%) during 1st exercise	59%±3.8%	57%±5.4%*
2nd Exercise EE (Kcal)	472.5±55.81	408.6±24.54
Carbohydrate utilization (%) during 2nd exercise	42%±2.1%[a]	60%±3.3%[b]
Fat utilization (%) during 2nd exercise	58%±2.1%[a]	40%±3.3%[b]
EB: EI - exercise EE (Kcal)	697.6±143.91	815.9±88.11

EI=energy intake; EE=energy expenditure; EB=energy balance

[a,b] Within the same variable, groups with different superscripts are significantly different ($p<.05$)

* Within HCX group, less carbohydrate and more fat were utilized during the 1st exercise than during the 2nd exercise ($p<.05$)

Figure 5-1a: Hunger responses to high-carbohydrate meals in sedentary (SED) and exercise before meals (EBM) trials

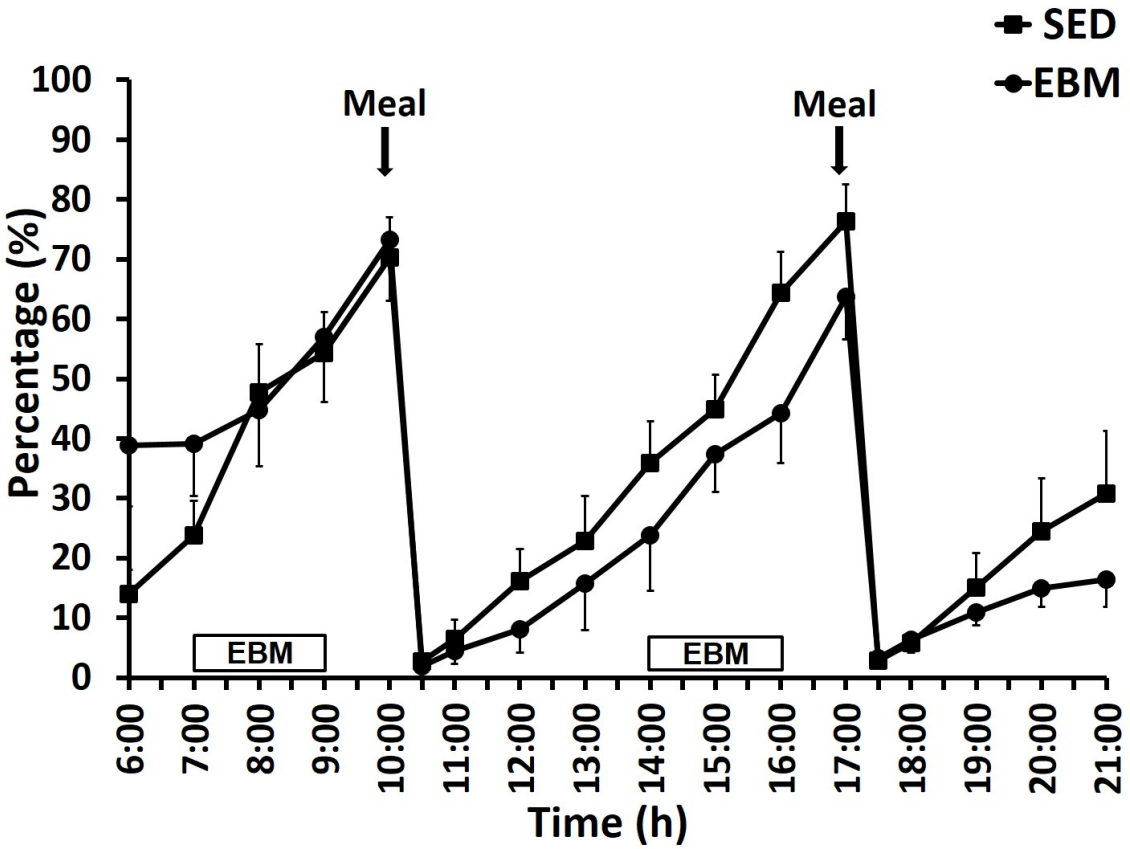
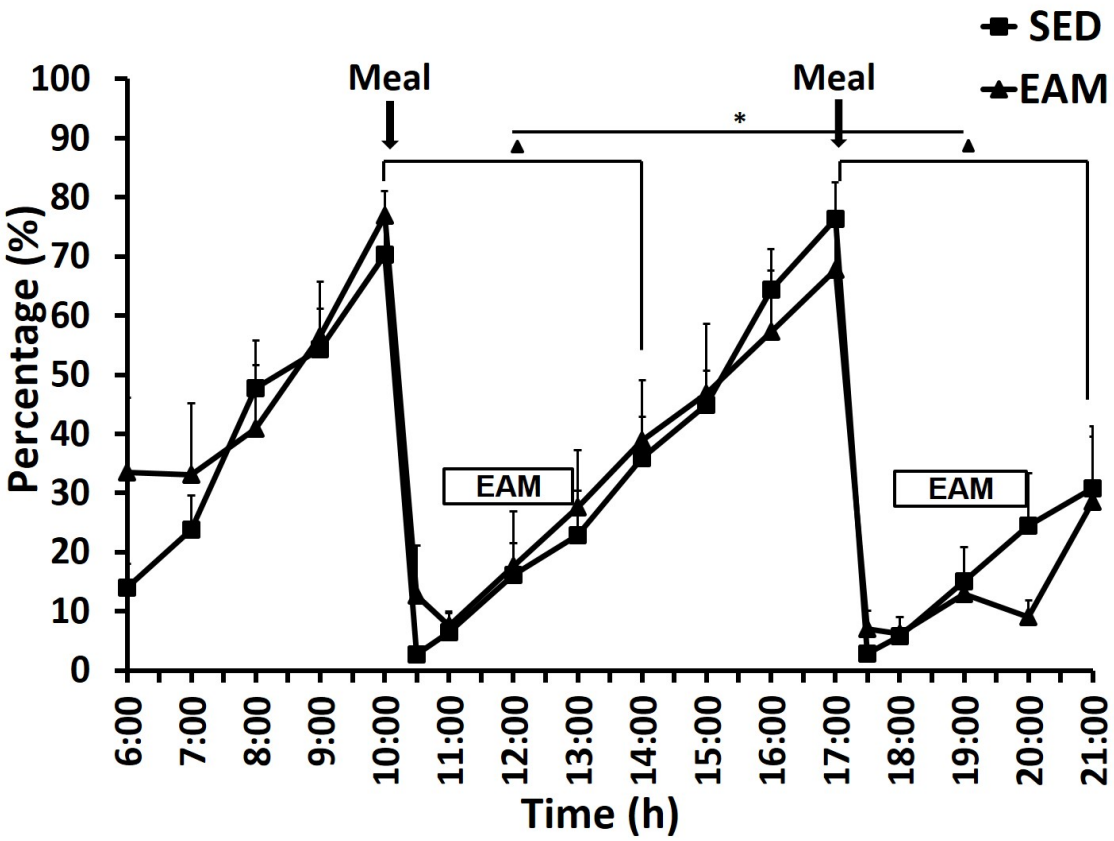
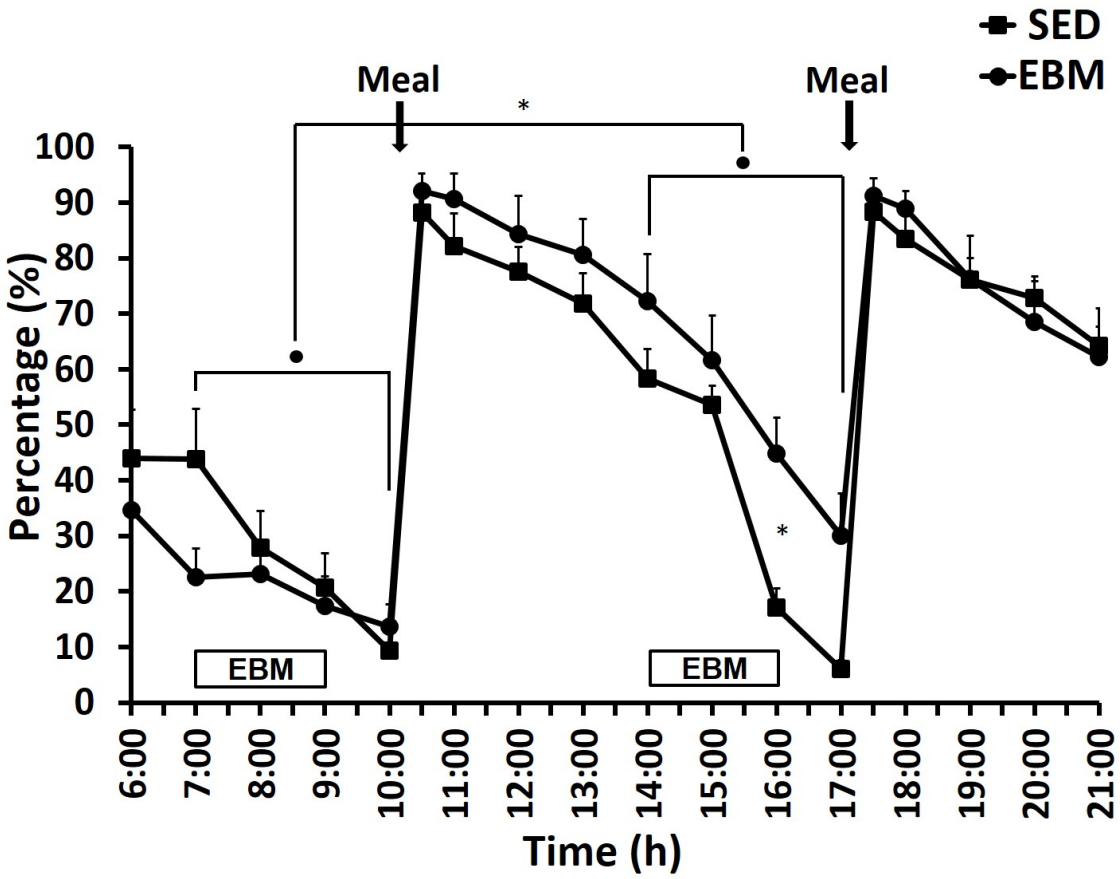


Figure 5-1b: Hunger responses to high-carbohydrate meals in sedentary (SED) and exercise after meals (EAM) trials



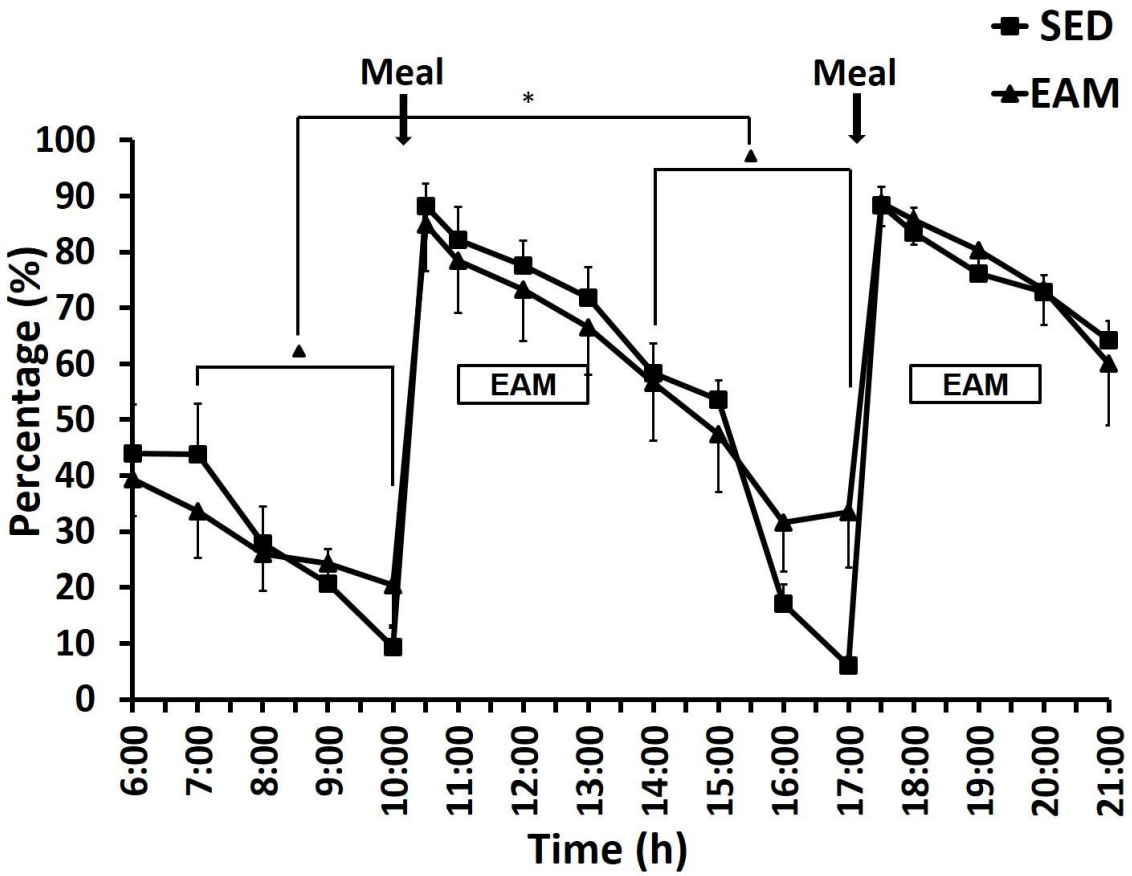
"*" indicates the statistical significance between two selected time periods.

Figure 5-2a: Fullness responses to high-carbohydrate meals in sedentary (SED) and exercise before meals (EBM) trials



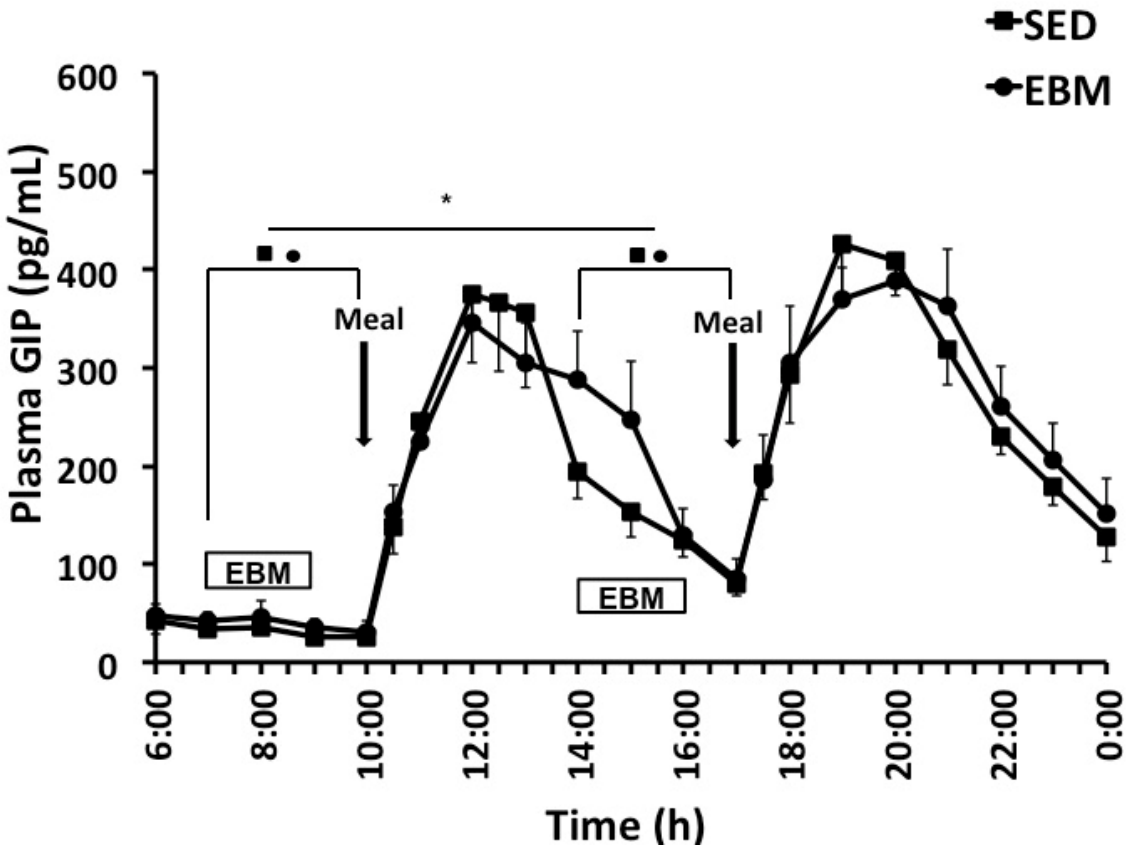
"*" indicates the statistical significance between two selected time periods/areas under the curve.

Figure 5-2b: Fullness responses to high-carbohydrate meals in sedentary (SED) and exercise after meals (EAM) trials



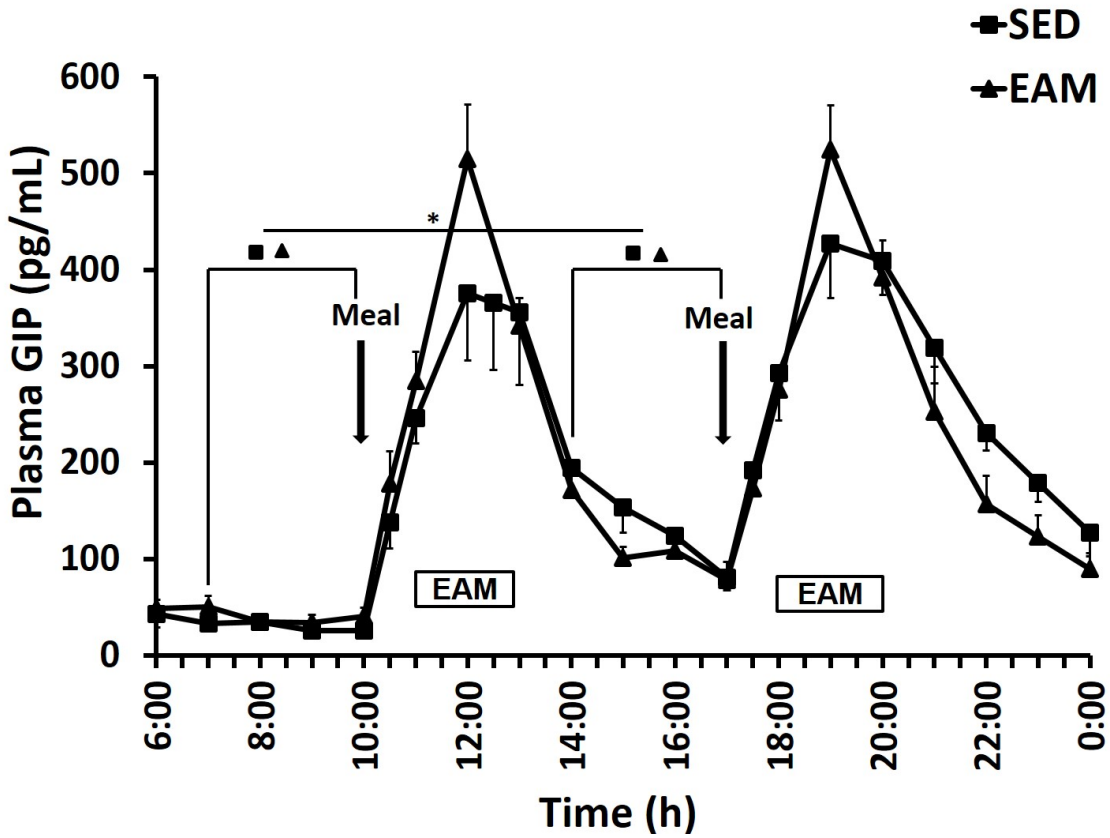
"*" indicates the statistical significance between two selected time periods.

Figure 5-3a: Plasma GIP responses to high-carbohydrate meals in sedentary (SED) and exercise before meals (EBM) trials



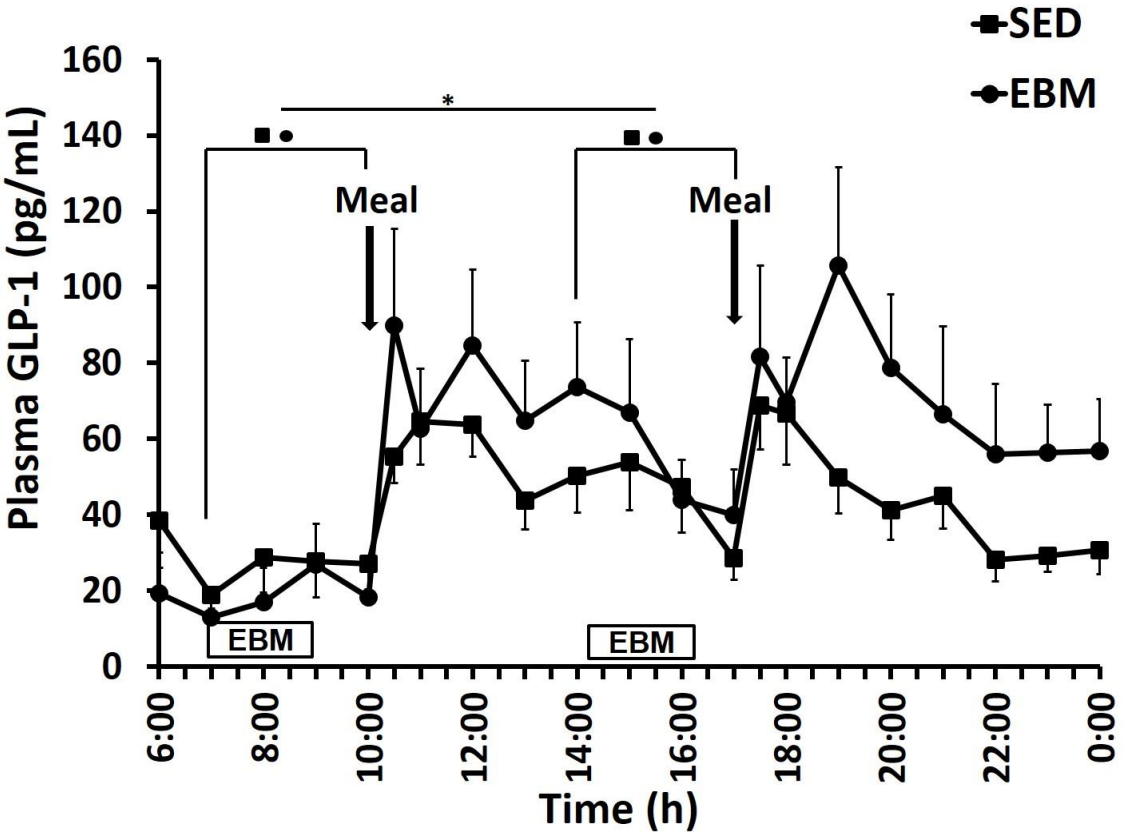
"*" indicates the statistical significance between two selected time periods.

Figure 5-3b: Plasma GIP responses to high-carbohydrate meals in sedentary (SED) and exercise after meals (EAM) trials



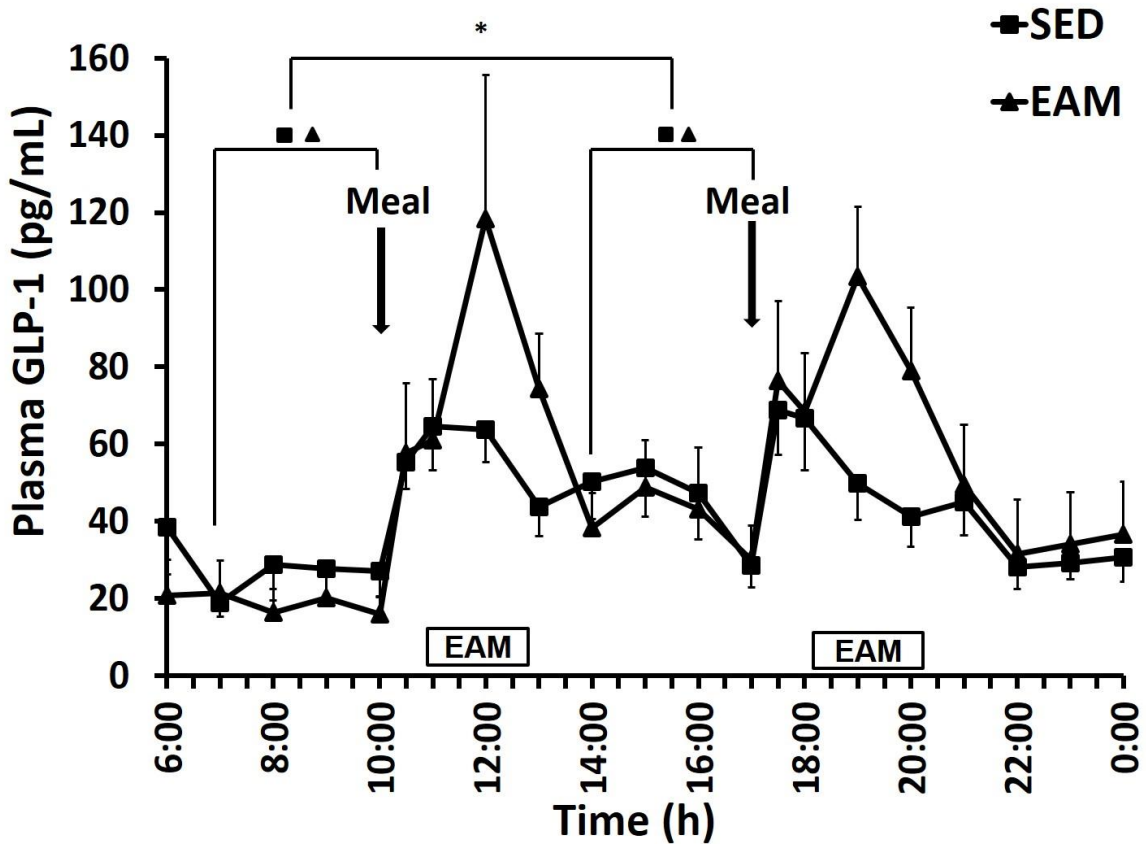
"*" indicates the statistical significance between two selected time periods.

Figure 5-4a: Plasma GLP-1 responses to high-carbohydrate meals in sedentary (SED) and exercise before meals (EBM) trials



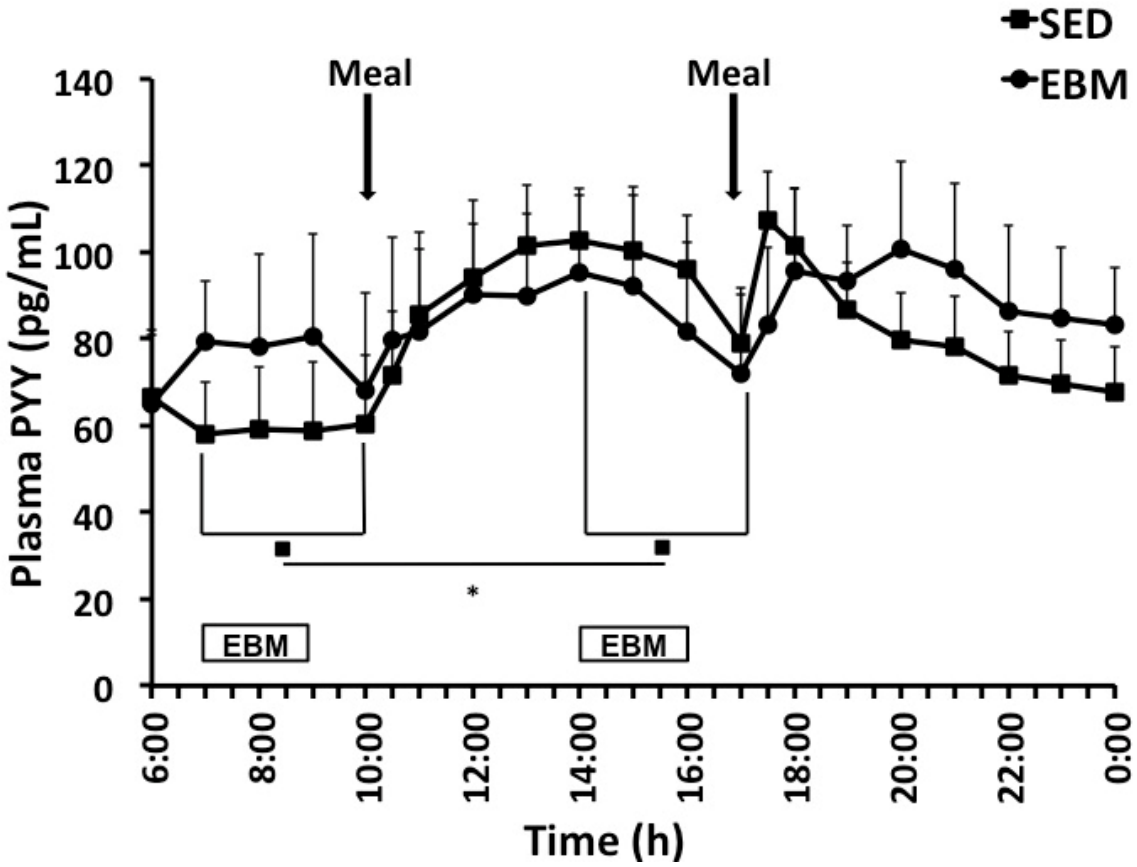
"*" indicates the statistical significance between two selected time periods.

Figure 5-4b: Plasma GLP-1 responses to high-carbohydrate meals in sedentary (SED) and exercise after meals (EAM) trials



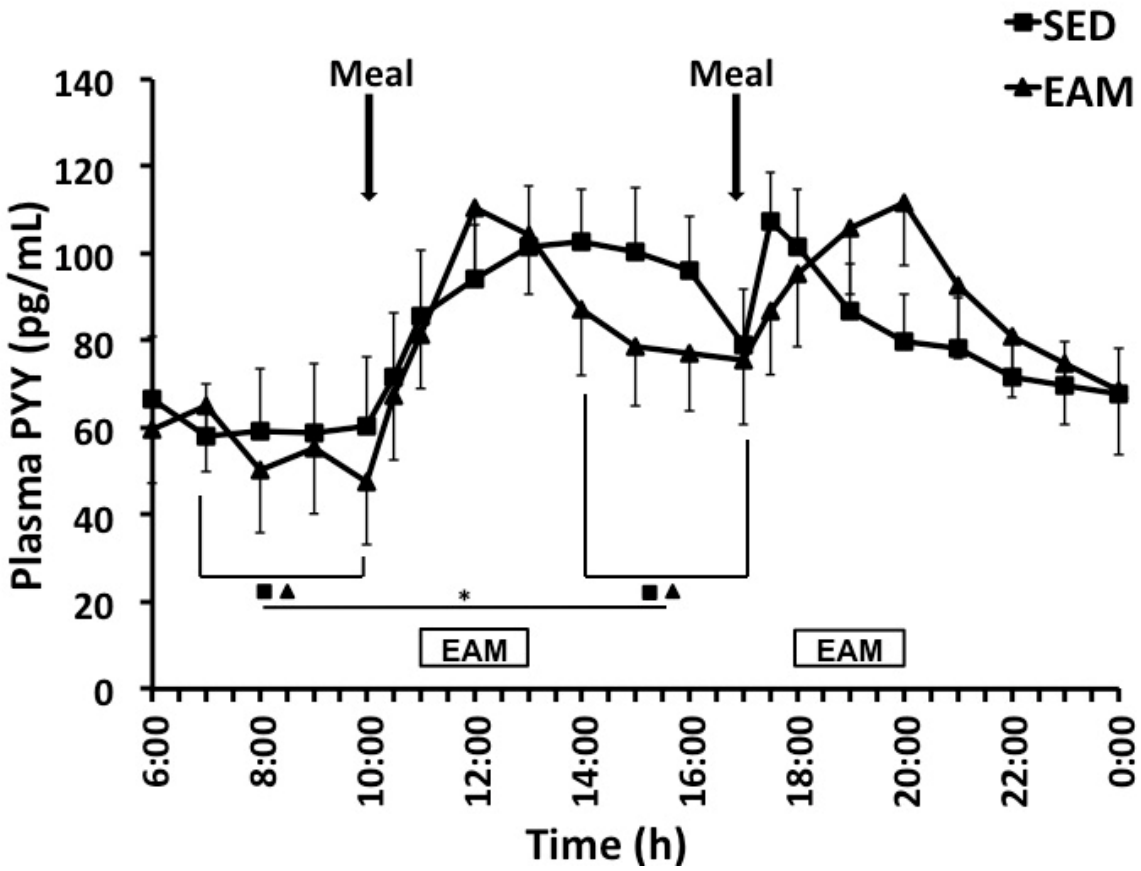
"*" indicates the statistical significance between two selected time periods.

Figure 5-5a: Plasma PYY responses to high-carbohydrate meals in sedentary (SED) and exercise before meals (EBM) trials



"*" indicates the statistical significance between two selected time periods.

Figure 5-5b: Plasma PYY responses to high-carbohydrate meals in sedentary (SED) and exercise after meals (EAM) trials



"*" indicates the statistical significance between two selected time periods.

Figure 5-6: Hunger responses to low-carbohydrate meals in sedentary (X0), 1-hour exercise (X1), and 2-hour exercise (X2) trials

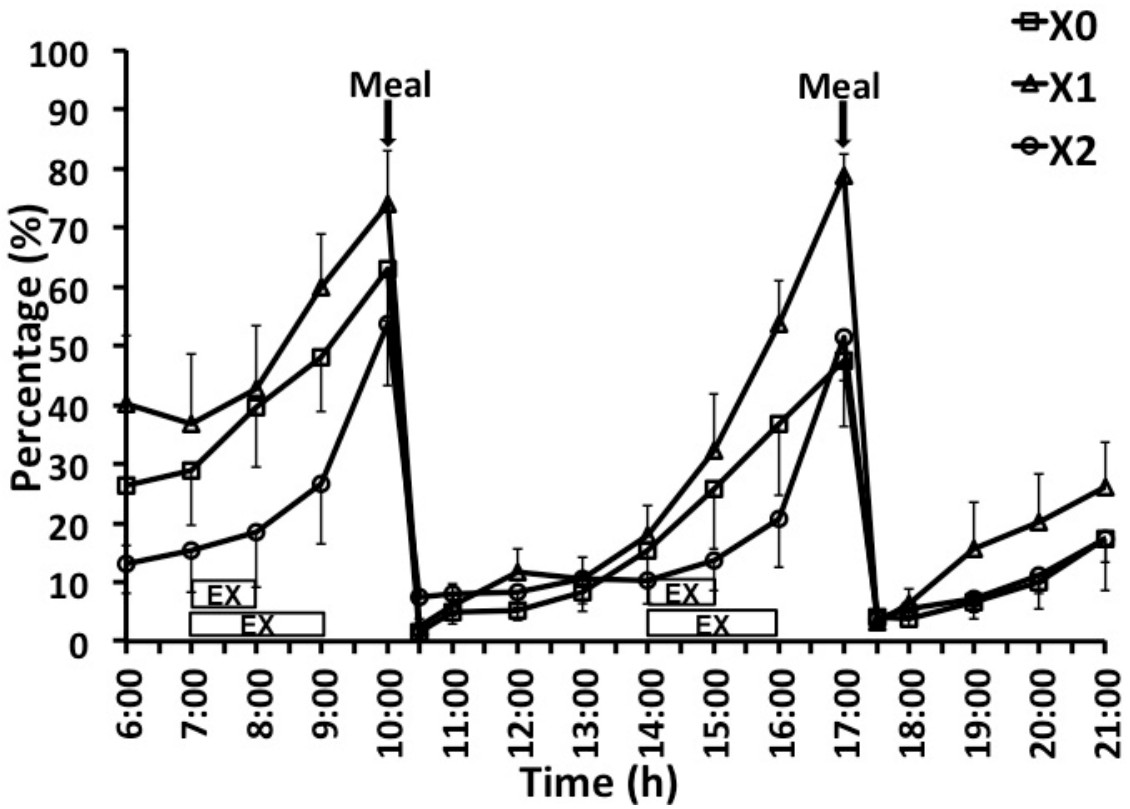
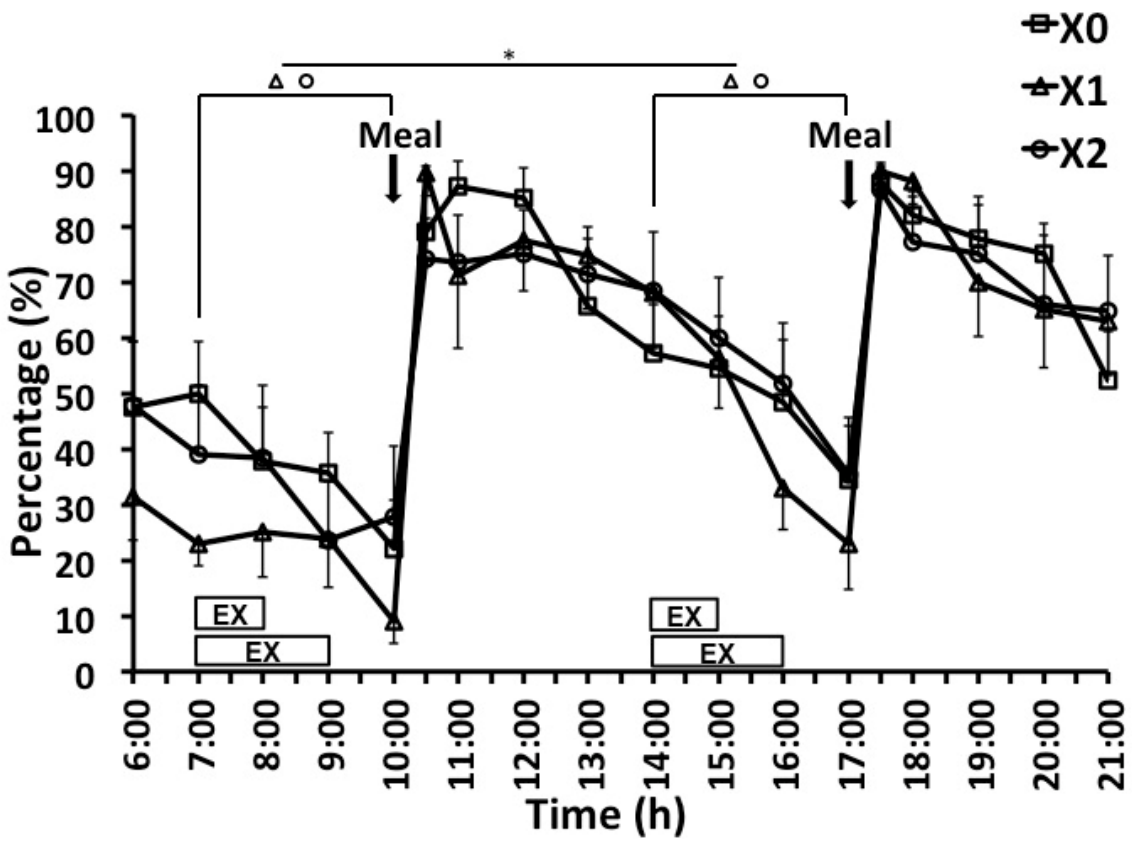
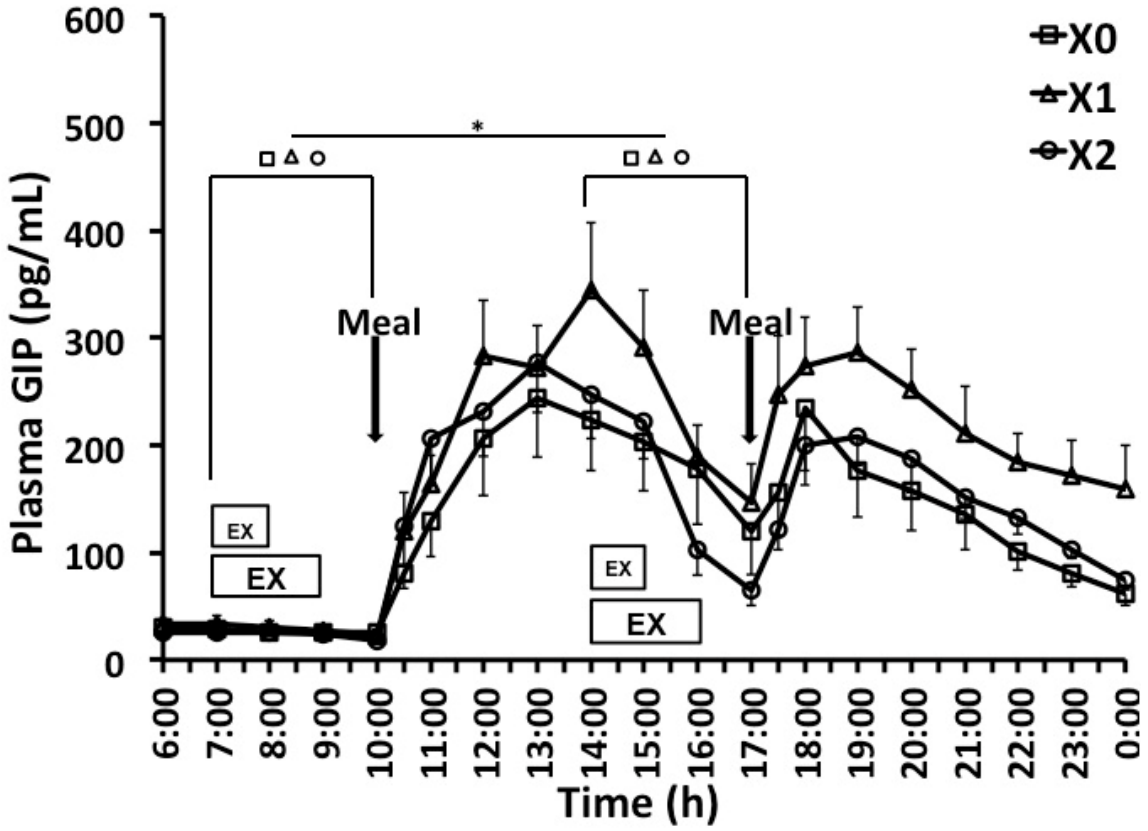


Figure 5-7: Fullness responses to low-carbohydrate meals in sedentary (X0), 1-hour exercise (X1), and 2-hour exercise (X2) trials



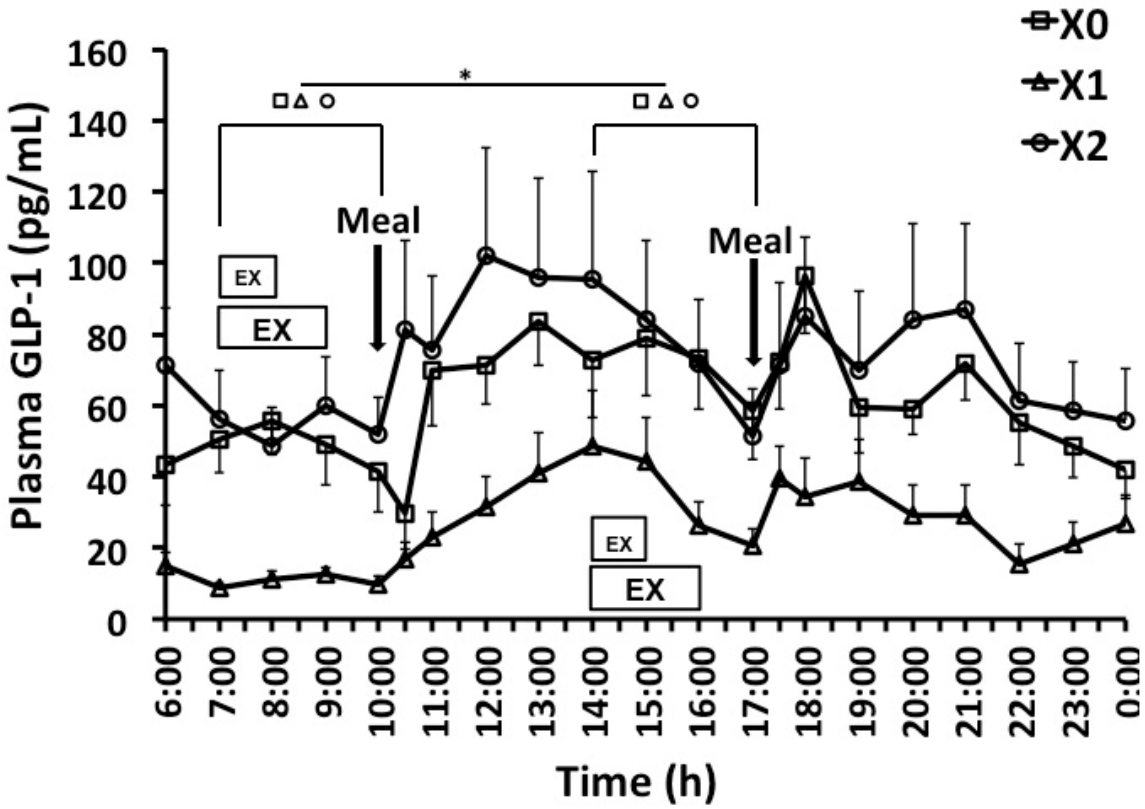
"*" indicates the statistical significance between two selected time periods.

Figure 5-8: Plasma GIP responses to low-carbohydrate meals in sedentary (X0), 1-hour exercise (X1), and 2-hour exercise (X2) trials



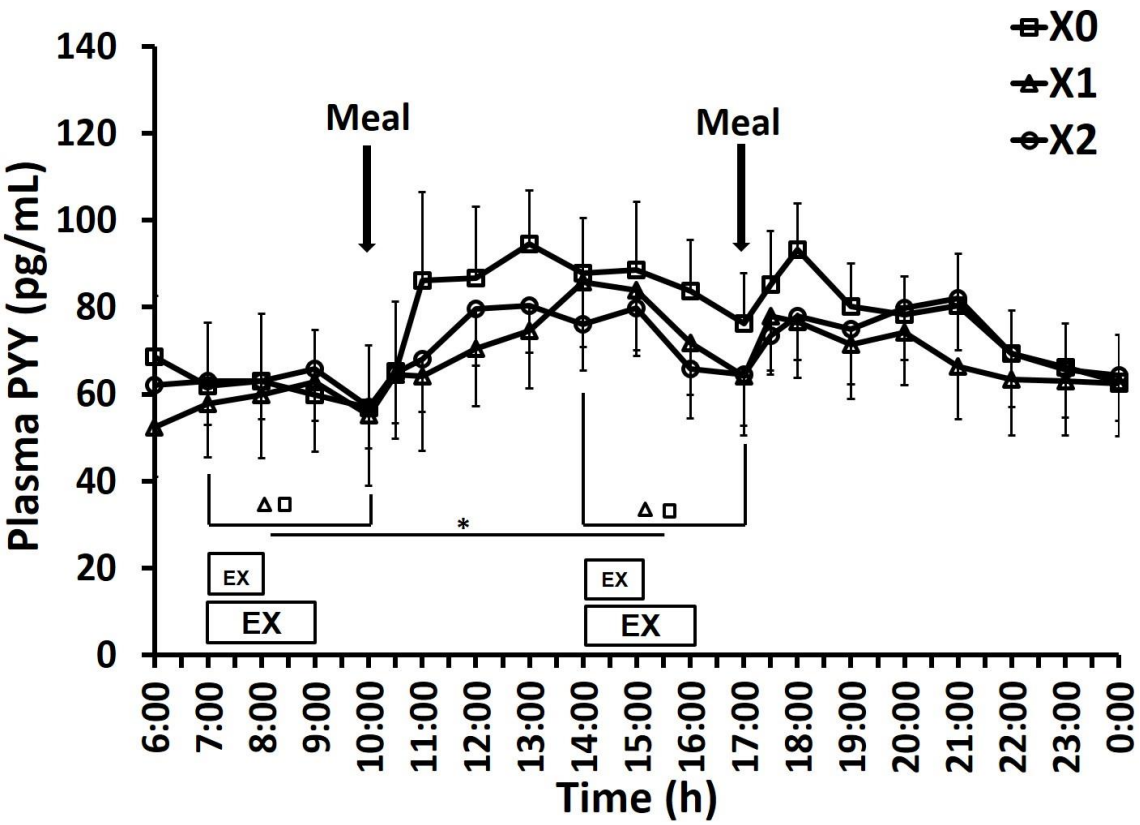
"*" indicates the statiostical significance between two selected time periods.

Figure 5-9: Plasma GLP-1 responses to low-carbohydrate meals in sedentary (X0), 1-hour exercise (X1), and 2-hour exercise (X2) trials



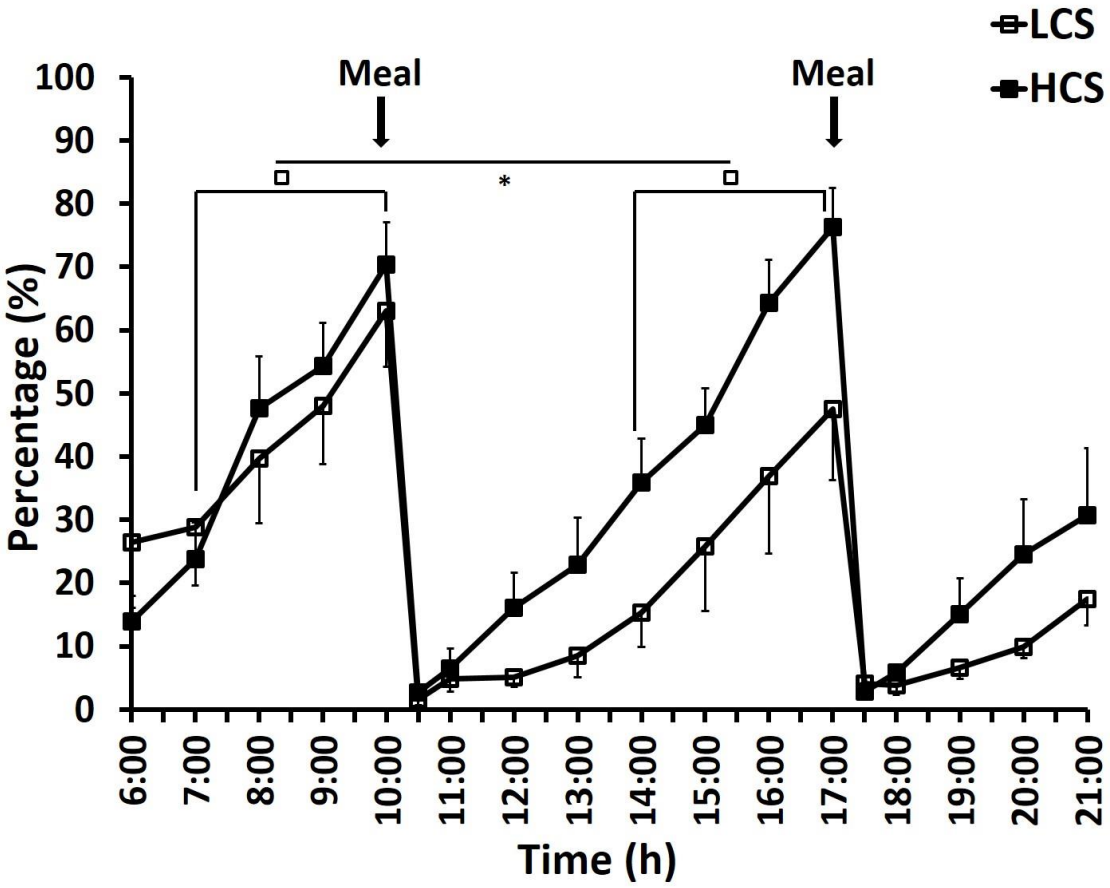
"*" indicates the statistical significance between two selected time periods.

Figure 5-10: Plasma PYY responses to low-carbohydrate meals in sedentary (X0), 1-hour exercise (X1), and 2-hour exercise (X2) trials



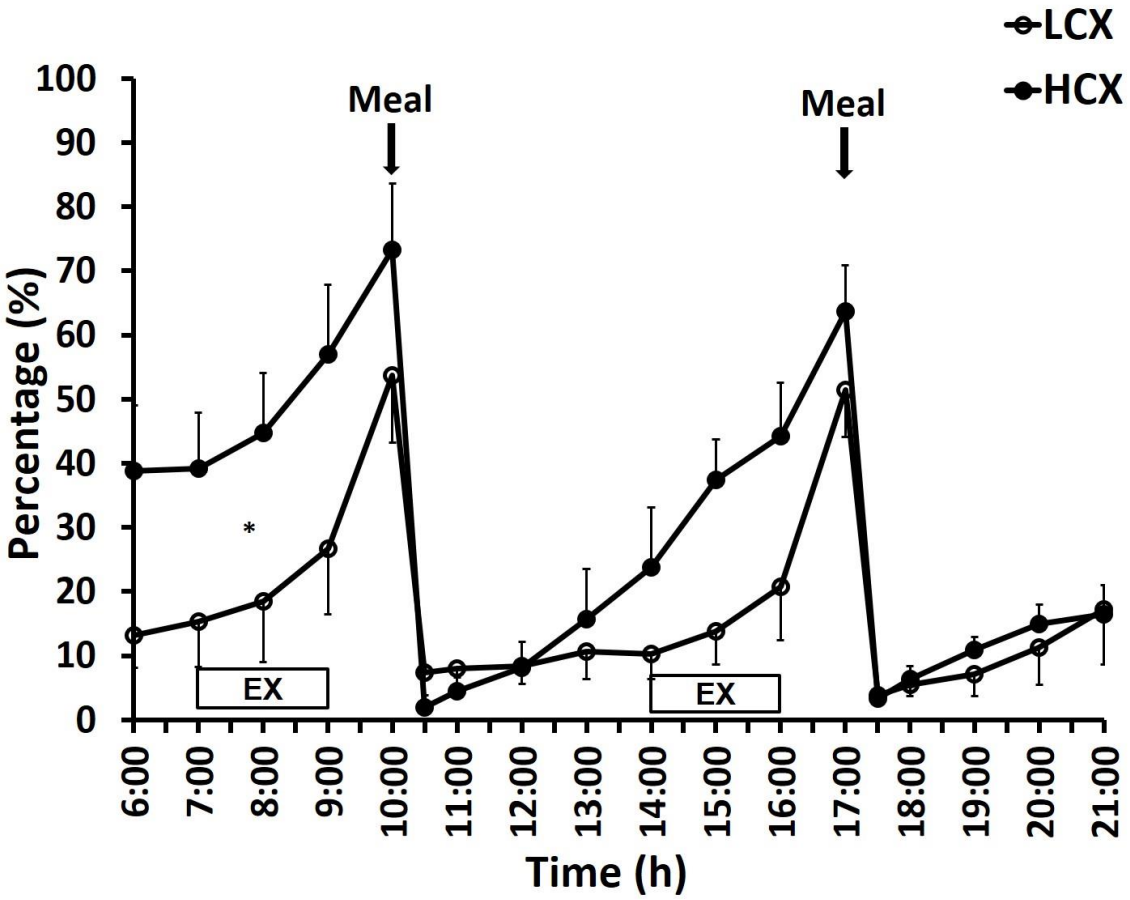
"*" indicates the statistical significance between two selected time periods.

Figure 5-11a: Hunger responses to low-carbohydrate (LCS) and high-carbohydrate (HCS) meals in sedentary condition



"*" indicates the statistical significance between two selected time periods.

Figure 5-11b: Hunger responses in exercise before low-carbohydrate meals (LCX) and before high-carbohydrate meals (HCX) trials



"*" indicates the statistical significance between LCX and HCX exercising groups in the specified areas under the curve.

Figure 5-12a: Fullness responses to low-carbohydrate (LCS) and high-carbohydrate (HCS) meals in sedentary condition

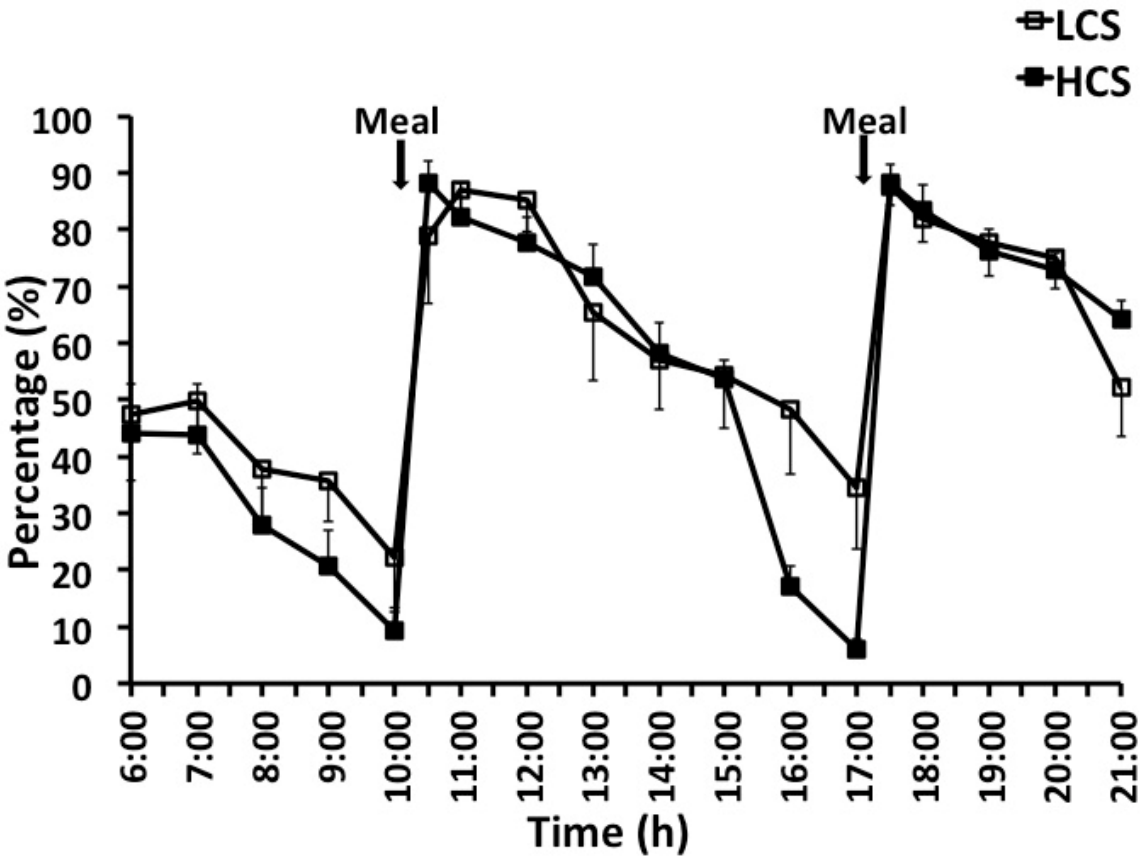
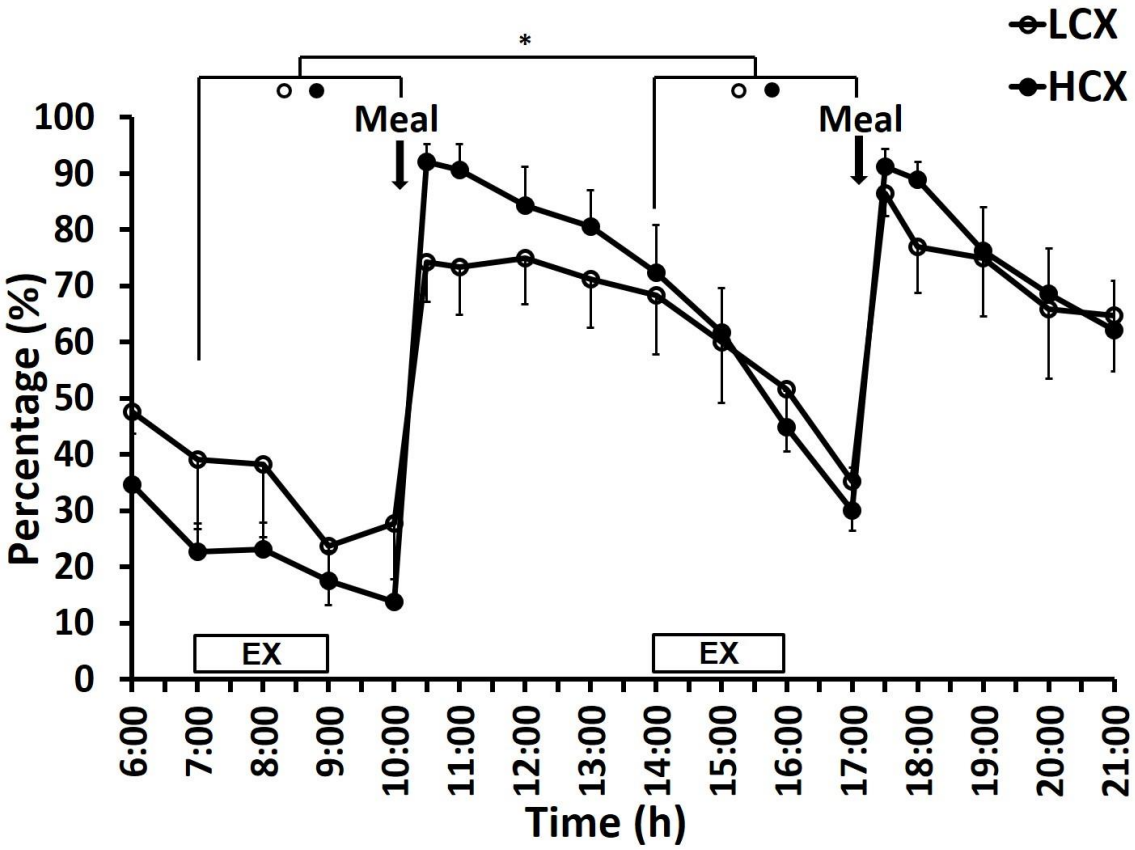
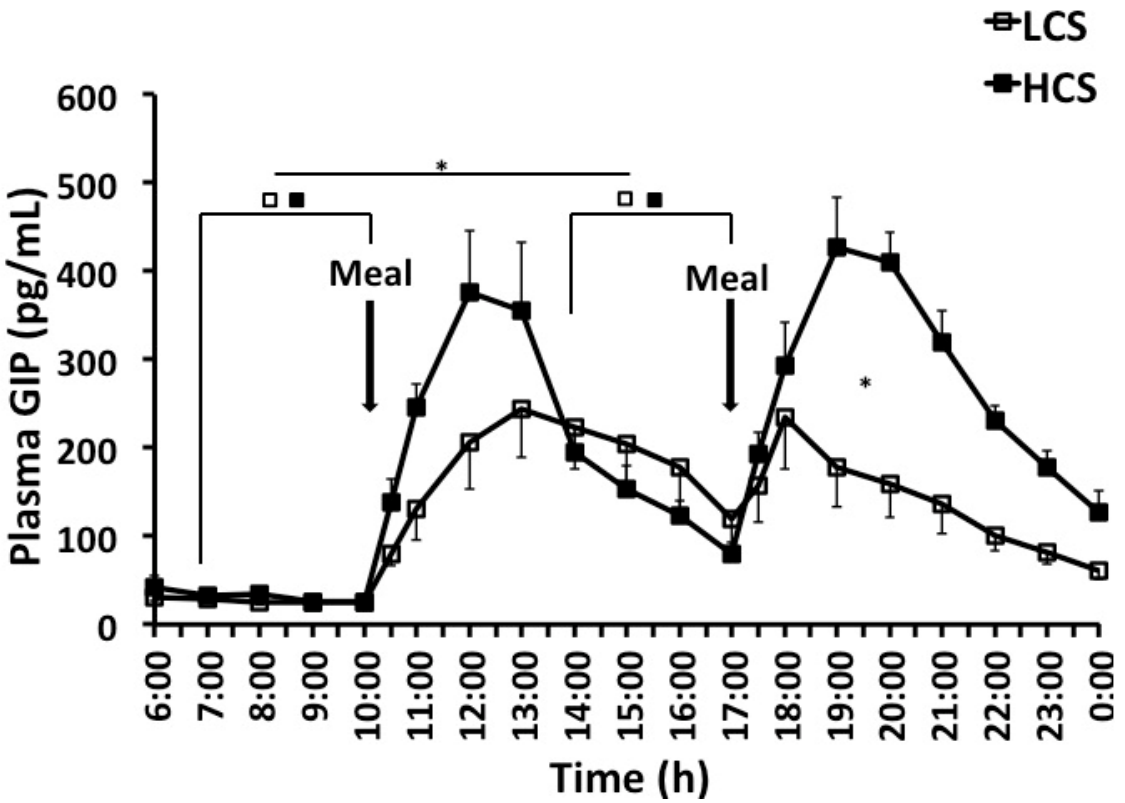


Figure 5-12b: Fullness responses in exercise before low-carbohydrate meals (LCX) and before high-carbohydrate meals (HCX) trials



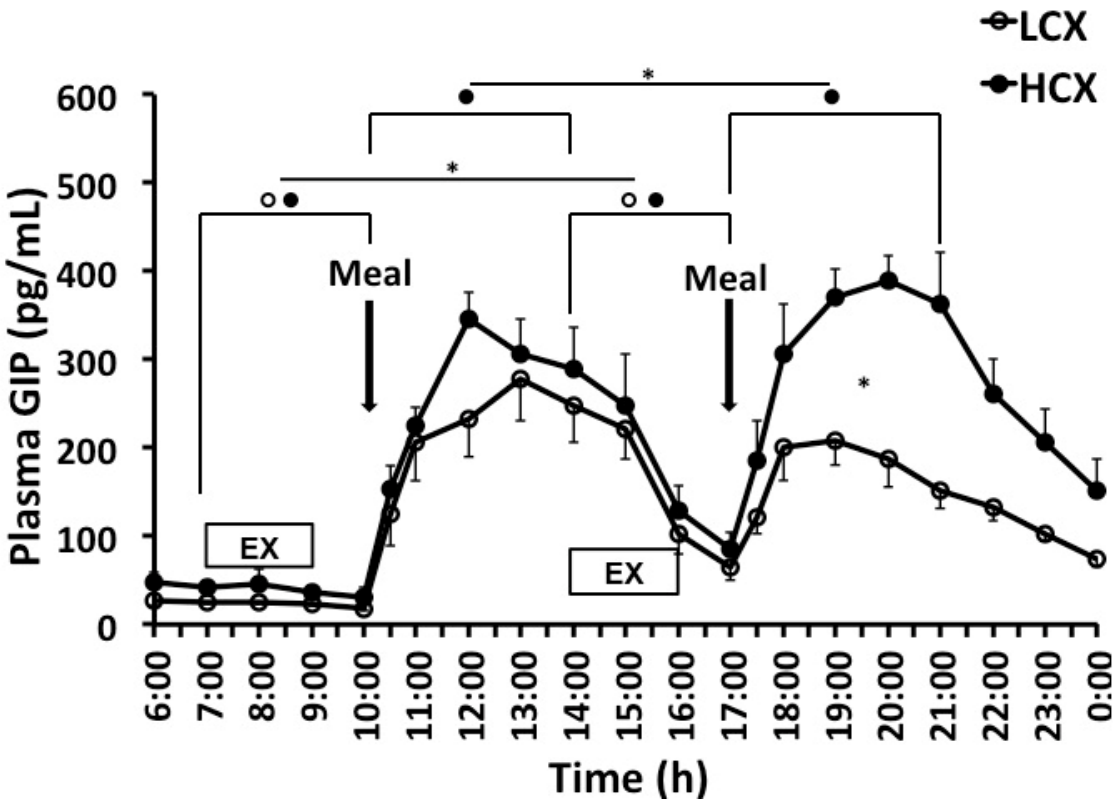
"*" indicates the statistical significance between two selected time periods.

Figure 5-13a: Plasma GIP responses to low-carbohydrate (LCS) and high-carbohydrate (HCS) meals in sedentary condition



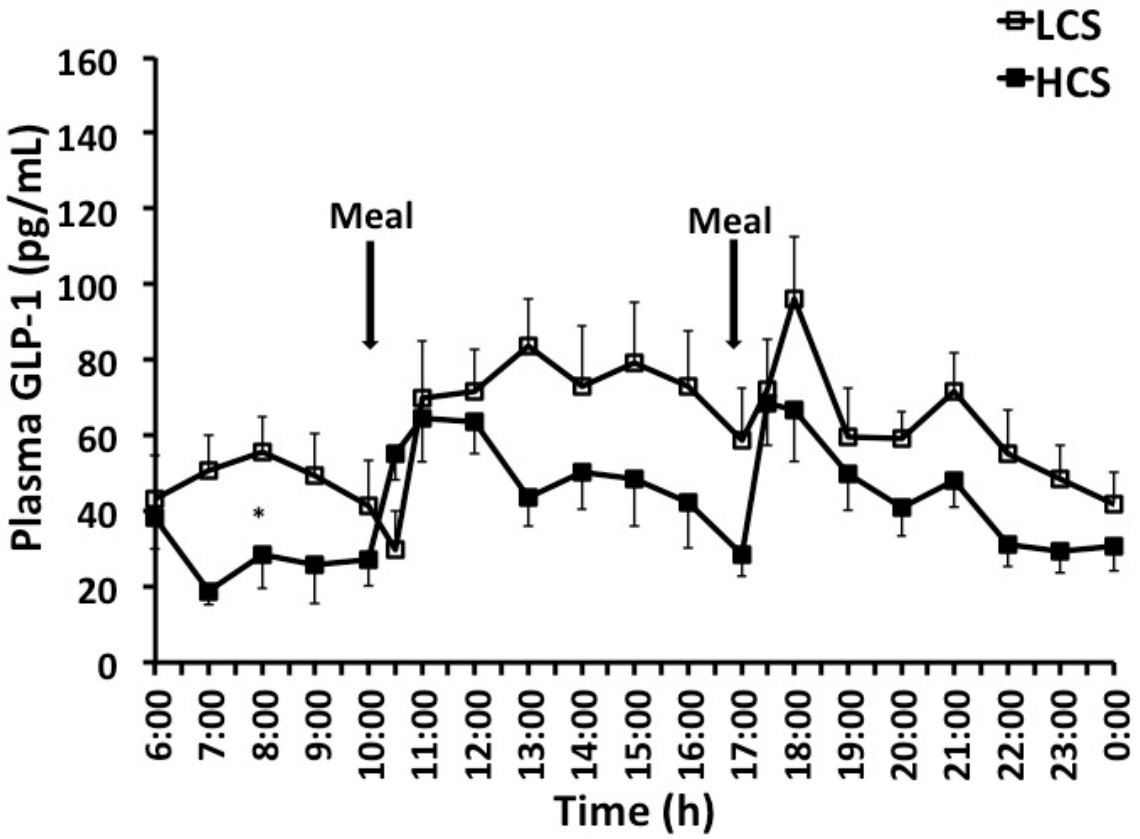
"*" indicates the statistical significance between two selected time periods/areas under the curve.

Figure 5-13b: Plasma GIP responses in exercise before low-carbohydrate meals (LCX) and before high-carbohydrate meals (HCX) trials



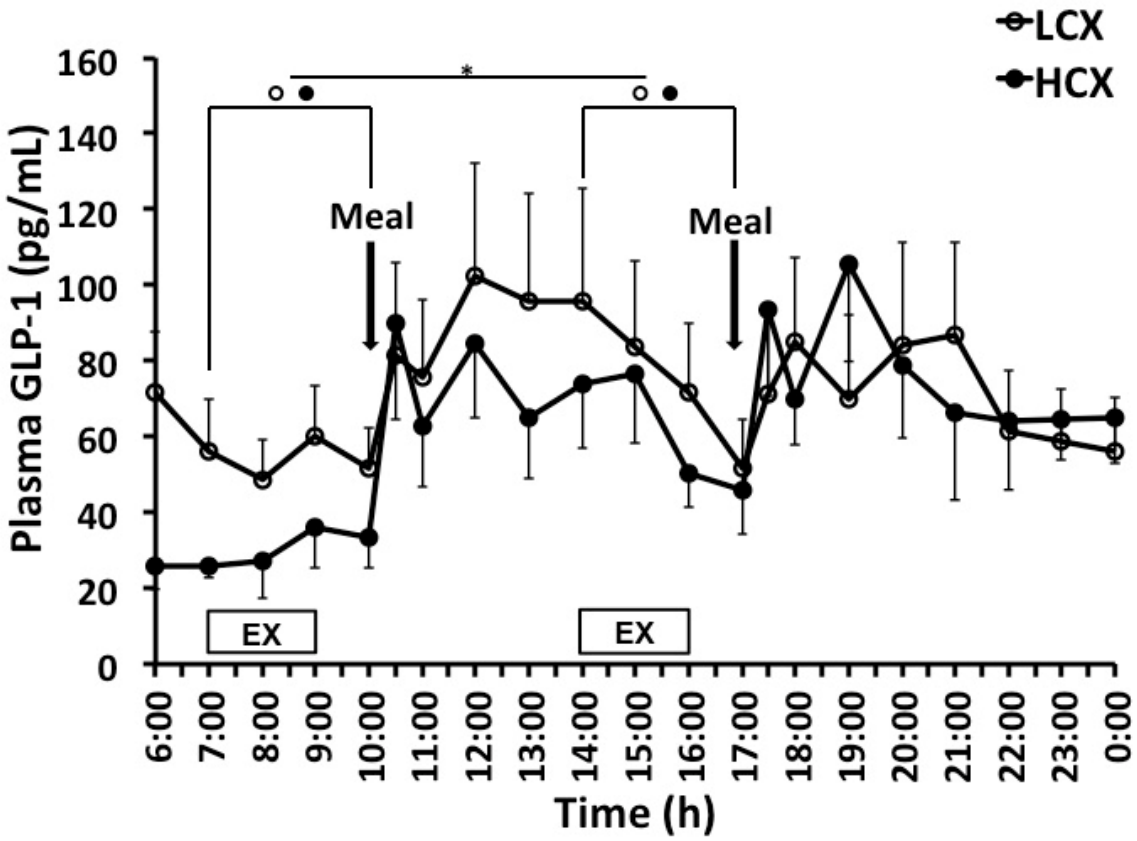
"*" indicates the statistical significance between two selected time periods/areas under the curve.

Figure 5-14a: Plasma GLP-1 responses to low-carbohydrate (LCS) and high-carbohydrate (HCS) meals in sedentary condition



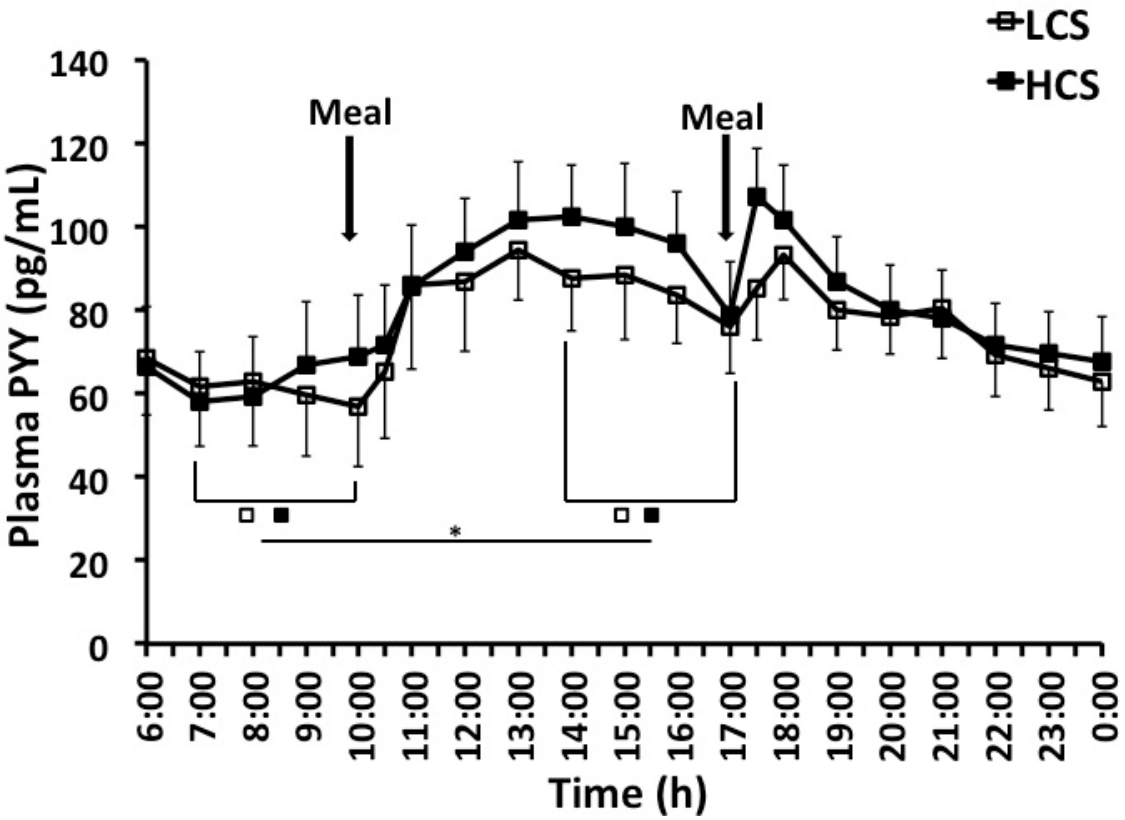
"*" indicates the statistical significance between LCS and HCS sedentary groups in the specified areas under the curve.

Figure 5-14b: Plasma GLP-1 responses in exercise before low-carbohydrate meals (LCX) and before high-carbohydrate meals (HCX) trials



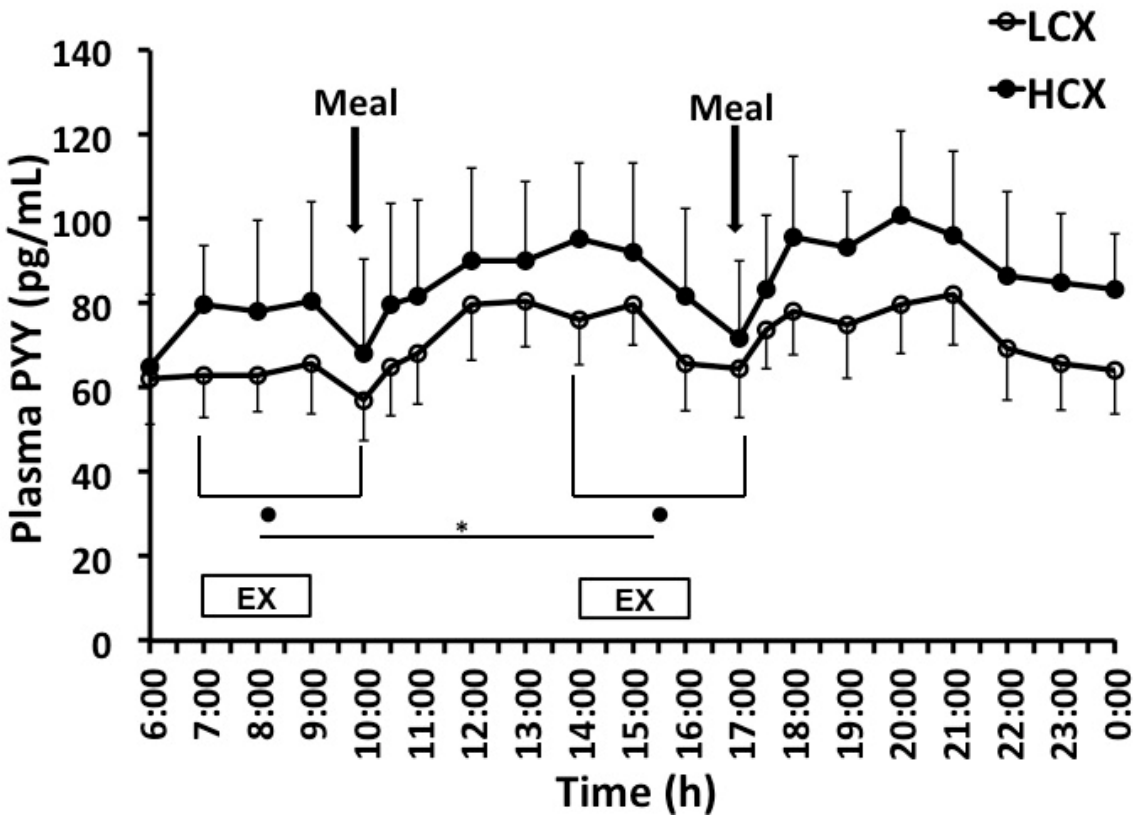
"*" indicates the statistical significance between two selected time periods.

Figure 5-15a: Plasma PYY responses to low-carbohydrate (LCS) and high-carbohydrate (HCS) meals in sedentary condition



"*" indicates the statistical significance between two selected time periods.

Figure 5-15b: Plasma PYY responses in exercise before low-carbohydrate meals (LCX) and before high-carbohydrate meals (HCX) trials



"*" indicates the statistical significance between two selected time periods.

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CHAPTER 6

Overall Discussion

Glycemic responses to food ingestion and exercise have been widely studied because protracted postprandial glucose elevation represents a diagnosis of insulin resistance in pre-diabetes and type 2 diabetes. Two behavioral interventions, meal eating and exercise, have been commonly studied for their transient disruptive effect on glucoregulation. In the tradition of classical physiology, they have typically been studied as single events isolated from other confounding variables. However, the condition of human life is to eat and/or exercise intermittently more than once within a day. Therefore it is important to examine the glycemic and insulin responses in the context of the timing of exercise relative to the meals because they will differ as a function of the prandial phase, and this will influence glucose and lipid utilization by the muscle, glucose production by the liver, and hunger and satiation ratings. Besides the variation in glucose and insulin responses as a function of the timing of human feeding and exercise behaviors, a circadian influence also affects glucoregulation and reduces afternoon glucose tolerance and insulin secretion compared to the same treatment applied in the morning. Examining the glucoregulatory responses to the intermittent timing of meals and exercise provides the opportunity to understand the interaction between these behavioral influences and the physiology of the circadian influence.

To address this knowledge gap, this dissertation has systematically implemented in Study 1 a repeat-event design (Figure 3-1) with two isocaloric meals, one in the morning and the other in the afternoon, and two substantial bouts of moderate-intensity exercise bout either completed 1 hour before the meals (EBM) or started 1 hour after the meals (EAM). This study design provides the opportunity to examine the influence of prandial states, fasting period before the first meal (first EBM exercise bout), early postprandial (PP) periods 1 hour after the meals (two EAM exercise bouts), and late PP period, 4 hours after the first meal (second EBM exercise bout), on glycemia, insulin and couterregulatory glucagon release, and the secretion of incretin or

insulin-stimulating gut peptides, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). Besides the study of the timing of repeat exercise with respect to two fixed daily meals, this dissertation also examined the possibility that currently prevailing high-carbohydrate (CHO) meals may be contributing to diurnal decline in glucose tolerance. The repeat meal design with repeat exercise before the meals was therefore carried out in Study 2 with meals that differed in CHO content by a factor of two. The metabolic and hormonal changes caused by intermittent timing of exercise with respect to meals can influence hunger and satiation or produce exercise anorexia; these psychophysical ratings can influence food intake and glucoregulation as well as be influenced by the secretion of satiating gut hormones, peptide tyrosine tyrosine (PYY) that was also studied along with GLP-1 in Study 3.

The repeat-event design in this dissertation on the effects of meals and exercise on glucoregulation revealed several striking, interesting and important differences from results obtained by the prevailing single-event research approaches. The first unanticipated finding was that exercise during late PP produced greater and more sustained reduction in plasma glucose to a high-CHO meal than did the well-known rebound or compensatory hypoglycemia that occurred when exercise was performed during early PP where a sugar drink was ingested within 1 hour of exercise [1-4]. Thus a repeat EBM exercise bout starting 4 hours after the morning meal had a more substantial glucose lowering effect despite the insulin concentration being at low, basal level than did the exercise performed during early PP when insulin concentration was at its peak. The second unanticipated finding from the repeat-event study design was absence of mitigating exercise effect on the high glycemia to high-CHO meals or the capacity of exercise to abolish the afternoon glucose intolerance. This finding contradicts a plethora of single-event experiments demonstrating the effectiveness of exercise to increase non-insulin-dependent glucose uptake by the muscle for use as a fuel [5-7] and to increase insulin-stimulated muscle glucose uptake for resynthesis of muscle glycogen [8-11]. The third unexpected finding with the repeat-event design that was not reported with a single-event design was the striking reduction in the afternoon, but not in the morning, PP insulin response, which was also accompanied by a parallel decline in the afternoon PP GIP response. Thus with the single variable approach, afternoon hyperglycemia was shown to be increased with increased CHO content and glycemic load of the meals [12]. PP glycemia increased by about 10%, insulin response by about 47%, and insulin resistance

estimated by HOMA by about 230% when the GI of 61% CHO meals was 84 compared to 34. The effect was amplified when the meals provided 60% as compared to 20% of daily energy [13]. All three examples of differences in glucoregulation studied with a repeat-event design as compared to single-event design points to the importance for the systematic study of intermittent meals and exercise for closing the knowledge gap regarding their interactions.

The new interesting and important findings of the present studies appear to be interconnected. Study 1 revealed that some metabolic circumstances resulting from two bouts of exercise separated by a high-CHO meal lead to a sustained hypoglycemia during late PP exercise. Unlike the compensatory hypoglycemia of exercise performed within 3 hours of a sugar drink [1-4] or a high-CHO meal (Study 1), late PP exercise resulted in hypoglycemia in the presence of low, basal insulin concentration. At least three other studies [14-16] have also reported a glucose lowering effect when a relatively higher intensity (60-70% VO_2max) but shorter duration (60-75 min) of exercise was performed 2 or more hours after the meal. Two of these studies [15, 16] provided strong evidence to support our findings as they found that exercise in fasted state had no impact on plasma glucose [15] but exercise during late PP, 3-5 hours after the meal, significantly lowered blood glucose by 38-43% in subjects with type 2 diabetes [16]. In addition, the glucose-lowering effect of exercise during late PP may not be specific to exercise with high-CHO meals because it was also seen when meals of 30% CHO content (Appendix B). This late-PP hypoglycemia appears to reflect reduced availability of CHO fuel and metabolic fuels in general because it is associated with a shift toward increased CHO utilization compared to pre-meal fasting exercise, and a large increase in FFA and ketone body concentrations [17-19] during exercise. The cause of this apparent shortage of metabolic fuel during late PP exercise most likely reflects incomplete muscle glycogen repletion after fasting exercise before the first meal suggested by the rise in ketone body concentration, a reflection of liver glycogen shortage that is not corrected before the muscle glycogen shortage. Late PP CHO fuel shortage appears to be mediated by the high-CHO content of the morning meal because it was manifest in study 2 during the late-PP EBM exercise in this dietary condition but not when the morning meal was low in CHO. Late PP was also the prandial state most likely to result in exercise anorexia. This paradoxical reduction of hunger and increased satiation during energy expending exercise was not associated with increased satiating gut peptide release as postulated by some authors [20, 21].

While the cause of exercise anorexia remains elusive, a better understanding of its association with circumstances leading to energy and CHO fuel shortage during late PP period may provide useful cues.

The importance of prandial events in modulating glucoregulation during exercise is further seen in the results of Study 2 which explored the role of CHO content of the meals. Here the most interesting and unexpected finding was that about 60g low (30%)-CHO meal in the morning produced an identical insulin response as a 120g high (60%)-CHO meal indicating relative refractoriness of insulin response to the actual quantity of absorbed glucose. Seven hours later, the same two meals produced dramatically different results. Insulin response to 60g CHO meal was now 39% lower than the insulin response to the 120-g CHO meal. The afternoon PP insulin response was stoichiometrically related to the quantity of absorbed glucose. Whether this rapid diet-induced change is a consequence of a parallel afternoon decline in PP GIP response requires additional direct tests of this hypothesis. A slight decline in the morning PP GIP AUC may support this hypothesis as a preamble to a more substantial afternoon decline. An association between GIP and insulin secretion was previously proposed by results of another study [22]. Two versions of the null hypothesis to disprove a functional association between exercise anorexia and satiating gut peptide secretion were applied: (1) that exercise anorexia would be found during fast at the time no gut peptides are released, and (2) that exercise anorexia would not be specifically associated with exercise during the PP period at the time gut peptide secretion is increased. Both null hypothesis premises were met and allowed rejection of the hypothesized exercise-anorexia-gut peptide hypothesis.

Consideration that the afternoon insulin-lowering effect of low-CHO may be caused by agents other than reduced afternoon GIP response was not found convincing. The decline in the afternoon PP insulin responses could be a consequence of the secretion and actions of counterregulatory hormones glucagon, cortisol and growth hormone (GH) rather than the gut peptide GIP. Glucagon secretion in Study 2 was uniformly higher after low-CHO than after high-CHO meals, but the afternoon PP concentration was not higher than in the morning and thus was unlikely to influence afternoon PP insulin response. Food ingestion during the day is inhibitory to GH secretion which exhibits a largest secretory peak at night [23] eliminating the likelihood of

the role of this hormone in the afternoon PP insulin counterregulation. Likewise, cortisol exhibits a strong diurnal decline in its concentration despite a mid-day peak that coincides with the mid-day meal [24, 25]. However, since peak cortisol concentrations are observed in the morning, and its circadian nadir in the evening, afternoon cortisol secretion cannot account for the afternoon decline in the PP insulin response. That a simple CHO withdrawal can reveal such a striking change in the afternoon PP insulin response to CHO brings up the possibility that this effect is related to the reported decline in insulin secretory capacity in the afternoon and the afternoon glucose intolerance [12, 22, 26-29], a phenomenon obviously in need of further elucidation.

Finally the two final unexpected findings of this study were the inability of 2 hours of endurance exercise or a reduction in CHO content of the meals by a factor of two to reduce PP glycemia overall and abolish the afternoon decline in glucose tolerance on either low-CHO or high-CHO diet. As mentioned previously, in single-event studies, exercise was shown to increase glucose tolerance [5-7] followed several hours later by increases in insulin sensitivity [8-11]. The hypothesis tested in Study 2 was that 2 hours of moderate-intensity exercise generating about 450 Kcal of energy expenditure 1 hour before the two meals will reduce overall glycemia and lead to disappearance of afternoon PP glucose intolerance. The possibility that exercise also could affect insulin secretory response by altering the postprandial responses of incretin gut hormones, GIP and GLP-1 and their insulinotropic actions [30, 31] was also entertained. As the results show, exercise did not alter the overall pattern of glycemic, insulinemic or GIP responses beyond what was effected by diets, and GLP-1 PP responses were unaffected by either diet or exercise. Exercise trials actually maintained higher afternoon PP hyperglycemia on low-CHO diets and thus removed the beneficial effect of low-CHO diet on the afternoon hyperglycemia seen in the sedentary trial. Although there is no obvious explanation for the absence of a hypoglycemic and hypoinsulinemic effect of exercise in this study, three possible contributing factor could be considered. The first factor is that the differences observed between morning and afternoon insulin responses to meals of different CHO composition were selectively influenced by postingestive effects of the two diets and therefore unaffected by the energy cost of preceding exercise. The second possibility is that the prolonged exercise of moderate intensity exerts less of an influence over subsequent PP insulin secretion than does the form of exercise of higher intensity and shorter duration, which typically deployed in studies showing glycemic and insulin

changes [32-37]. The third possible explanation is the study population that postmenopausal women chosen in present study while other studies typically recruited young men. Gender, age, fitness level of subjects could critically interfere the effect of exercise and diet on glycemic and insulin responses.

The final unexpected finding was an unanticipated absence of a PP hypoglycemic and hypoinsulinemic response to low-CHO meal could possibly be a function of the large about 800-Kcal meals in present study. Afternoon hyperglycemia was shown to be increased with increased size of the meals [12] especially when the meals have high-CHO content and glycemic load. PP glycemic and insulinemic responses could be greatly affected by manipulating the glycemic index of meals as Morgan et al. (2012) reported a 10% increase in glucose, a 47% increase in insulin, and more than 2 fold increased in insulin resistance when the GI of high (61%)-CHO meals was 84 compared to 34. The effect was amplified when the high-CHO meals provided 60% as compared to 20% of daily energy [13]. Since we offered close to weight-maintenance amount of energy in only two rather than customary three meals, it is possible that even 60 g of CHOs in the afternoon low-CHO meal taxed the afternoon insulin-secretory capacity.

Collectively, this dissertation extends the body of scientific knowledge on how the interactions between exercise and meals affect glucoregulation. It shows that the prandial state at the time of exercise determines whether blood glucose during exercise will be maintained or will decline. Based on the findings of this dissertation, blood glucose declined only when the exercise took place during late PP period, but not in the fasting state or during early PP period. To what extent changes in muscle and liver glycogen and in muscle sensitivity to insulin account for or how counterregulatory hormones, other than glucagon, play a role for this effect requires further study. However, a clear definition of the prandial state in which the phenomenon occurs should inform studies where exercise is performed at random prandial periods of the importance of controlling this variable. This finding also provides the helpful information to people who would like to use exercise as a strategy to better control their plasma glucose level if they perform their daily exercise during late PP period, 4-6 hours after the meal. Athletes whose physical training depends more on CHO may need to consider CHO supplements if the training is taking place during this time of the day.

Next, this dissertation shows that the CHO content of the meals plays exerts a strong influence on PP insulin response to repeat meals. While the association with the parallel decline in the PP concentration of the incretin hormone GIP, the possible causal relationship between the two will require experimental design and methods other than those used in this study. Tests of the significance of PP insulin change in the context of sensitivity to its action will require appropriate tests, not available in this study. In addition, it will be important to determine whether the effect is a consequence of repeat exposure to the diet and will persist with additional meals, or whether it is, like afternoon glucose intolerance, a circadian rhythm effect. It is possible that this low-CHO effect may reduce chronic PP insulin over-secretion that can lead to pancreatic beta cell exhaustion in prediabetes and type 2 diabetes.

Finally, the results of Study 3 again point to the importance of the late PP period as the state conducive to elicitation of exercise anorexia, but the data of Study 3 did not support the hypothesis of the functional association between the gut peptide secretion and this psychophysical response.

Overall, the results of this dissertation demonstrate that is important to study the interactions of exercise and diet with repeat-event experimental designs as the intermittent meal taking and exercise that more related to human living condition. The repeat-event experimental design provides the opportunity to explore the different effect of the same exercise performed in different prandial states on glucoregulation and appetite responses. Reducing CHO content of meals could be a practical strategy to prevent afternoon insulin over-secretion.

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APPENDIX A

Additional Analyses for Study 1 (Chapter 3):

Effects of the glycemic index of morning meals and exercise in different prandial states

In Study 1, equicaloric high-carbohydrate (CHO) meals of same macronutrient composition resulted in higher afternoon postprandial (PP) glucose response than after the same type of meal consumed in the morning. This effect was independent of exercise. High-CHO diets can differ in their glycemic index (GI), a measure of glycemic effect of CHO in food relative to the effect of an equal amount of glucose [1]. A low-GI meal eaten the night before the trial improved glucose tolerance to a morning meal [2]. Afternoon PP glycemia and insulin response were lower after eating a morning low-GI meal [3, 4]. PP glycemia increased by about 10%, insulin response by about 47%, and insulin resistance estimated by HOMA by about 230% when the GI of 61% CHO meals was 84 compared to 34. The effect was amplified when the meals provided 60% as compared to 20% of daily energy [5]. A recent meta-analysis has revealed that risk of diabetes was 50% higher in individuals who consumed a diet low in cereal fiber but containing a high glycemic load (GL, a product of the GI and the quantity of CHO in the meal) [6]. A high-GI meal, compared to a low-GI meal, consumed before exercise produces higher PP glycemic responses and leads to a greater decline in blood glucose concentrations in response to exercise [7]. This is due to the combined hypoglycemic effects of increased insulin responses to the high-GI meal as well as increased insulin-independent, exercise-associated muscle glucose uptake during exercise. High-GI meal leads to greater muscle glycogen repletion and higher PP glycemia and insulin responses during the 24-hour recovery from glycogen-depleting exercise, than does the low-GI trial [8, 9]. In addition to promoting greater glycogen storage, a high-GI meal, relative to a low-GI meal, also leads to greater utilization of muscle glycogen during subsequent exercise [10]. Therefore, this supplemental study examined high-GI vs. low-GI of the morning meal on PP glycemic and insulinemic responses to repeated exercise bouts completed

either 1-hour before meals or started 1-hour after the meals. The hypothesized outcomes were that low-GI morning meal would lower both morning and afternoon PP glucose and insulin responses to a greater extent than the high-GI morning meal regardless of the timing of exercise before or after the meals.

Methods

Nineteen subjects matched by body weight and BMI were assigned to exercise before meals (EBM) trials: 13 of them followed low-GI (LGI) morning meals (LGI-EBM) and 6 of them followed high-GI (HGI) morning meals (HGI-EBM). Another 19 body-weight and BMI-matched subjects were assigned to exercise after meals (EAM) trials. Again, 13 of them were in LGI-EAM and 6 of them were in HGI-EAM group. The LGI meals were the same as used in Study 1 with the GI of 58 and 68 for the morning and afternoon high-CHO meal, respectively (Tables 3-3 and 3-4). The HGI high-CHO meals were provided in the form of French toast with pancake syrup and margarine spread, Cheese omelet, cereal, skim milk, and orange juice in the morning (Table A-1) and wheat rolls with butter, rice, turkey breast with gravy, corn, soda drink, and ice cream with chocolate syrup in the afternoon (Table A-2). The GI was 74 and 61 for the morning and the afternoon HGI high-CHO meal. The timing of meals and exercise bouts based on the assigned trials and the same metabolites and hormones were measured as in Study 1 (Chapter 3).

Results

Subject characteristics and energy intake and exercise energy expenditure were presented in Tables A-3 and A-4. No group difference was seen in either EBM or EAM trials.

Plasma Glucose (Figures A-1a, A-1b)

The afternoon PP glucose AUC was significantly higher than the morning PP glucose AUC in LGI-EBM ($t=3.87$, $p=.0012$) but not in HGI-EBM group (Figure A-1a). Afternoon PP glucose was also significantly higher in both EAM groups (LGI-EAM: $t=4.25$, $p=.0005$; HGI-EAM: $t=2.31$, $p=.0339$, Figure A-1b). In addition, plasma glucose was significantly lower during exercise in late PP (second EBM exercise bout), 4-hours after HGI morning meal than during exercise in fasting state before the first meal ($t=-2.39$, $p=.0288$). This effect also was present with the LGI morning meal but did not quite reach statistical significance ($t=-2.07$, $p=.0544$).

Plasma Insulin (Figure A-2a, A-2b)

GI of the morning meal did not affect PP insulin AUCs when exercise took place during the early PPs (Figure A-2b), or late PPs (Figure A-2a).

Plasma Glucagon (Figure A-3a, A-3b)

Glucagon responses were significantly higher when exercise was performed during the late PP, 4-hours after the first meal compared to exercise during the fasting period in both LGI-EBM ($t=5.88$, $p<.0001$) and HGI-EBM ($t=2.24$, $p=.0389$) groups. The GI of the morning meals had no differential impact on glucagon AUCs within either PP or within either exercise period. The GI of the morning meal did not affect glucagon responses when exercise was performed during early PPs, either.

Discussion

The GI manipulation of the morning meal produced largely negative results most likely because the GI of the morning meal differed by only 22% (58 vs. 74) in the two high-CHO diets. Within each diet, the difference between the morning and afternoon LGI meals was only 17% increase (GI 58 in the morning vs. GI 68 in the afternoon). In the HGI meals, the afternoon GI value was 18% lower than the morning one (GI 74 in the morning vs. GI 61 in the afternoon). Given that manipulation of morning meal GI produced no substantial glycemic or insulin differences, it is unlikely that the modest GI difference between morning and afternoon meals could have contributed to diurnal changes in the afternoon PP hyperglycemia. Substantial effect on afternoon glycemia and insulin response were shown when there was a 60% GI difference between two high-carbohydrate diets (24). While it is not ideal study to examine the diurnal change in postprandial glycemia and hormonal responses with non-identical morning and afternoon meals, a 22% higher GI of morning meal and the17-18% GI difference between morning and afternoon meals did not affect postprandial glycemia and insulin responses cannot explain increased afternoon postprandial glucose intolerance documented in Study 1 (Chapter 3). This study also confirms the phenomenon of glucose lowering with exercise during late PP before the second high-carbohydrate meal, also documented in Study 1 (Chapter 3).

Table A-1: Menu template for the morning meal in high-carbohydrate (CHO) and high-glycemic index (GI) groups

Meal Composition: 60% CHO, 15% protein (PRO), 25% fat, GI=74

Food items	**Wt (g)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
French toast, 2 medium slice-4 ½" x 4 ¼" x ½"	118 g	27.8	9.4	9.2	235
Pancake syrup 1.5 packet-approx. 3 TB	88.5 g	61.6	0	0.09	227
Margarine spread	4 g	0.03	<0.1	2.7	24
Cheese omelet 1.3 large eggused:1 whole egg or 2 egg whites	92.5 g	1	12.7	13	175
Cheerios cereal	17.7	13.2	2	1	65
Skim milk	226.8 g	11.2	7.6	0.2	77
Orange juice	113.4 g	12.5	0.8	0.2	53
Total‡		**127.3**	**32.5**	**26.3**	**856**
Percentage (%)		**56.8**	**15.9**	**27.2**	

‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-half of the subject's daily caloric need. Subjects' daily caloric need=25kcal/kg BW

Table A-2: Menu template for the afternoon meal in high-carbohydrate (CHO) and high-glycemic index (GI) groups

Meal composition: 60% CHO, 15% protein (PRO), 25% fat, GI=61

Food items	**Wt (g)**	**CHO (g)**	**PRO(g)**	**FAT(g)**	**Kcal**
Turkey breast, skin removed	56.7 g	0	17.1	0.7	79
Yellow corn, cooked	65 g	12.5	1.7	0.4	53
Gravy, chicken or turkey, prepared from dry mix	50 g	2.7	0.4	0.5	17
Wheat rolls	65 g	30.9	7.1	2.4	173
Regular butter, unsalted	15 g	0	0.1	12.2	108
Ice cream and frozen desserts, regular, vanilla or other flavors	65 g	15.3	2.3	7.2	135
Chocolate syrup, nonfat	35 g	12.1	0.5	0.3	54
Soda pop, Sprite	231 g	23.4	0.1	0.05	92
White rice, cooked	100 g	28.2	2.7	0.3	130
Total‡		**125.2**	**32.0**	**24.0**	**840**
Percentage (%)		**59.1**	**15.4**	**25.3**	

‡ This menu template is calculated for a study participant weighing 64.5 kg. Total calories and portion sizes should be adjusted based on subject's body weight. This meal will contribute one-half of the subject's daily caloric need. Subjects' daily caloric need=25kcal/kg BW

Table A-3: Subjects' characteristics and energy balance in exercise before low-glycemic index (LGI-EBM) vs. high-glycemic index (HGI-EBM) morning meal

Groups	LGI-EBM (n=13)	HGI-EBM (n=6)
Age (years)	**59.7±0.93**	**58.7±1.36**
Weight (Kg)	**68.8±2.69**	**69.2±2.38**
Percentage of Body Fat (%)	**38.3±2.37**	**33.7±2.68**
BMI (Kg/m^2)	**24.9±0.79**	**24.3±0.66**
Fitness level (VO_2/min×Kg)	**23.8±1.81**	**30.7±4.91**
EI in meal 1 (Kcal)	**836.7±35.74**	**777.3±47.31**
EI in meal 2 (Kcal)	**839.7±38.85**	**848.8±31.21**
1st Exercise EE (Kcal)	**423.6±30.90**	**519.1±73.18**
CHO utilization (%) during 1st exercise	**39%±3.6%**	**37%±4.7%**
Fat utilization (%) during 1st exercise	**61%±3.6%**	**63%±4.7%**
2nd Exercise EE (Kcal)	**420.8±25.87**	**520.6±70.30**
CHO utilization (%) during 2snd exercise	**59%±2.4%**	**52%±4.9%**
Fat utilization (%) during 2nd exercise	**41%±2.4%**	**48%±4.9%**
EB: EI - exercise EE (Kcal)	**832.0±79.30**	**586.3±154.34**

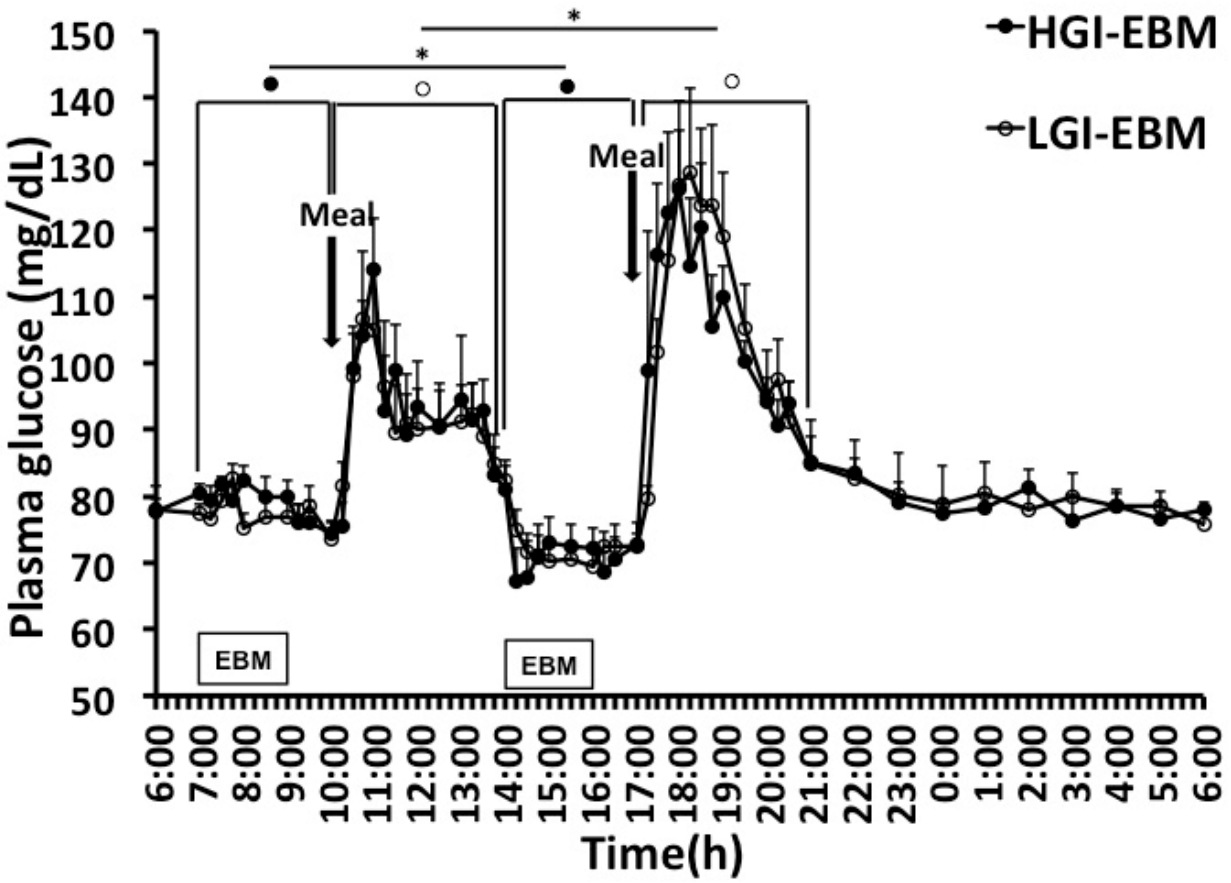
EB=energy balance; EE=energy expenditure; EI=energy intake

Table A-4: Subjects' characteristics and energy balance in exercise after low-glycemic index (LGI-EAM) vs. high-glycemic index (HGI-EAM) morning meal

Groups	LGI-EAM (n=13)	HGI-EAM (n=6)
Age (years)	**65.6±2.75**	**56.8±0.87**
Weight (Kg)	**63.7±2.10**	**66.8±2.78**
Percentage of Body Fat (%)	**35.5±2.47**	**35.5±2.67**
BMI (Kg/m^2)	**23.4±0.97**	**23.5±0.76**
Fitness level (VO_2/min×Kg)	**24.7±1.73**	**27.7±4.08**
EI in meal 1 (Kcal)	**784.9±36.15**	**756.5±42.03**
EI in meal 2 (Kcal)	**767.3±53.32**	**825.6±37.65**
1st Exercise EE (Kcal)	**501.0±48.58**	**486.5±57.97**
CHO utilization (%) during 1st exercise	**64%±3.7%**	**63%±5.8%**
Fat utilization (%) during 1st exercise	**36%±3.7%**	**37%±5.8%**
2nd Exercise EE (Kcal)	**471.6±40.84**	**491.2±58.86**
CHO utilization (%) during 2nd exercise	**68%±3.5%**	**67%±3.7%**
Fat utilization (%) during 2nd exercise	**32%±3.5%**	**33%±3.7%**
EB: EI - exercise EE (Kcal)	**462.2±103.29**	**604.4±119.46**

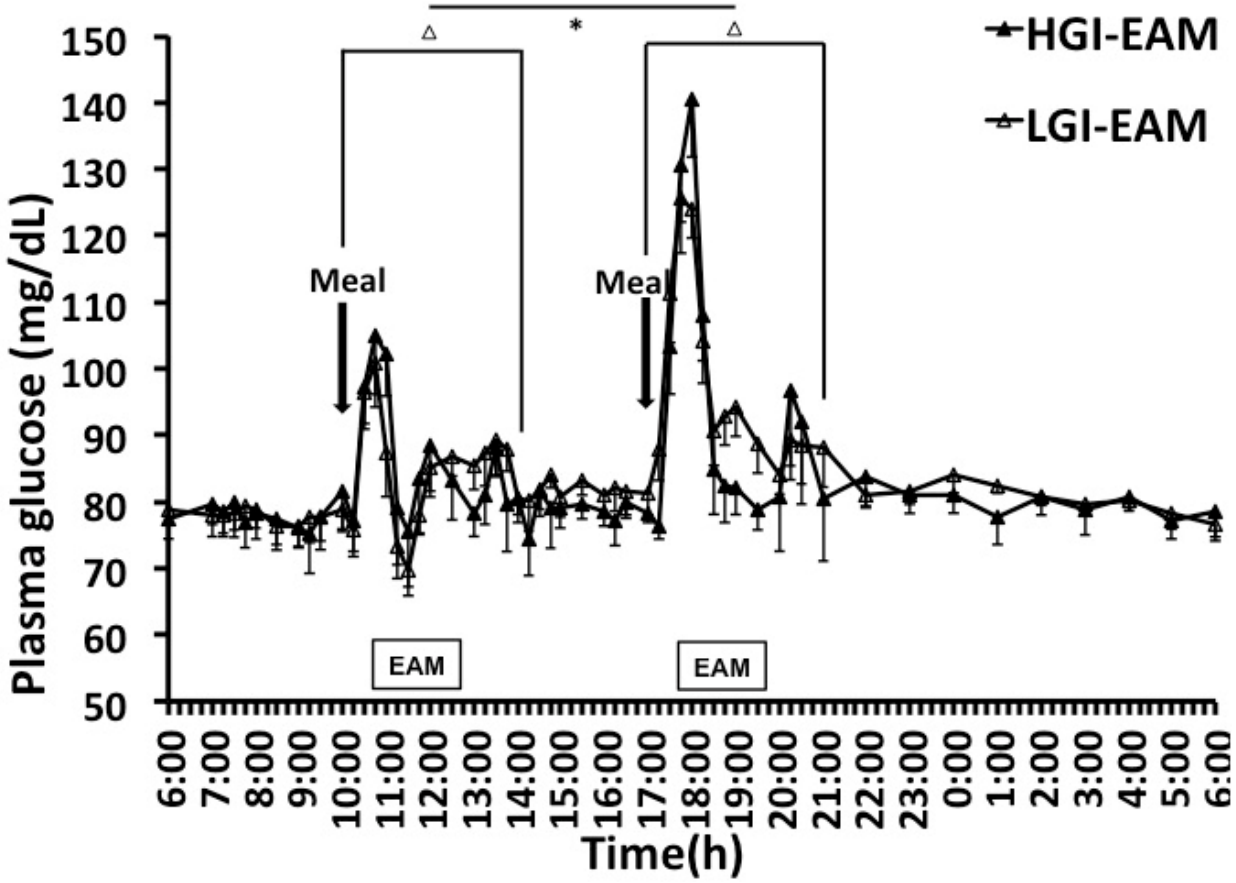
EB=energy balance; EE=energy expenditure; EI=energy intake

Figure A-1a: Plasma glucose responses in exercise before the low-glycemic index (LGI-EBM) vs. high-glycemic index (HGI-EBM) morning meal



"*" indicates the statistical significance between two selected time periods.

Figure A-1b: Plasma glucose responses in exercise after the low-glycemic index (LGI-EAM) vs. high-glycemic index (HGI-EAM) morning meal



"*" indicates the statistical significance between two selected time periods.

Figure A-2a: Plasma insulin responses in exercise before the low-glycemic index (LGI-EBM) vs. high-glycemic index (HGI-EBM) morning meal

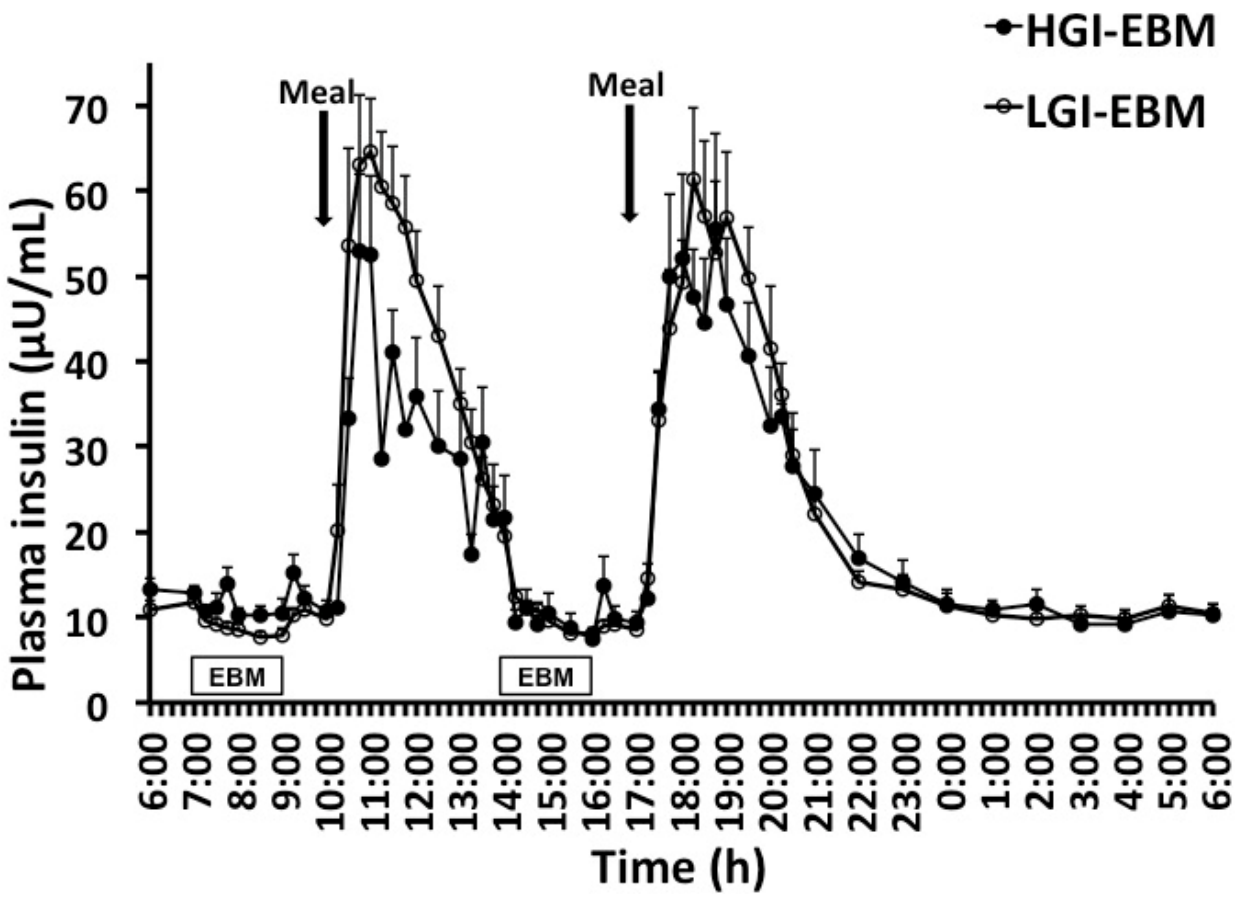


Figure A-2b: Plasma insulin responses in exercise after the low-glycemic index (LGI-EAM) vs. high-glycemic index (HGI-EAM) morning meal

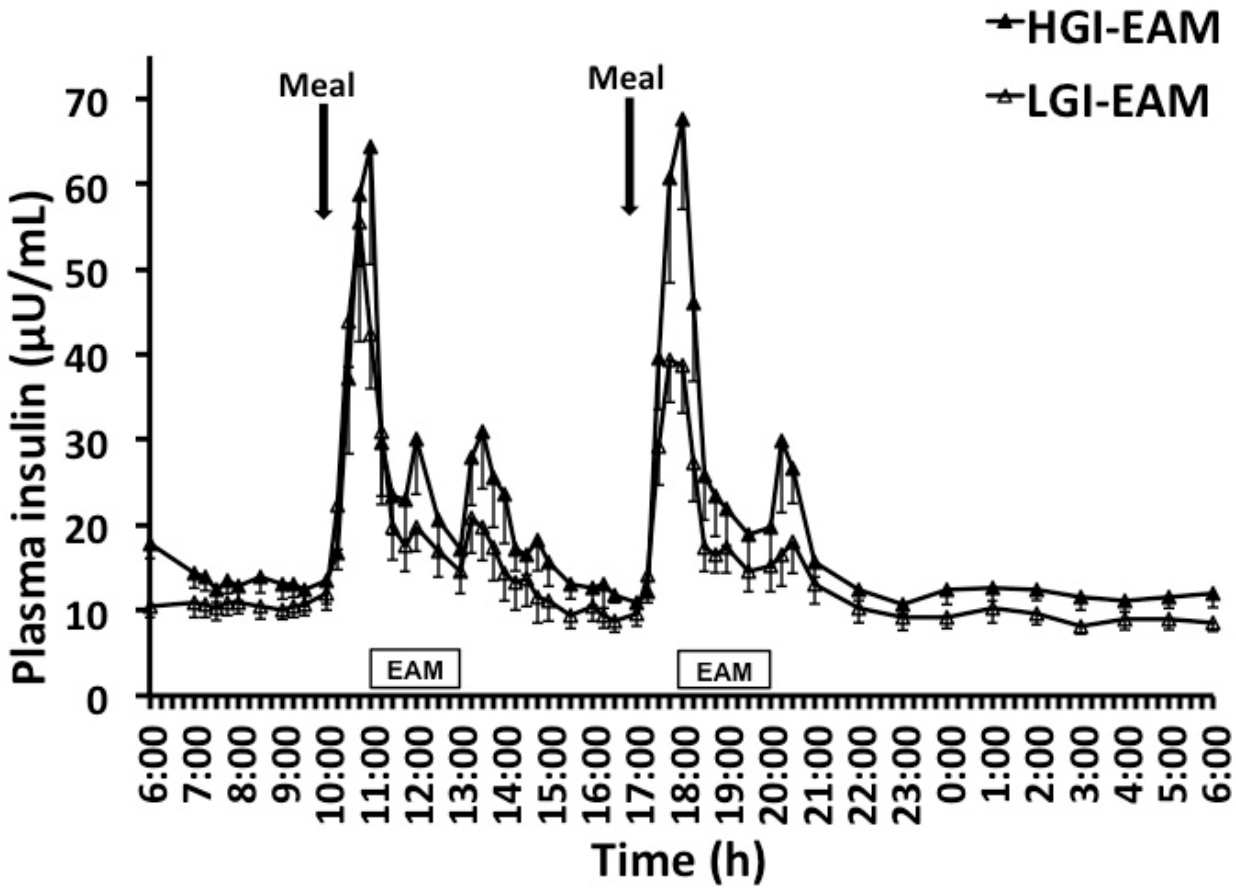
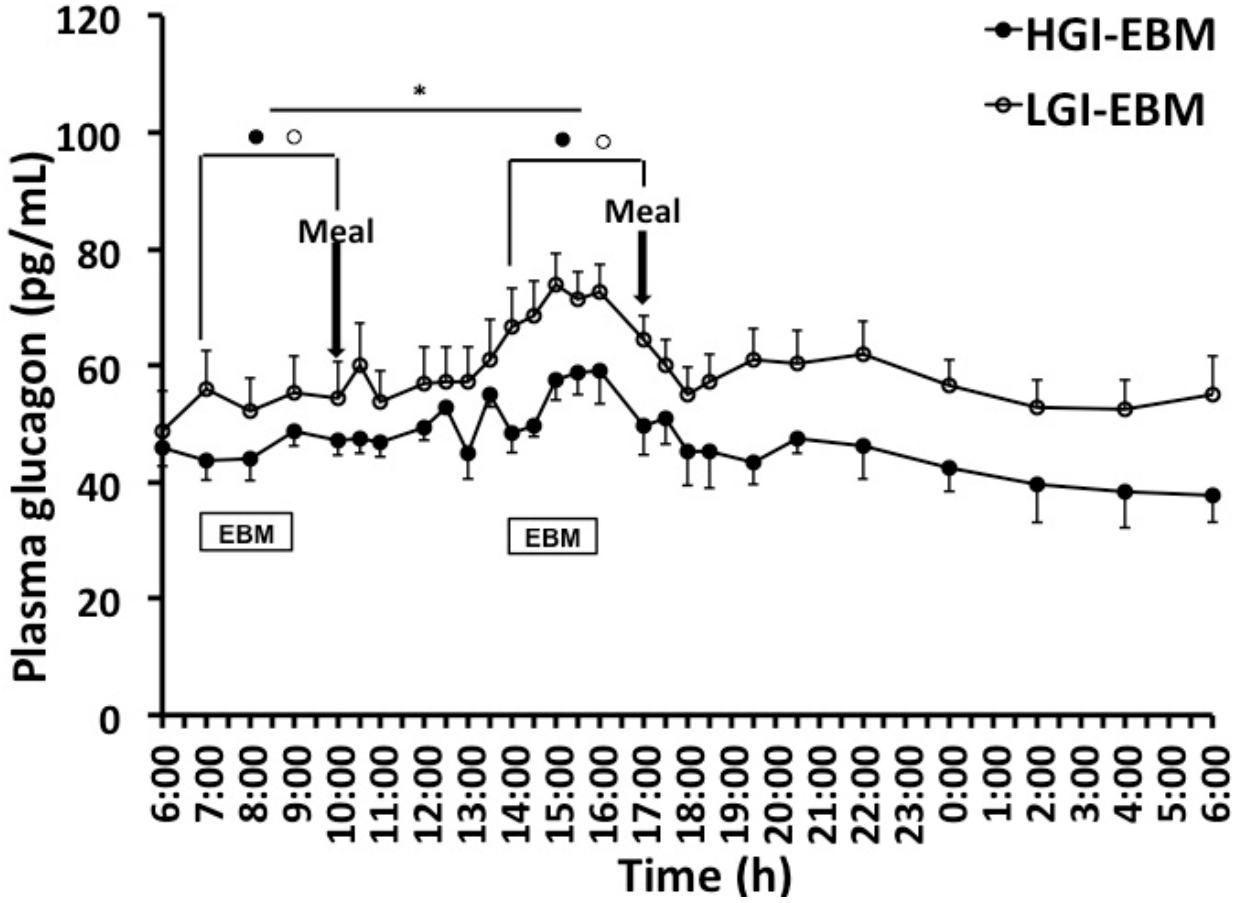
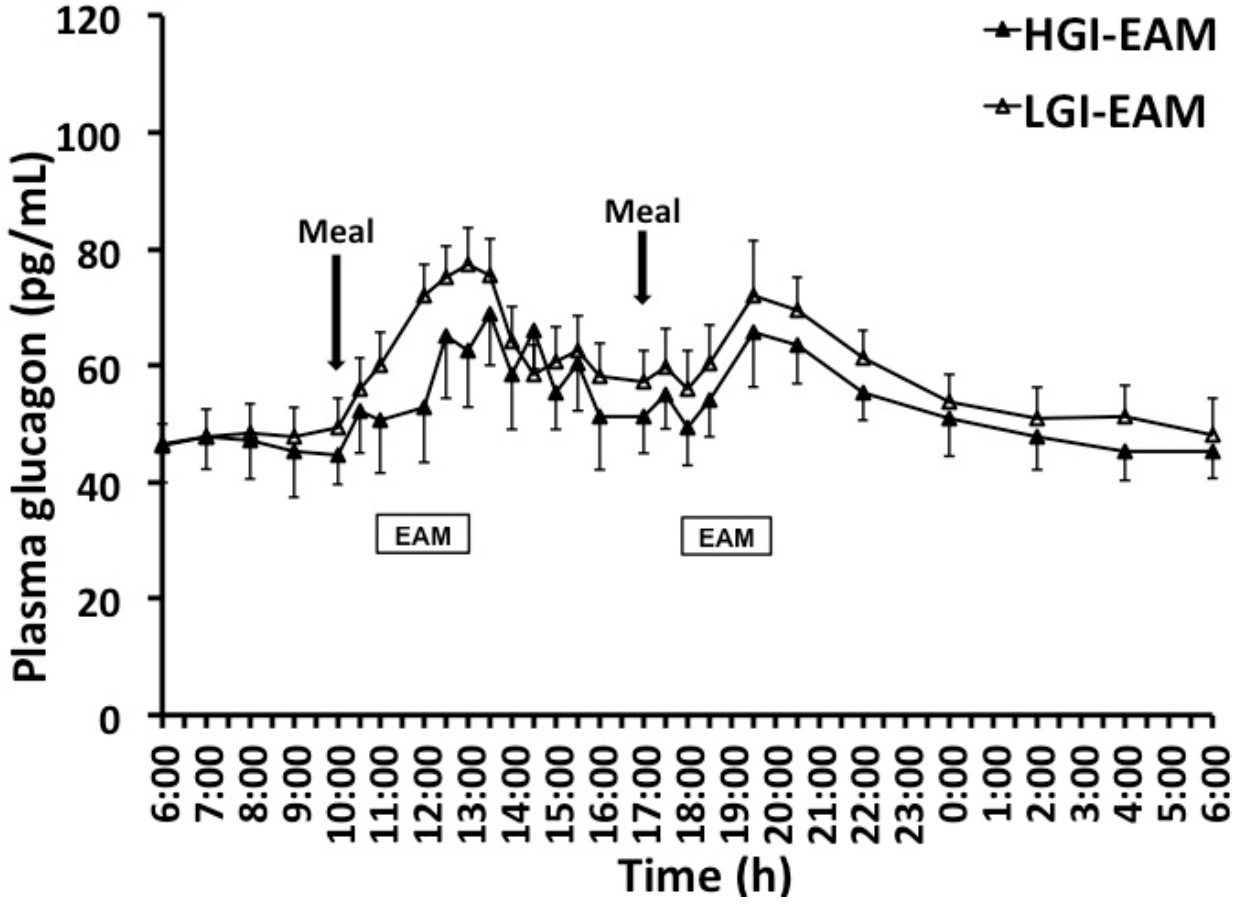


Figure A-3a: Plasma glucagon responses in exercise before the low-glycemic index (LGI-EBM) vs. high-glycemic index (HGI-EBM) morning meal



"*" indicates the statistical significance between two selected time periods.

Figure A-3b: Plasma glucagon responses in exercise after the low-glycemic index (LGI-EAM) vs. high-glycemic index (HGI-EAM) morning meal



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APPENDIX B

Additional Analyses for Study 2 (Chapter 4):

Effects of exercise duration on glycemic and insulinemic responses to low-carbohydrate meals

One of the main findings of Study 2 was a 39% reduction in the afternoon postprandial (PP) insulin response to low-carbohydrate (30% CHO) meals independently of a 2-hour pre-meal exercise. An important finding of Study 1 was a sustained reduction in plasma glucose when exercise was performed during late PP following a high-CHO meal consumed 4 hours before. Glucose lowering effects have been known to be positively related to the magnitude of energy expenditure during exercise [1-3]. To determine whether either of the above effects in studies 1 and 2 were related to the magnitude of exercise energy expenditure, this supplemental study tested the hypotheses that (1) reducing the duration of moderate-intensity exercise from 2 to 1 hour would attenuate the afternoon PP insulin decline by half and (2) blunt the glucose-lowering effect of late PP exercise with low-CHO meals. The expectations were that in trials with low-CHO meals (1) reducing the duration of exercise by half will attenuate afternoon PP insulin decline by half and (2) similarly attenuate he glucose-lowering effect of late PP exercise by half.

Methods

Twenty-four subjects mean age of 57.2±0.89 years; body mass index (BMI) of 25.4±0.55 kg/m^2 met the study criteria and were matched by body weight and BMI to one of three 36-hour-long study trials: sedentary (X0) trial, 1-hour-exercise-before-meals (X1) trial, and 2 hour-exercise-before-meals (X2) trial, 8 each. Two isocaloric low-carbohydrate (CHO) meals (Tables 4-3 and 4-4) were provided at fixed time, 1000 h and 1700 h. Two exercise bouts were performed during 0700- 0800 h and 1400-1500 h in X1 group and during 0700-0900 h and 1400-1600 h in X2 group. Same hormone and metabolite measurements and statistical analyses were used as in Study 2.

Results

Subjects' characteristics are summarized in Table B-1. The groups did not differ in age, weight, percent body fat, BMI, or fitness level. There was a trend for both X1 (t=2.3, p=.0275) and X2 (t=2.09, p=.0487) groups to consume more calories during the second meal than the sedentary X0 group. There also was a trend for the total energy intake (t=2.24, p=.0363) and energy intake per kg of body weight (t=2.39, p=.0264) to be higher in X1 relative to X0 group but these differences were lost after Bonferroni correction. Energy expenditure during the two hours of exercise in X2 was significantly higher than during 1-hour of exercise in X1 group (F=13.81, p=.0023), which made energy balance in X2 group 50.2% and 46.3% lower, respectively, relative to X0 (t=3.47, p=.0023) and X1 (t=3.12, p=.0052) groups, in part also because exercise energy expenditure was not replaced with extra food.

Metabolism and fuel utilization (Table B-1)

The X2 group also utilized more carbohydrate (F=9.04, p=.0094) and less fat (F=8.49, p=.0113) during exercise than the X1 group. The resting metabolic rate on the discharging day was significantly higher it was measured on the study day in the X2 group (t=2.44, p=.0238), but no such difference was seen in X0 or X1 group. However, the fuel utilization during morning resting period in X1 group was different from the study day to the discharging day that less carbohydrate and more fat were utilized during the morning resting period on the discharging day (t=3.12, p=.0052), compared to the same time period on the study day.

Plasma glucose (Figure B-1)

Glucose AUC was significantly lower when 2-hour exercise (X2) was performed during the late PP, 4 h after the first meal than when it was performed in fasted state (t=-2.96, p=.0075). This effect was not seen when the exercise bout was only 1-hour long.

Plasma insulin (Figure B-2)

Both exercising X1 and X2 groups and the sedentary X0 group had significantly lower insulin responses during the second than the first PP (X0: t=-5.91, p<.0001; X2: t=-3.25, p=.0038; X2: t=-4.31, p=.0003). There only was a trend for the insulin AUC to be higher in X0 than in X1 group during the first exercise period (t=-2.27, p=.0341).

Plasma glucagon (Figure B-3)

Plasma glucagon AUCs in both X1 and X2 exercising groups were significantly higher when exercise took place during the late PP, than during the fasting period (X1: t=3.52, p=.002, X2: t=3.96, p=.0007).

Plasma FFAs (Figure B-4)

Exercise of both durations significantly and similarly increased FFA concentration both during the first (X1>X0: t=2.12, p=.0463; X2>X0: t=2.24, p=.0359) and the second (X1>X0: t=3.49, p=.0022; X2>X0: t=4.62, p=.0001) exercise bouts relative to the sedentary trial. FFA concentration during total 24-hour period was significantly and similarly greater in the two exercising groups than during the sedentary trial (X1>X0: t=2.91, p=.0083; X2>X0: t=3.98, p=.0007). Higher FFA AUCs in the exercising trials also extended to the PPs. The FFA AUCs were significantly higher during both PPs in the 2-hour exercising group (X2) than during the sedentary X0 trial (PP1: t=3.18, p=.0045; PP2: t=3.82, p=.001) while this difference reached significance in the 1-hour X1 trial only during the second PP (t=2.59, p=.0172).

Plasma D-3-hydroxybutyrate (Figure B-5)

Ketone body AUCs were significantly higher in the 2-hour X2 than during 1-hour X1 or sedentary X0 trials during both PPs (PP1: t=3.68, p=.0014; PP2: t=3.55, p=.0019) and there was a trend for the same effect during exercise periods (t=2.74, p=.0122 and t=2.11, p=.0466, respectively). In addition, increased ketone body in X2 group was significantly greater when exercise was performed during late PP than during the morning fasting exercise (t=2.5, p=.0207) and also during the second PP than the first one (t=2.64, p=.0152).

Discussion

Findings from this supplemental study confirmed the findings of Study 2 that low-CHO diet containing only 30 % of CHO lowers afternoon PP plasma insulin concentration to a similar extent in sedentary (X0) as well as during exercise ranging in duration between 1 (X1) and 2 (X2) hours. While duration of exercise did not affect the afternoon PP insulin lowering effect, only 2 hours of exercise was effective in lowering glycemia during late-PP exercise below that of the sedentary control. The findings from present study did not support by previous studies as

Hostmark et al. [4] and Nygaard et al. [5] who reported that glucose lowering effects were dose dependent on exercise duration. In these studies, a single event light- to moderate-intensity walking or cycling immediately after a high-glycemic breakfast decreased the glycemic response to a meal in healthy women in a dose-respondent manner, suggesting that this blood glucose lowering effect was mediated by the magnitude of energy expenditure during exercise. In another study single-event study a dose-response relationship between glucose lowering and duration of exercise was reported [6]. Forty-five minutes of moderate-intensity exercise improved insulin action better than 30 minutes of the same-intensity exercise in individuals with metabolic syndrome [6]. It is probable that lowering rather than increasing the duration of exercise was not the ideal test of the effect of exercise duration on glycemia. In additio, the small number of subjects in each group could have provided another limitation to statistical power needed to reach significance. In conclusion, low-CHO meals containing about 61 g or 30% CHO lowered afternoon PP insulin response to a same extent with exercise bouts lasting 1 or 2 hours. However, longer, 2-hour long moderate-intensity exercise was necessary to lower blood glucose during late-PP exercise suggesting that hypoglycemia in that prandial state may be responsive to the magnitude of energy expended during exercise.

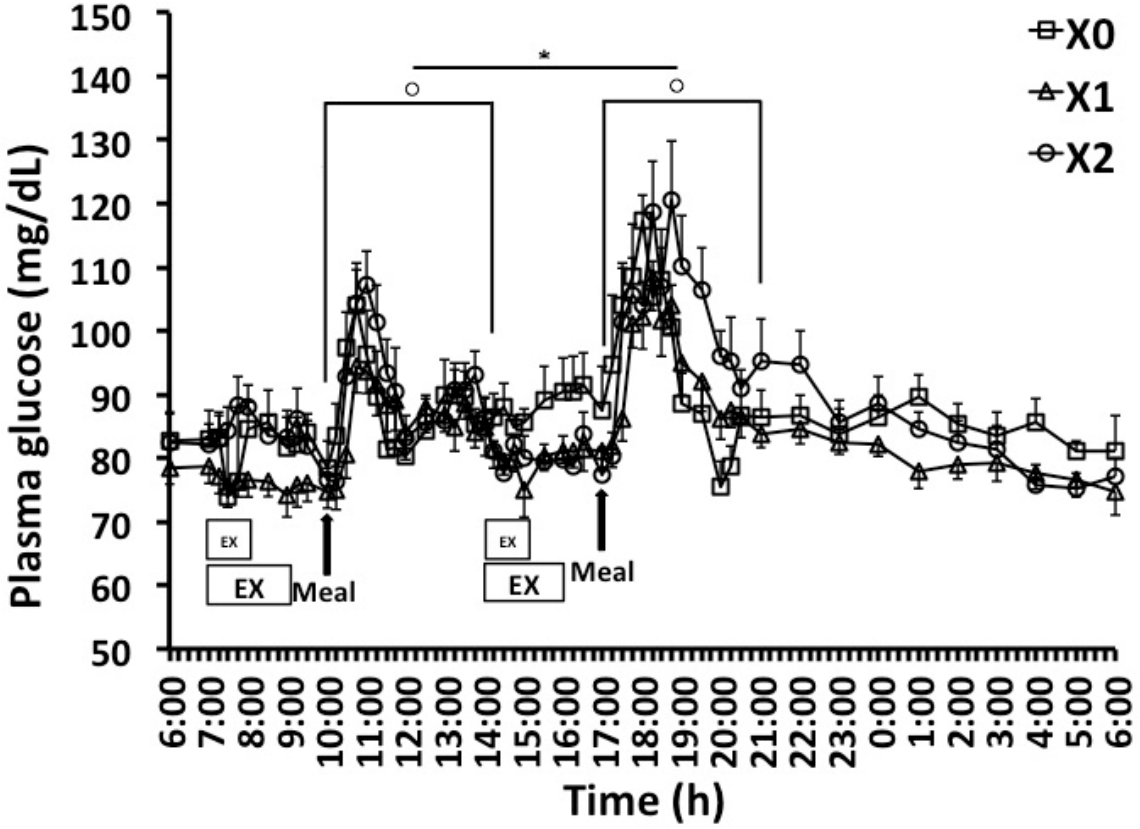
Table B-1: Subjects' characteristics in (X0) no exercise, (X1) one hour of exercise, and (X2) two hours of exercise trials

Groups	X0 (n=8)	X1 (n=8)	X2 (n=8)
Age (years)	56.9±1.54	55.4±1.48	59.3±1.46
Weight (Kg)	69.9±3.41	70.3±2.97	71.8±2.76
Percentage of Body Fat (%)	38.0±1.65	38.9±2.25	39.0±3.30
BMI (Kg/m^2)	25.4±0.75	25.2±1.01	25.7±1.16
Fitness level ($VO_2/min \times Kg$)	24.7±2.49	20.7±1.39	26.2±3.60
EI in meal 1 (Kcal)	751.8±59.69	913.8±55.08	782.1±58.24
EI in meal 2 (Kcal)	648.6±112.98	902.8±55.25	873.2±38.00
1^{st} Exercise EE (Kcal)	NA	239.5±22.16	485.2±63.28
Carbohydrate utilization during 1^{st} exercise	NA	54%±6.2%[a]	41%±3.8%[b]
Fat utilization during 1^{st} exercise	NA	46%±6.2%[a]	59%±3.8%[b]
2^{nd} Exercise EE (Kcal)	NA	247.0±22.48	472.5±55.81
Carbohydrate utilization during 2^{nd} exercise	NA	58%±1.9%[a]	42%±2.1%[b]
Fat utilization during 2^{nd} exercise	NA	42%±1.9%[a]	58%±2.1%[b]
EB: EI - exercise EE (Kcal)	1400.4±167.50	1330.1±113.42	697.6±143.91

EI: energy intake; EE: energy expenditure; EB: energy balance

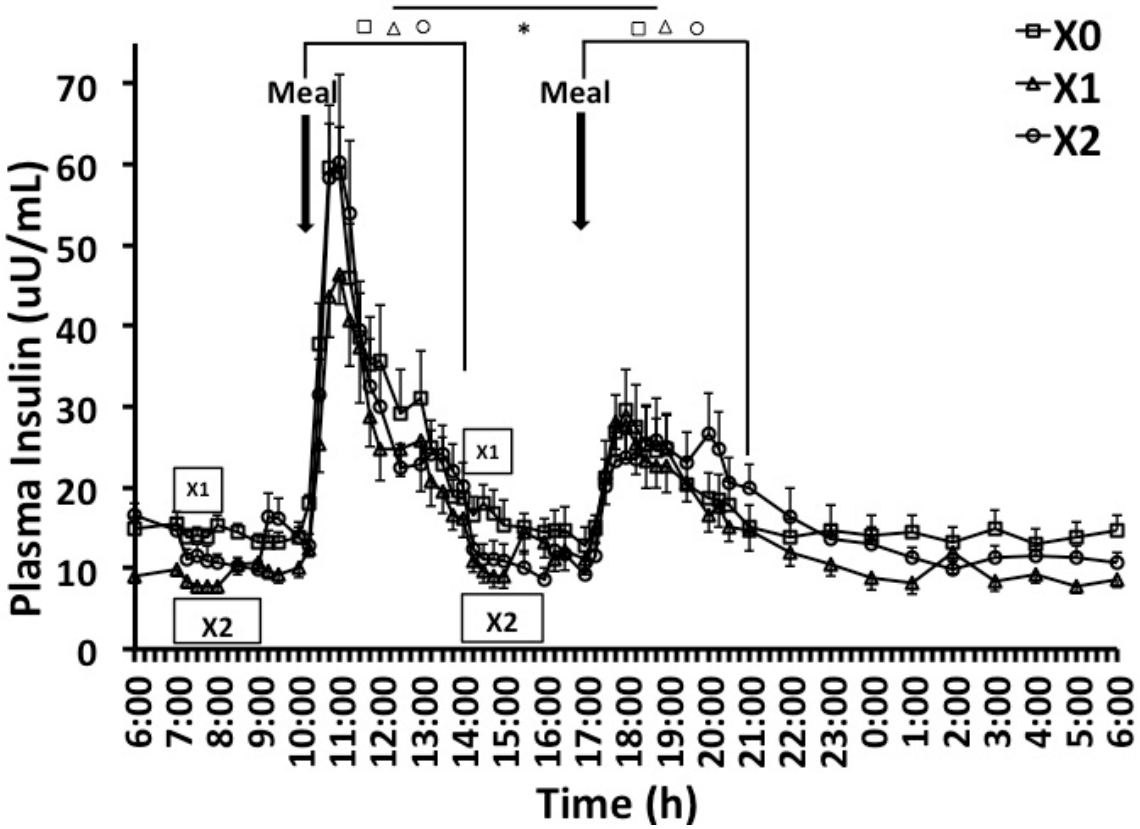
[a,b] Within the same variable, groups with different superscripts are significantly different ($p<.05$)

Figure B-1: Plasma glucose responses in in (X0) no exercise, (X1) one hour of exercise, and (X2) two hours of exercise trials



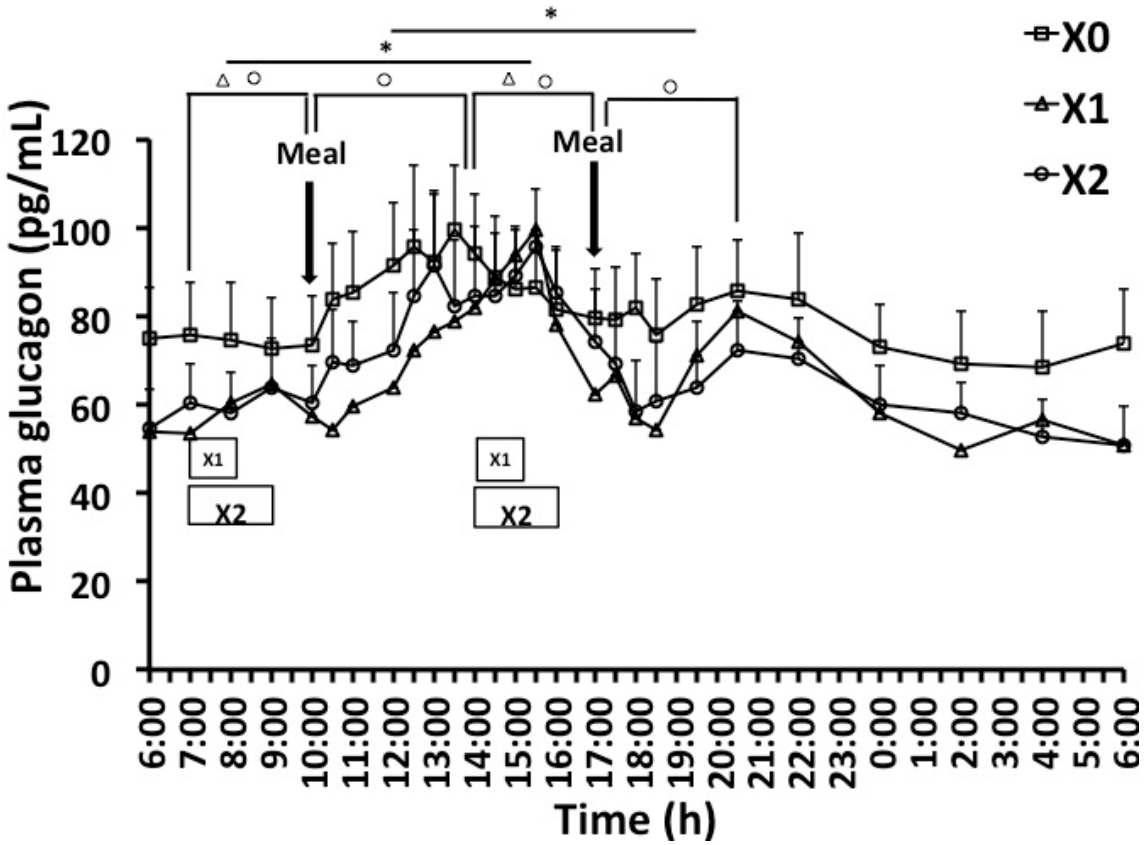
"*" indicates the statistical significance between two selected time periods.

Figure B-2: Plasma insulin responses in (X0) no exercise, (X1) one hour of exercise, and (X2) two hours of exercise trials



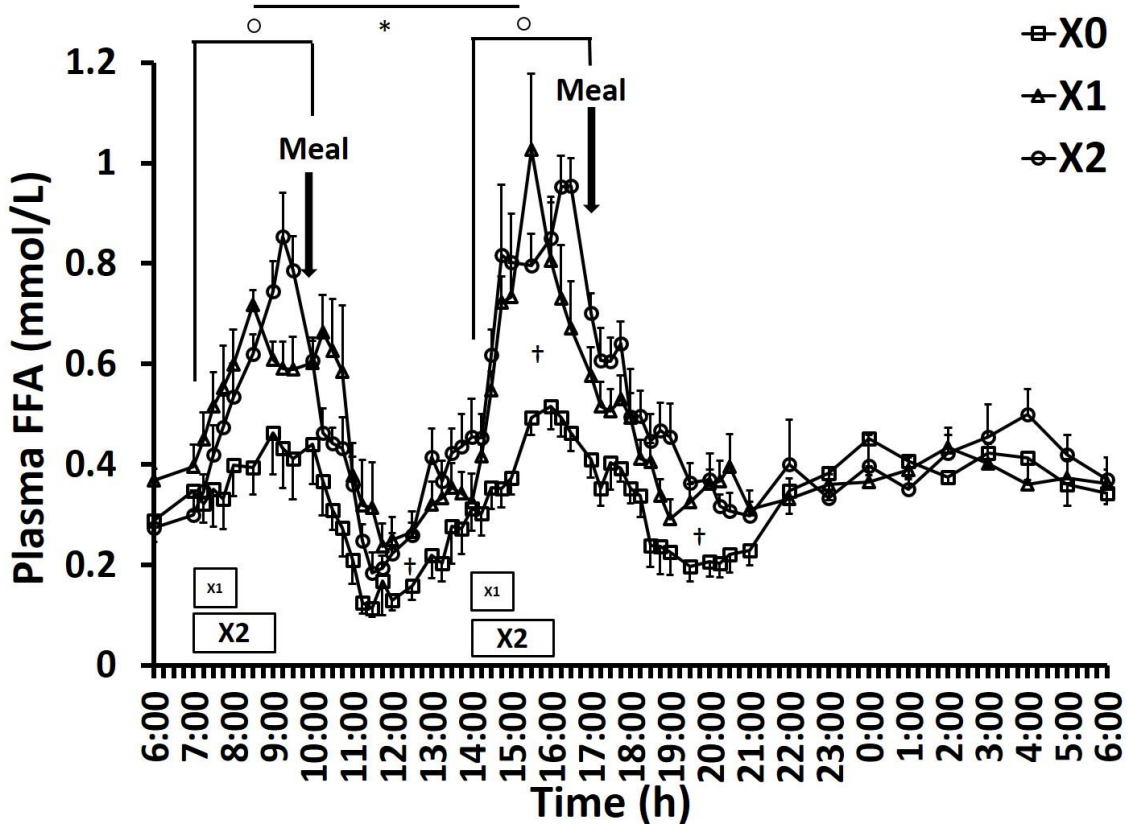
"*" indicates the statistical significance between two selected time periods.

Figure B-3: Plasma glucagon responses in (X0) no exercise, (X1) one hour of exercise, and (X2) two hours of exercise trials



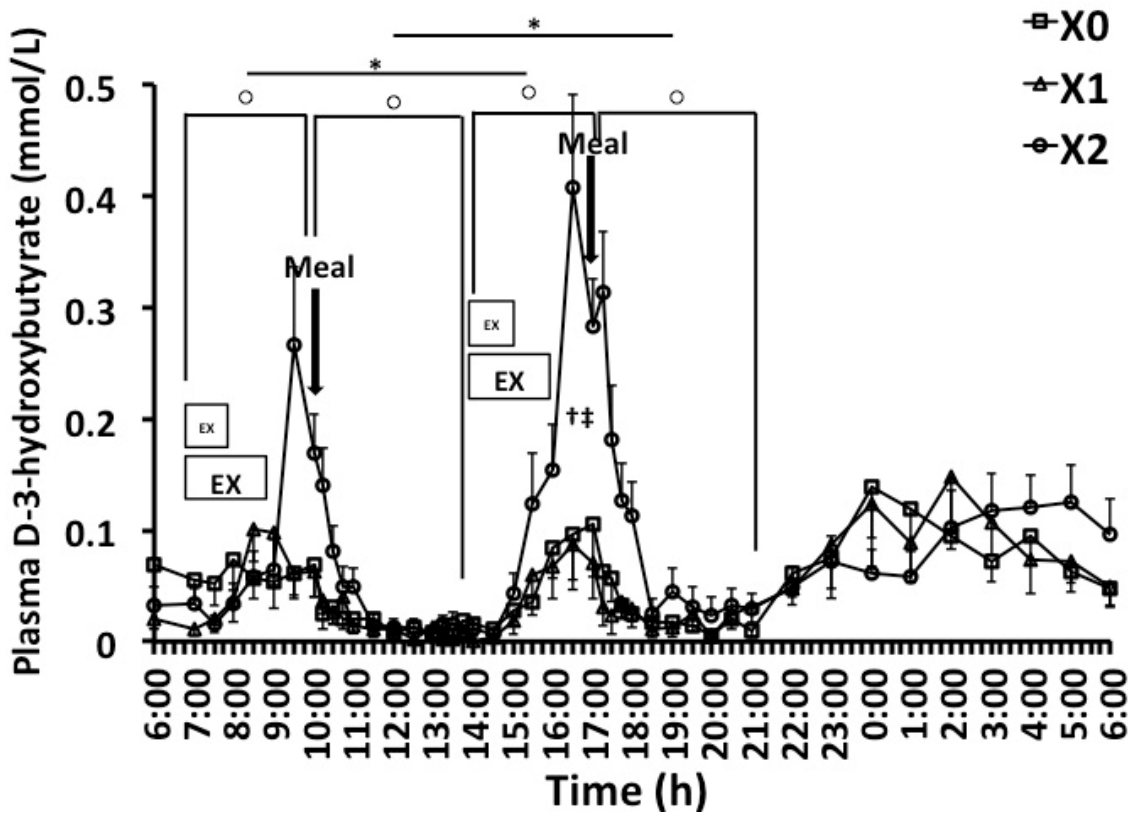
"*" indicates the statistical significance between two selected time periods.

Figure B-4: Plasma free fatty acids (FFAs) responses in (X0) no exercise, (X1) one hour of exercise, and (X2) two hours of exercise trials



"*" indicates the statistical significance between two selected time periods.
"†" indicates the statistical significance when compared to no exercise X0 group during the specified area under the curve.

Figure B-5: Plasma D-3-hydroxybutyrate ketone body responses in (X0) no exercise, (X1) one hour of exercise, and (X2) two hours of exercise trials



"*" indicates the statistical significance between two selected time periods.

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