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THE EFFECT OF POST-EXERCISE MEAL COMPOSITION ON INSULIN ACTION

A Thesis Presented

by

KAILA A. HOLTZ

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

September 2007

Department of Kinesiology

THE EFFECT OF POST-EXERCISE MEAL COMPOSITION ON INSULIN ACTION

A Thesis Presented

By

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ABSTRACT

THE EFFECT OF POST-EXERCISE MEAL COMPOSITION ON INSULIN ACTION

SEPTEMBER 2007

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INTRODUCTION: Exercise increases insulin stimulated glucose uptake (insulin action) if expended energy (kcal) is withheld following exercise, but the effect is blunted when expended energy is replaced as carbohydrate. Restricting carbohydrate and replacing expended energy as fat maintains increased insulin action in rodents; however, this effect has not been evaluated in humans. In humans, restricting carbohydrate intake following exercise may be a useful strategy to maximize the effect of individual exercise bouts on insulin action and promote gains in metabolic health over time. Therefore, the purpose of this study was to determine if carbohydrate restriction following exercise (carbohydrate deficit) increased insulin action in sedentary, overweight adults as hypothesized. **METHODS:** Ten healthy, sedentary, men and women, aged 21±2 years, body fat 37.3±3.1%, and VO_{2peak} 34.6±1.2ml×kg^{-1}×min^{-1} completed three, two-day experimental conditions in random order: 1) a no-exercise baseline condition (BASE), 2) exercise followed by a high-carbohydrate meal (HIGH-CHO= 76.3±2.5% CHO), and 3) exercise followed by a low-carbohydrate meal (LOW-CHO=17.8±0.1% CHO). On DAY 1, subjects came to the laboratory (early evening) and expended 30% of total daily energy expenditure on a cycle ergometer at

70% of VO_{2peak}. Following exercise, an isocaloric meal (HIGH-CHO or LOW-CHO) was consumed to refeed the expended energy during exercise and venous blood samples were taken to record the insulin and glucose responses to the meals. Twelve hours later (Day 2), whole-body insulin action (steady-state glucose uptake per unit insulin) was measured using a continuous infusion of glucose with stable isotope tracers. A paired t-test was used to detect differences between exercise bouts and the glucose and insulin responses to the post-exercise meals. A one-way repeated measures ANOVA was performed to evaluate the effect of experimental condition on insulin action ($p<0.05$, for all tests). **RESULTS:** Intensity (VO_{2peak}), duration (minutes) and energy expenditure (kcal) were similar between exercise bouts. After exercise, plasma glucose and insulin concentrations were significantly higher following the HIGH-CHO meal compared to the LOW-CHO meal ($p<0.001$, respectively). The next morning, insulin action was similar between experimental conditions ($p=0.30$). Non-oxidative glucose disposal was increased during the glucose infusion in Low-CHO compared to BASE (27.2±3.2 vs. 16.9±3.5$\mu M \times kg^{-1} \times min^{-1}$, $p<0.05$). Carbohydrate oxidation was reduced in Low-CHO (8.6±1.3$\mu M \times kg^{-1} \times min^{-1}$) compared to High-CHO (12.2±1.2$\mu M \times kg^{-1} \times min^{-1}$), and to BASE (17.1 ± 2.2 $\mu M \times kg^{-1} \times min^{-1}$), $p<0.05$ respectively. Resting fat oxidation was increased in Low-CHO compared to BASE (109.8 ± 10.5 $mg \times min^{-1}$ vs. 80.7 ± 9.6 $mg \times min^{-1}$, $p<0.05$) and remained elevated during the glucose infusion.

CONCLUSION: Limiting carbohydrate, but not energy intake after exercise (carbohydrate deficit) resulted in increased non-oxidative glucose disposal, decreased carbohydrate oxidation and increased fat oxidation during the glucose infusion, compared to baseline, indicating a favorable shift in energy metabolism. Creating a

carbohydrate deficit, by withholding expended carbohydrate but not energy following exercise may be a sensible strategy to promote favorable gains in insulin action that requires further evaluation.

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CHAPTER 1

DEVELOPMENT OF THE PROBLEM

1.1 Introduction

Type 2 diabetes is preceded by insulin resistance, a condition that is characterized by decreased insulin action (37). Insulin action is defined by the effectivity of insulin to stimulate peripheral glucose uptake from the blood, decrease whole body lipolysis of adipose tissue, and suppress hepatic glucose output. Regular physical activity increases insulin action and slows the progression from insulin resistance to type 2 diabetes (41).

An acute bout of exercise increases insulin action (3, 7, 18, 51, 57). Creating an energy deficit through caloric restriction also increases insulin action (2). Following exercise, the nutritional environment created by energy intake, or lack of energy intake, affects the degree insulin action is increased. Combining exercise with an energy deficit (not replacing expended energy after exercise) significantly increases insulin action. This effect is not observed when energy is re-fed as dietary carbohydrate to energy balance (energy expenditure = energy balance) (5, 54).

When dietary carbohydrate is restricted after exercise in rodents, insulin action remains elevated until carbohydrate is re-fed (11, 21). The mechanism relating carbohydrate restriction to increased insulin action following exercise is poorly understood but may be a function of maintaining low glycogen concentrations by inadequate carbohydrate intake (12). Low muscle glycogen regulates enzymatic activity

that promotes glycogen storage (16, 35, 39) which is associated with increased insulin action (50).

Carbohydrate restriction in rodents can be accomplished in two ways. Either expended energy is withheld following exercise (creating both energy and carbohydrate deficits), or energy is re-fed without carbohydrate (creating a carbohydrate deficit only). It has been well established that restricting carbohydrate and refeeding expended energy as fat maintains increased insulin action in rodents (11, 21). However, this effect has not been systematically evaluated in humans. Maximizing the effectiveness of every exercise bout could result in the greatest long-term gains in metabolic health.

Summary: An energy deficit after exercise clearly increases insulin action. It remains unclear if refeeding expended energy, but not carbohydrate to a carbohydrate deficit, affects insulin action compared to the match of carbohydrate expenditure with intake (carbohydrate balance). Therefore, the purpose of this study is to evaluate the effect of carbohydrate availability following exercise on insulin action.

1.2 Statement of the Problem

Energy intake following acute exercise affects insulin action in ways that are poorly understood. Regular exercise is a viable way for sedentary, overweight individuals with impaired insulin action to improve their metabolic health though the nutritional environment following exercise may moderate its effect. Fasting after exercise increases insulin action, yet individuals most often eat soon after an exercise session. It has been demonstrated that re-feeding expended energy after exercise as

dietary carbohydrate eliminates exercise-induced increases in insulin action (5, 7, 58). It is unclear whether energy or carbohydrate moderates enhanced insulin action reversal following a post-exercise meal high in carbohydrate. Evidence for restricting carbohydrate and replacing expended energy with fat (to maximize the effectiveness of exercise on insulin action) appears strong in animal models (11, 21), but conclusions from animal models are limited and need to be evaluated systematically before being applied to humans.

1.3 Purpose and Hypotheses

The purpose of this study is to evaluate how varying carbohydrate availability after exercise affects insulin action in sedentary, overweight adults. A strong relationship between post-exercise carbohydrate availability and insulin action is expected. Increases in insulin action will be largest when post-exercise carbohydrate availability is low and smallest following high post-exercise carbohydrate availability.

It is hypothesized: 1) high carbohydrate availability following an acute bout of exercise will nullify the sensitizing effect of exercise on insulin action and return insulin action toward baseline, and 2) low carbohydrate availability following exercise will significantly increase insulin action compared to both baseline, and a condition of high-carbohydrate availability.

1.4 Significance of the Study

Athletes increase carbohydrate intake immediately after exercise in order to maximize glycogen re-synthesis and optimize performance (29). However, low

glycogen concentrations are associated with increased insulin action (17, 50); individuals with impaired insulin action might benefit from minimizing post-exercise carbohydrate intake. In addition, low carbohydrate availability maintains increased activity of oxidative enzymes compared to high carbohydrate availability (48). Maintaining the activity of oxidative enzymes may be important for the accrual of exercise benefits on metabolic health. Identifying ways to combine exercise and diet to maximize increases in insulin action will benefit individuals at risk for type 2 diabetes.

1.5 Limitation

Conclusions from this study will be limited to the effect of post-exercise carbohydrate availability on insulin action at the whole body level. While experimental conditions may yield muscle and liver glycogen concentrations that vary, it is impossible to relate changes in muscle glycogen concentrations and cellular regulators to changes in insulin action without muscle biopsies. Insight regarding the molecular mechanisms underlying the regulation of insulin action by carbohydrate availability on insulin action will be speculative; however, it is expected that results from this study will provide new information regarding the mediation of whole body insulin action by carbohydrate availability following exercise.

1.6 Definition of Terms

Carbohydrate availability: the amount (g) of digestible carbohydrate in a meal.

Carbohydrate deficit: the difference between carbohydrate expended during exercise (g) and carbohydrate consumed after exercise (g).

Carbohydrate balance: carbohydrate expenditure during exercise matched with carbohydrate intake after exercise.

Energy balance: a period when energy intake (kcal) equals energy expenditure (kcal).

Energy deficit: a period when energy expenditure (kcal) exceeds energy intake (kcal).

Insulin action: the effectivity of insulin to stimulate peripheral glucose uptake in insulin responsive tissues (skeletal muscle, the liver and adipose tissue).

Insulin resistance: a condition of reduced glucose uptake in response to physiological insulin that precedes the development of type 2 diabetes.

Metabolic health: the combined markers (insulin action, fat oxidation and plasma lipids) measured in this study.

CHAPTER 2

UPDATED LITERATURE REVIEW

2.1 Introduction

Approximately 8% of the adult population in the United States is diagnosed with type 2 diabetes (27, 45). Increasing incidence of type 2 diabetes parallels increasing incidence rates of obesity (45)since impaired insulin action and obesity share a common origin (49): a family history of type 2 diabetes (25) combined with a sedentary lifestyle (42).

The Diabetes Prevention Program (DPP) demonstrated that lifestyle interventions increase physical activity and decrease energy intake and significantly reduce the risk of type 2 diabetes (41). The results of the DPP and others (55) suggest that exercise be viewed as a "drug" for metabolic health. As defined in the Food and Drugs Act, a drug includes "any substances for use in the diagnosis, cure, mitigation, treatment, or prevention of disease intended to affect the structure or any function of the body". Exercise fits this description of a drug because it affects cellular structure and function, and has been associated with the prevention or treatment of cardiovascular disease (19), osteoporosis (15), mental dysfunction (32), and type 2 diabetes (41, 55).

Like pharmacological drugs, exercise interacts with the nutritional environment proximate to its application. Exercise increases insulin action, but the nutritional environment following an exercise session may influence the magnitude of this effect. There is evidence to suggest that any benefit of exercise on insulin action in sedentary, overweight individuals with impaired insulin action may be reversed by carbohydrate

consumed after exercise completion (5, 7). Thus, research to find sensible exercise and diet prescriptions for a sedentary, overweight population is necessary to maximize the positive effect of each exercise dose and minimize the negative interactions between exercise and certain nutritional prescriptions.

Maximizing the effect of a single exercise dose is important for two reasons. First, habitually sedentary individuals may exercise sporadically as they contemplate beginning an exercise program; sensible nutritional prescriptions are necessary to ensure benefits are not reversed by nutritional choices. Second, exercise results are most favorable when repeated over time through applications of individuals exercise sessions; if each exercise dose is maximized (by nutritional prescriptions) the cumulative effect of exercise on metabolic health may be greatest.

2.2 Current Exercise and Diet Prescriptions

A growing population of sedentary, overweight/obese individuals with poor metabolic health has prompted several national health bodies (ACSM, IOM, CDC) to announce exercise prescriptions for weight loss and health improvement. The duration and intensity of exercise required to elicit weight loss and/or increase metabolic health remains controversial; however, it is agreed that exercise (regardless of duration) is better than no exercise at all (6). While more sedentary overweight Americans may be starting exercise programs, these exercise prescriptions do not have a specific diet component as recommendations for athletes do.

2.2.1 Athletes

Research in the late 1970's and early 1980's first demonstrated the importance of carbohydrate intake during and following endurance exercise to maximize performance (9, 12, 34). Consuming high-glycemic dietary carbohydrate following exercise quickly increases blood glucose and insulin concentrations and replenishes muscle glycogen (9). Athletes do this because high levels of muscle glycogen are associated with increased exercise performance (29). Research in the area of post-exercise nutrition has largely focused on generating recommendations to promote glycogen re-synthesis in athletes, agreed upon to be 5-7g/kg body weight/day during moderate training (36).

2.2.2 Sedentary, Overweight Individuals

Sedentary, overweight individuals have different metabolic profiles than physically active, lean individuals (athletes). Two markers of poor metabolic health are: impaired insulin action (23) and decreased fat oxidation (40), both of which can be improved with exercise (8, 14). In contrast to athletes, the post-exercise dietary recommendations for overweight, sedentary individuals are not nutrient specific. Generally, the 2005 Dietary Guidelines for Americans state: "Control of caloric intake is advisable (for overweight/obese individuals) ... The higher a person's physical activity level, the higher his or her energy requirement." Given the rising levels of obesity in the United States, current recommendations understandably promote exercise and diet as a means to regulate body weight. However, the benefits of exercise on metabolic health are apparent before changes in body weight occur (1, 5). Moreover, increased metabolic

health is observed regardless of body composition when cardiorespiratory fitness is matched (22) (overweight individuals are as “metabolically healthy” as lean individuals when they are aerobically fit). Maximizing the effect of exercise on metabolic health with nutritional recommendations following exercise may be as important, if not more so, than promoting weight loss.

Summary: Current exercise and diet prescriptions for the sedentary, overweight population are general and do not include specific strategies to maximize the effect of each exercise session on metabolic health. This population may not benefit from following the same exercise and diet prescription given to athletes – because their metabolic profile is different and their goals should be to improve metabolic health and reduce the risk of disease, not to maximize endurance performance.

2.3 Exercise and Insulin Action

One exercise session increases insulin action compared to baseline in both lean fit (7, 30, 51) and overweight sedentary (3, 10, 18, 28, 57) individuals. There are two signaling pathways of glucose uptake: the insulin-stimulated pathway and the contraction-mediated (exercise) pathway. Following exercise the pathways interact to stimulate glucose uptake in a manner greater than the sum of the two pathways working independently (13). Moreover, increased insulin-stimulated glucose uptake (following exercise) results largely from one exercise bout and is not an adaptation to exercise training (31, 51). Individuals with impaired insulin action may therefore seek to

immediately target the contraction-mediated pathway of skeletal muscle glucose uptake to overcome their reduced ability to stimulate it with insulin (24).

2.4 Interaction between Exercise and Diet to Influence Insulin Action

2.4.1 Effect of Energy Intake Following Exercise

Recently, the Energy Metabolism Laboratory at the University of Massachusetts paired an energy deficit with exercise to evaluate its combined effect on insulin action. To test the hypothesis that manipulating energy balance following exercise affects insulin action, Black et al. separated sedentary, overweight subjects into two groups: an exercise-induced energy deficit group (DEF) and an energy balance group (BAL) (5). The BAL group was re-fed expended energy during and immediately after exercise over a 6 day period; the DEF group was not re-fed any energy after exercise. Insulin action was not increased in the BAL group (compared to baseline); however, the DEF group (having an energy deficit of -481 kcal), increased insulin action by 40% (compared to baseline).

The finding from Black et al. – that restoring energy balance by re-feeding expended energy reverses increased insulin action toward baseline – confirmed findings of longer term training studies (52, 54). Ross et al. in a study matching energy expenditure with intake maintained a stable body mass and demonstrated no improvement in insulin action after 14 weeks of exercise training (52). Similarly, Segal et al, during 12 weeks of training, added a carbohydrate supplement to subjects' diets equivalent to the amount of expended energy due to exercise. Segal et al. subjects

successfully maintained body weight and saw no increase in insulin action following exercise training (54).

The results of Black et al. and longer exercise training studies suggest that creating an energy deficit after exercise is necessary to improve insulin action. However, two factors that may influence insulin action were not controlled in the Black, Segal and Ross studies. First, expended energy was re-fed immediately during, and after exercise; meal timing may influence post-exercise insulin action (33) and this will not be addressed by this study. Second, post-exercise meals were composed mainly of high-glycemic carbohydrate; consuming high-glycemic carbohydrate following exercise affects insulin action (5, 7). Evaluating the effect of varying post-exercise carbohydrate intake on insulin action is the focus of this study.

2.4.2 The Effect of Dietary Carbohydrate Intake Following Exercise

Carbohydrate feeding following intense exercise reverses insulin action toward baseline (5, 7, 11, 21, 58). It was first demonstrated in rodents (11, 21, 58); in humans, Bogardus et al. also demonstrated a reversal of increased insulin action after exercise with carbohydrate feeding (7). Bogardus et al. exercised subjects and afterwards they were fasted or fed 100 grams of carbohydrate. The 100g of carbohydrate did not replace all of the expended energy but blunted the effect of exercise (to increase insulin action). This finding suggests that carbohydrate intake following exercise affects insulin action, independent of energy deficit, in a manner that requires further exploration.

2.4.2.1 Potential Mechanism

The majority of carbohydrate consumed after exercise is stored as muscle glycogen (9). Insulin action and muscle glycogen have a poorly understood inverse relationship (50), but low muscle glycogen correlates well with increased insulin action (16). Mechanistically, glucose transporters (GLUT4), glycogen synthase activity and AMPK activity are all high after exercise, and all contribute to increased insulin action when muscle glycogen concentrations are low (56). When muscle glycogen is super-compensated, insulin action is attenuated (21, 39). Kawanaka et al. found glycogen super-compensation resulted in a significant reduction in GLUT4 concentrations at the muscle cell membrane (compared to baseline), a reduction in PKB phosphorylation (involved in the pathway of insulin signaling and glycogen synthesis) and a reduction of AMP kinase (an energy sensing kinase also involved in insulin signaling and glucose uptake) (39). They suggested a negative feedback mechanism by which an influx of glucose into the muscle cell, after the absorption of carbohydrate, affects cellular metabolism to down-regulate further glycogen synthesis. A follow-up study revealed glycogen may be one contributing factor, but not solely responsible for moderating insulin action (38); it has been suggested that the molecular regulation of insulin action by carbohydrate intake may depend also on the insulin response to carbohydrate intake before glycogen can be synthesized (48).

2.4.3 The Effect of Fat Intake Following Exercise

In rodents, restricting carbohydrate intake after exercise by increasing fat intake prolongs increased insulin action (11, 21). To obtain information regarding the length of

time this effect persists, Cartee et al. (11), exercised and separated the animals into one of four meal conditions: standard chow (60% carbohydrate), lard (100% fat), 50/50 corn-starch and glucose (100% carbohydrate), and a control (fasted). Insulin action was reversed toward baseline within 18 hours in the carbohydrate fed group but remained significantly increased in the fat-fed group for 48 hours following the cessation of exercise. Similar results were produced by Garcia-Roves et al., who evaluated the hypothesis that skeletal muscle maintains its capacity to promote glycogen synthesis as long as glycogen super-compensation is prevented. Again, insulin action remained elevated as long as muscle glycogen levels were kept low by fasting or fat-feeding. Thus (in rodents) a carbohydrate deficit by restricting carbohydrate intake after exercise may increase insulin action as effectively as an energy deficit.

Human studies evaluating the effect of post-exercise fat feeding on insulin action also supports that fat intake does not decrease insulin action. Schenk et al. (53), following exercise, infused a lipid and heparin solution overnight which created an energy surplus of ~1100 fat calories. Insulin action the next morning was similar to the control condition of a post-exercise saline infusion. In another study by the same research group, Fox et al. fed subjects a high-fat vs. a low-fat diet following exercise, of equal carbohydrate content (~350 grams), and demonstrated similar insulin action the next morning between conditions (20). Moreover, the high-fat condition restored 24 hour energy balance, while the low-fat condition created an energy deficit of ~ 1500kcals which did not affect insulin action (both groups had similar insulin action).

Summary: Rodent data demonstrates that exercise-induced increases in insulin action were prolonged with fat intake. Both the Schenk and Fox studies, conducted in humans, demonstrated that insulin action following exercise was not negatively affected by fat intake but carbohydrate was not restricted in those studies. It remains unclear though, how of post-exercise carbohydrate restriction combined with increased fat intake may be a useful nutritional strategy to maximize insulin action in humans.

2.5 Methodologies for Metabolic Measurements

2.5.1 Measures of Insulin Action

In response to increased glucose concentrations in the plasma, an insulin release is triggered by the islet cells of the pancreas to clear glucose into peripheral tissues, inhibit lipolysis and decrease hepatic glucose production. Measurements of the effectivity of insulin to stimulate glucose uptake fall into two categories: the open loop and closed loop approaches (4). The open loop approach breaks the feedback mechanism between glucose concentrations and the pancreas by infusing glucose and insulin to euglycemic and hyperinsulinemic conditions. An advantage of the open-loop approach is its sensitivity and tissue specificity – the hyperinsulinemic, euglycemic clamp is the gold standard measure of insulin action in research. A disadvantage of the open loop approach is that the physiological conditions during insulin action's measurement do not simulate "real-world" scenarios. During the hyperinsulinemic, euglycemic clamp, specifically, insulin concentrations remain artificially elevated throughout the entire 2 – 4 hour measurement, while glucose is infused intravenously to

match increased glucose uptake in response to the heightened insulin concentrations. The clamp is in direct contrast to a “real-world” scenario where carbohydrate is ingested and blood glucose/ insulin concentrations increase, then decrease over time.

The closed loop approach does not disrupt the feedback mechanism between glucose concentrations and the pancreas. For example, the oral glucose tolerance test (OGTT) of insulin action is a closed loop test; a glucose challenge of 75g is ingested within 5 minutes and blood glucose/ insulin concentrations are sampled every 30 minutes over the next two hours. From the glucose/ insulin concentrations, an area under the curve (AUC) is generated. In response to an intervention, if the AUC of both glucose and insulin increase, insulin action has decreased. Conversely, if the AUC for both glucose and insulin decreases, insulin action has increased.

Compared to the clamp, advantages of this measure are: the cost of an OGTT is significantly less in terms of resources and time, and using the C-ISI index (proposed by Matsuda and DeFronzo) is a measure of insulin action that correlates well with clamp values (43). A disadvantage of the OGTT, compared to the clamp, is that sensitivity and tissue specificity may be lost. An OGTT assesses whole body insulin action, while the clamp (especially if stable isotopes are included in the glucose infusate) allows researchers to make inferences about the rate of glucose disposal, storage, oxidation and hepatic glucose production.

2.5.2 Continuous Infusion of Glucose with Stable Isotope Tracers

The continuous infusion of glucose with stable isotope tracers (CIG-SIT) evaluates insulin action by combining the open and closed loop approaches was

developed by the Energy Metabolism Laboratory at the University of Massachusetts and has been used successfully (5). Glucose is infused to stimulate plasma glucose/ insulin concentrations similar to a mixed meal ("real world" scenario) while allowing researchers to measure non-oxidative glucose disposal, oxidation and hepatic glucose production (tissue specific). Insulin action values generated during the CIG-SIT correlate well with the OGTT ($r=0.78$, $p<0.001$) meaning the test accurately assesses insulin action relative to other known measures.

CHAPTER 3

METHODS

3.1 Proposed Study Design

To test the hypothesis that post-exercise meal composition affects insulin action, it was initially proposed 10 lean, trained subjects would go through a period of de-training and overeating (2.5 days), which has been previously demonstrated by our laboratory to decrease baseline insulin action (26). Following the period of de-training and overeating subjects would complete, in random order, four experimental conditions: high carbohydrate, galactose (an alternative moderate glycemic index carbohydrate), low-carbohydrate, and a no-exercise control – separated by a one-week washout period. Subjects would come into the laboratory in the early evening and complete an intense exercise session designed to expend 30% of total daily energy expenditure (TDEE) at 65% of VO_{2peak}. Thirty minutes following the completion of exercise subjects would consume the post-exercise meal and blood was to be drawn for one hour to measure glucose and insulin concentrations. Blood glucose and insulin measurements were to be taken to quantify the glucose and insulin response to the post-exercise meal. After blood sampling, subjects would return home, fast overnight, and report back to the lab the next morning for a measurement of insulin action.

3.2 Actual Study Design

Experimental the hypotheses and study design were streamlined to be more cost effective and scientifically sound. Sedentary, overweight subjects will be recruited

instead of physically fit, lean individuals, and one of the experimental conditions (galactose, the alternate form of moderate glycemic index carbohydrate) will be eliminated. Thus, the experimental conditions will allow us to focus specifically on the effect of high-carbohydrate availability vs. low-carbohydrate availability following exercise in a relevant subject population to which we hope to apply our findings.

An addition to the proposed study design is the regulation of food intake during the day of exercise, also making the methods stronger scientifically. In all conditions subjects will consume the same foods prior to exercise or the standardized evening meal. Since energy imbalance has an independent effect on insulin action, the regulation of pre-exercise food intake is important to minimize confounding variables in this study.

The actual experimental protocol (Figure 1A) is similar to the one proposed. Subjects will come into the lab in the early evening and expended 30% of total daily energy expenditure at 65% of VO_{2peak}. Fifteen minutes following the completion of exercise, a background blood draw will be taken. Thirty minutes following the completion of exercise, subjects will consume an isocaloric meal (either high or low in carbohydrate) to replace expended energy and blood sampling will occur every fifteen minutes thereafter for one hour to quantify the glucose and insulin response to the post-exercise meal. After the completion of blood sampling, subjects will be instructed to consume only water overnight and returned home. The next morning (12 hours after the meal) subjects will return to the laboratory, fasted, for a measure of insulin action using the continuous infusion of glucose with stable isotope tracers (CIG-SIT) method.

3.3 Subject Characteristics

Ten healthy, previously sedentary subjects will be recruited from the Amherst, Massachusetts area for this study. All included subjects will be between the ages of 18-45 yrs, healthy (self-report as per physician evaluation within the last year), sedentary (<1hr per week of structured physical activity) and overweight (body fat >25%). Individuals that have a history of cardiovascular disease, Type 2 diabetes, respiratory disease and metabolic disorders, actively trying to lose weight, taking any supplements or medications that would interfere with testing, trying to get pregnant, and who smoke or use tobacco products will be excluded. Participation in this study is voluntary and subjects will be required to sign an informed consent approved by the Institutional Review Board for human subjects at the University of Massachusetts upon inclusion in the study.

3.4 Pre-Treatment Testing

Before starting the experimental conditions, subjects will undergo measurements of body composition (DXA), resting energy expenditure, and maximal aerobic capacity (VO_{2peak}).

3.4.1 Body Composition

Body composition will be measured by dual energy X-ray absorptiometry (DXA) (Lunar, Madison, Wisconsin). The DXA scan will measure 1) percent body fat and 2) lean body mass. Lean body mass will be used in the calculations of isotope infusion rate during the measure of insulin action.

3.4.2 Total Daily Energy Expenditure

Resting energy expenditure (REE) will be measured after an overnight fast. Subjects will be familiarized with the equipment: nose piece, head gear, two-way mouthpiece, and the metabolic cart. After being oriented to the equipment, subjects will lie supine and rest quietly for 15-20 minutes. After resting, expired gases will be collected for 20 minutes using the TrueMax2400 Metabolic Measurement System (Paromedics, Salt Lake City, Utah). Total daily energy expenditure (TDEE) will be calculated by multiplying REE by an appropriate activity factor for young, sedentary individuals (46).

3.4.3. Peak Aerobic Capacity

Peak aerobic capacity (VO_{2peak}) will be measured on the cycle ergometer. Subjects will warm-up for five minutes at a self-selected pace with low resistance. After warming-up subjects will cycle at a self-selected "brisk" pace. Every two minutes the resistance will be increased by 25 watts, until the subject can no longer maintain their selected pace and voluntarily stops the test. Respiratory gases will be collected throughout the test and recorded using a metabolic cart, (TrueMax2400, Paromedics, Salt Lake City, Utah). Heart rate will be monitored throughout the test, (Polar Advantage).

The test will be considered a valid measure of VO_{2peak} if two of the three criteria are met; 1) respiratory-exchange ratio (RER) >1.10 2) heart rate is within 15 beats of the predicted maximum (220-age), or 3) VO_2 remains constant as the resistance is increased by 25 watts.

3.5 Exercise Sessions

Subjects will be instructed to not engage in strenuous physical activity three days prior to each experimental condition. Subjects will report to the lab in the evening for a session of intense exercise. Exercise will be performed on cycle ergometer, the same equipment used for the test of VO_{2peak}. The exercise session is designed to expend 30% of estimated total daily energy intake at 65% VO_{2peak}.

To ensure subjects are exercising at 65% VO_{2peak} respiratory gases will be collected during the first 15 minutes of exercise, and resistance will be adjusted until the desired steady-state intensity is achieved. At steady-state, workload (watts), RPM and heart rate will be recorded. Subjects will be instructed to maintain the recorded RPM and researchers will monitor heart rate for the rest of the exercise session. Total exercise time will be calculated based on the following equations:

1. Desired energy expenditure (EE) in the exercise bout (kcal) =

30% TDEE (kcal) - EE to steady-state (kcal)

2. EE (kcal)/ minute at steady-state = EE during 10min of steady-state/10 minutes

3. Duration of exercise bout (minutes) =

Desired EE in exercise bout (kcal) / (kcal/min at steady-state)

Half-way through the exercise session, respiratory gases will be collected to ensure subjects are still cycling at the desired intensity. Adjustments will be made to the workload (watts) and RPM if the subject's VO_2 has deviated from the goal of 65% VO_{2peak}. Finally, in the last ten minutes of exercise, subjects will perform five, forty-five second sprints alternating with a one minute and fifteen seconds rest to deplete muscle glycogen as much as possible.

3.6 Pre-Exercise Meals

Before each experimental condition, subjects will be instructed not to skip a meal in the three days prior to exercise and to consume all three macronutrients (carbohydrate, protein, and fat) at every meal as part of their normal diet.

All food will be provided to subjects on the day of the exercise session. Subjects will come to the laboratory in the morning (8:00am) to eat breakfast and pick-up their meals for the day. Subjects will be provided lunch, and a high-carbohydrate snack to be consumed 3 hours before returning to laboratory for exercise. Subjects will consume the same meals for each experimental condition, except for the last meal of the day exercise which will be provided following exercise and vary in carbohydrate content. For the no-exercise control condition, subjects will be provided a standardized meal, equal to 30% of their estimated total daily energy intake to be consumed at home.

3.6.1 Post-Exercise Meals

Thirty minutes following the completion of the intense exercise session, subjects will be instructed to consume a post-exercise meal and will be given 10 minutes to complete it. Post-exercise meals will differ in macronutrient content will be designed to vary carbohydrate availability (high or low). The high carbohydrate meal (High-CHO) will consist of sugary Kool-aid® (8% carbohydrate) and a lightly buttered bagel. The low carbohydrate meal (Low-CHO) will consist of Half and Half cream, mixed with Nesquick®, and an Atkins® bar. The caloric value of both post-exercise meals is designed to replace exercise energy expenditure (30% TDEE) and differ in carbohydrate content. In addition, the high carbohydrate meal will replace the carbohydrate (g)

expended during exercise (carbohydrate balance), and the low carbohydrate meal will not (carbohydrate deficit).

3.7 Glucose and Insulin Measurements

Fifteen minutes following the completion of exercise an indwelling superficial catheter will be inserted into one of the subject's forearm veins and a background blood sample will be taken. After the background blood draw, the post-exercise meal will be consumed and blood sampling will resume 15 minutes after the meal's completion. Blood samples will be collected every fifteen minutes thereafter for one hour. Once each blood is collected it will be transferred into vacutainers containing sodium fluoride and potassium oxalate (a glycolytic inhibitor), and EDTA (an anti-coagulant). Blood samples will immediately be centrifuged for 10 minutes at 3000g, plasma aliquots will be stored in 2ml cryotubes at -80°C.

3.8 Assessment of Insulin Action

Subjects will report, fasted, the next morning to the laboratory for a measure of insulin action. Insulin action will be measured using a continuous infusion of glucose with stable isotope tracers (CIG-SIT). Indwelling superficial venous forearm catheters will be inserted into each arm of the subject. One catheter will be used as the site of infusion and the other for blood collection. Baseline blood samples will be collected to determine naturally occurring levels of isotopic enrichment prior to the infusion. A priming bolus of 200mg [6,6-^{2}H] glucose will be given followed by a 90 minute infusion of 2.0% [6,6-^{2}H] glucose and [1,1,2,3,3 – D_5] glycerol isotope at a rate of

2.5mg/min delivered by a peristaltic infusion pump (Harvard Apparatus Pump 22, Holliston MA). Resting blood samples will be collected at 75 and 90 minutes. Breath samples will be taken from minutes 75-85 to determine resting substrate oxidation rates using indirect calorimetry (TrueMax2400, Paromedics, Salt Lake City, Utah). At minute 90, the infusate will be changed to 20% dextrose containing 2.0% [6,6-^{2}H] glucose delivered at a rate of 8.45mg/kg FFM/min for 60 minutes. Steady-state blood samples will be collected at 50, 55 and 60 minutes. Again, breath samples will be taken from minutes 50-60 to determine steady-state substrate oxidation rates using indirect calorimetry.

Venous blood will be collected in heparinized syringes and immediately transferred to vacutainers containing sodium fluoride and potassium oxalate (a glycolytic inhibitor), and EDTA (an anti-coagulant). Blood collected in vacutainers containing a glycolytic inhibitor will be used for the analysis of plasma glucose and glucose isotopic enrichment. Vacutainers containing an anti-coagulant will be used for the analysis of insulin, glycerol, triglycerides and free fatty acid (FFA) concentrations. After collection, samples will be centrifuged for 10 minutes. Following centrifugation, plasma aliquots will be separated and stored in 2ml cryotubes and stored at -80°C until biochemical analysis.

3.9 Biochemical Analysis

Plasma glucose concentrations will be determined by the glucose oxidase method with a GL5 Analox Analyzer (Analox Instruments, Lunenberg, MA). Insulin concentrations will be determined using a radioimmunoassay kit specific for human

insulin (Millipore Research Corp., Billerica, MA). Glycerol and triglycerides concentrations will be determined using reagents from the serum triglyceride kit using the enzymatic hydrolysis by lipase of triglycerides to glycerol (Sigma Aldrich, St. Louis, MO). Free fatty acid (FFA) concentrations will be determined with an NEFA enzymatic colorimetric assay kit (Wako Chemicals USA Inc., Richmond, VA).

3.9.1 Plasma Isotopic Enrichment

Glucose isotopic enrichment will be measured by high-performance liquid chromatography, mass spectrometry (HPLC/MS) using a protocol adopted from (44). 1 ml of acetone (CH_3COCH_3) will be pipetted into a labeled microcentrifuge tube. 0.3ml of plasma will be added to the acetone to de-proteinate the sample. Samples will then be vortexed, incubated in the freezer at -20°C for 10 minutes. After chilling, the sample will be centrifuged for 2 minutes at 17000g and 4°C. Following centrifugation, the sample supernatant will be removed using a 1ml luer lock plastic syringe, and transferred through a polyethersulfone filter (4mm, 0.45µm) into a 200µl plastic insert and then placed into a glass HPLC vial and capped (Fisher Scientific Inc., Pittsburg, PA).

After the completion of sample preparation, the HPLC vials will be loaded into the autosampler compartment of the HPLC (Agilent 1100 Series; Agilent Technologies, Santa Clara CA). The HPLC method will be controlled using the Chemstation Software package (Agilent Technologies, Santa Clara CA) with the following conditions: isocratic mobile phase 75:25 - acetonitrile (ACN): water (H_2O), flow rate 1.0 mL/min,

injection volume 10µL, column temperature 35°C (Shodex Asahipak NH2P-50, 4.6 x 250mm, Showa Denko America Inc., New York NY), and a 10 minute run time.

Following equilibration, the samples will be injected into the system and sample compounds will be separated using isocratic reverse phase chromatography. After compounds are separated in the HPLC they will be ionized by an electrospray source in the mass spectrometer (Esquire 6000, Bruker Daltonics Inc., Billerica MA) using Esquire Control software and the mass to charge ratio of the positively ionized sample will be detected following conditions: capillary -5500V, endplate offset -500V, nebulizer 30.0 psi, dry gas 10.0 L/min, and dry temperature 300ºC, over a scan range of 100 to 210 m/z.

Chromatographs will be analyzed using Bruker Data Analysis software (Bruker Daltonics Inc., Billerica MA). The glucose peaks (elution time ~7.5 minutes) will be isolated, then integrated and the average mass to charge ratio will be generated. Isotopic enrichment of the dideuterated glucose (mass to charge ratio = 205) will be expressed as a percentage of the total glucose species (mass to charge ratio = 203 + 204 + 205):

$$\% \text{ isotopic enrichment} = \frac{205}{(203 + 204 + 205)}$$

3.10 Calculations

The necessary variables (R_a and R_d) will be calculated by:

Glucose rate of appearance (R_a) in mg/min=

$$\frac{F - V[(C_1 + C_2) / 2][(IE_2 - IE_1) / (t_2 - t_1)]}{[(IE_2 + IE_1) / 2]}$$

Glucose rate of disappearance (R_d) in mg/min = $Ra - V[(C_2 - C_1)/ (t_2 - t_1)]$

F represents the isotopic infusion rate. IE_1 and IE_2 are the isotopic enrichments (ratio of labeled [6,6-^{2}H] glucose to total plasma glucose) at time-points t_1 and t_2 respectively. C_1 and C_2 are the concentrations of plasma glucose at t_1 and t_2, and V is the estimated volume of distribution for glucose (180 ml/kg).

3.10.1 Whole Body Insulin Action

Whole body insulin action will be calculated by dividing the glucose rate of disappearance by the steady-state plasma insulin concentration (Rd/SSPI). Steady-state plasma insulin concentrations will be determined by averaging the insulin concentrations of minutes 50, 55, and 60 of the 20% glucose + isotope infusion.

3.10.2 Hepatic Insulin Action

Hepatic insulin action is defined as the percent suppression of fasting hepatic glucose output. % hepatic suppression:

$$= 1 - \left(\frac{HGO_{clamp}}{HGO_{fasting}}\right) * 100$$

HGO_{clamp} is the average R_a at minutes 50, 55, and 60 of the glucose infusion minus the infusion rate. $HGO_{fasting}$ is the R_a at minutes 75 and 90. The right side of the equation represents the amount of endogenous glucose coming from the liver during the steady-state glucose infusion. When subtracted from 1 and multiplied by 100, the result is percent hepatic suppression. Percent hepatic suppression is a measure of how effectively endogenous glucose production is lowered by increased insulin concentrations during the glucose infusion.

3.10.3 Substrate Partitioning during the Measure of Insulin Action

3.10.3.1 Non-oxidative Glucose Disposal and Carbohydrate Oxidation

Non oxidative glucose disposal ($R_d$nox) is calculated by subtracting the rate of carbohydrate oxidation from the glucose rate of disappearance (R_d).
The rate of carbohydrate oxidation ($R_d$ox) will be calculated using: 4.585 VCO_2 (L/min) – 3.226 VO_2 (L/min) (47).

3.10.3.2 Fat Oxidation

Rates of fat oxidation will be calculated using: 1.695 VO_2 (L/min) – 1.701 VCO_2 (L/min) (47).

3.11 Statistics

A commercially available software package, (SAS, Version 8, SAS Institute, Cary, NC) will be used to perform statistical analysis of the data. A paired t-test will be used to compare the two exercise sessions and the glucose and insulin responses to the

two post-exercise meals. The caloric value and composition of the post-exercise and standardized meals will be evaluated a one-way repeated measures ANOVA. The effect of carbohydrate availability following exercise on insulin action, hepatic insulin action, and whole body lipolysis will also be evaluated using one-way repeated measures ANOVA. A two-way (time x experimental condition) repeated measured ANOVA will be used to evaluate if there is a significant effect of time (rest to steady-state) and experimental condition on: plasma glucose, insulin, non-oxidative glucose disposal, carbohydrate oxidation, fat oxidation, free fatty acids, triacylglycerol and glycerol. For all statistical tests, significance will be set a $p<0.05$. If there is a significant effect of time or experimental condition with the ANOVA, Tukeys post-hoc analysis will be performed to make pair wise comparisons between group means.

A linear regression analysis ($p<0.05$) will be performed to evaluate a potential relationship between carbohydrate availability in the post-exercise meal and changes in insulin action (compared to baseline) the following morning.

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CHAPTER 4
CONDENSED MANUSCRIPT

4.1 Introduction

Insulin stimulated glucose uptake (insulin action) is increased following an acute bout of exercise (1, 3, 9, 29, 40). The insulin-sensitizing effect of exercise (7), among its many benefits, has led it to be characterized as a “drug” to improve metabolic health because it reduces the risk of type 2 diabetes with repeated application (20, 36). Metabolic health outcomes, like insulin action, are affected by the nutritional environment following exercise. Fasting following exercise significantly increases insulin action (2, 3); however, when expended energy is refed as dietary carbohydrate, insulin action does not increase from baseline values (2, 3, 34).

Following exercise, it is unknown whether the reversal of enhanced insulin action is mediated by refeeding expended energy or by refeeding expended energy as carbohydrate. In rodents, refeeding expended energy without carbohydrate (carbohydrate restriction) prolongs increased insulin action until carbohydrate is refed (5, 12, 41); supporting that the carbohydrate may be responsible for blunting the effect of exercise. Carbohydrate restriction (following exercise) may be a sensible strategy to enhance insulin action in humans, but its effect has not been systematically evaluated. Therefore, the purpose of this study was to evaluate the effect of carbohydrate availability following exercise on insulin action in sedentary, overweight adults. It was hypothesized that, relative to baseline (BASE), carbohydrate restriction following an exercise bout (Low-CHO) would increase whole-body insulin action but that replacing the expended energy as carbohydrate (High-CHO) would not.

4.2 Methods

4.2.1 Subjects

Subject characteristics are shown in Table 1. Ten volunteers - five men and five women – participated in the study. Subjects were young (age=22.2±1.0 years), overweight (BMI=26.1±3.7 kg × m^{-2}) and had a low level of cardiorespiratory fitness, (VO_{2peak}=34.6±3.8 mL × kg^{-1} × min^{-1}).

For inclusion, subjects completed a health and fitness questionnaire and had preliminary measurements taken. Subjects were sedentary (<1 hr leisure time physical activity per week for 3 months), weight stable for the past 6 months, and had a body fat % > 25. None of the subjects had type 2 diabetes, cardiovascular disease, respiratory disease, metabolic disorders or used tobacco products, nor were they taking any drugs or supplements that would interfere with the measure of insulin action (e.g. anti-diabetic drugs, blood pressure medication, statins, insulin, inhaled steroids, chromium, and ephedra). The experimental protocol was approved by the Institutional Review Board for human subjects at the University of Massachusetts and prior to participation, written consent from subjects was obtained. If subjects were non-compliant with study protocol (1 subject) or unable to successfully perform preliminary measurements (1 subjects), they were excluded from the study and their data were not analyzed.

4.2.2 Preliminary Measurements

Resting Energy Expenditure: Subjects reported to the laboratory the morning following an overnight fast. After familiarization with the laboratory and its equipment,

subjects rested quietly for 25 minutes, lying supine. After resting, respiratory gases were collected for a minimum of 20 minutes using the TrueMax2400 Metabolic Measurement System (Parvomedics, Salt Lake City, Utah) and indirect calorimetry determined resting energy expenditure. An estimate of total daily energy expenditure (TDEE) was calculated by multiplying measured resting energy expenditure by an activity factor representative of sedentary physical activity patterns (25). Body Composition: Fat mass, fat-free mass, and % body fat was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar, Madison, Wisconsin). Peak Oxygen Consumption: All subjects performed a progressive, incremental cycling test to exhaustion to measure peak oxygen consumption (VO_{2peak} $mL \times kg^{-1} \times min^{-1}$) on an electronically braked cycle ergometer, (SensorMedics 800, Yorba Linda, CA). The test was considered valid if: 1) respiratory-exchange ratio (RER) >1.10, 2) heart rate was within 15 beats of the predicted maximum (220-age), or 3) if VO_2 did not continue to increase with an increased resistance of 25 watts.

4.2.3 Experimental Protocol

Subjects completed three experimental conditions (each lasting two days) in random order: 1) a baseline measure of insulin action (BASE), 2) exercise followed by a high carbohydrate meal (High-CHO) and 3) exercise followed by a Low-CHO meal (Low-CHO). A minimum of one-week separated each experimental condition.

Figure 1 illustrates the two-day testing protocol timeline. On Day 1 of the exercise conditions, subjects reported to the laboratory in the early evening (1700h) and expended 30% of their TDEE, at 65% of VO_{2peak} on a cycle ergometer. Fifteen minutes

following the completion of exercise, a background blood draw was taken. Thirty minutes following the completion of exercise, subjects consumed a meal (either high or low in carbohydrate) that replaced all of the expended energy and blood sampling occurred every fifteen minutes thereafter for one hour (to quantify the glucose and insulin response to the post-exercise meal). After the completion of blood sampling, subjects were instructed to consume only water overnight and were sent home. The next morning (Day 2), approximately 12 hours after the post-exercise meal, subjects returned to the laboratory for a measure of insulin action using a continuous infusion of glucose with stable isotope tracers (2).

4.2.4 Pre-Exercise Meals

On Day 1 in all conditions, subjects consumed similar meals prior to exercise or the standardized evening meal, in the BASE condition. Subjects came to the laboratory in the morning (8:00am) to eat breakfast and receive pick-up the other meals for the day. Subjects were given lunch and an afternoon snack to eat on their own at given times (1200h and 3hr before exercise, respectively). As a measure of compliance, subjects were instructed to bring all empty food containers back to the laboratory. In the BASE condition, subjects were also given a standardized evening meal (724.3 ± 35.2kcal; 54.1 ± 1.6 % carbohydrate, 32.7 ± 0.7 % fat and 9.2 ± 1.1 % protein) to be eaten at home 12 hours prior to measurement of insulin action.

4.2.5 Exercise Sessions

Subjects were instructed, and agreed, not to engage in moderate physical activity at least three days prior to each experimental condition. On Day 1 of the Low-CHO and High-CHO experimental conditions, subjects reported to the laboratory at ~ 1700h for exercise on a cycle ergometer (Lifefitness, Champaign, IL) at 65% of VO_{2peak}.

Respiratory gases were collected during the first 15 minutes of exercise bout, until desired steady-state VO_2 ($mL\times kg^{-1}\times min^{-1}$) was achieved. At steady-state, the workload (watts), RPM and heart rate were recorded. Energy expenditure per minute at steady-state (kcal/min) was recorded and subjects cycled for the time necessary to expend 30% TDEE.

Half-way through the exercise session, respiratory gases were collected to verify subjects were cycling at the desired intensity. Adjustments were made to the workload and RPM if the subject's VO_2 ($mL\times kg^{-1}\times min^{-1}$) had deviated from the goal of 65% of VO_{2peak}. Exercise time was also re-calculated if necessary. In the last ten minutes of exercise, subjects performed five 45 second sprints alternating with 75 seconds rest to deplete muscle glycogen as much as possible.

4.2.6 Post-Exercise Meals and Blood Sampling

Fifteen minutes following exercise completion an indwelling catheter was inserted into a superficial forearm vein and a background blood sample was taken. Thirty minutes following exercise completion subjects were given a meal either high (76.6±2.5%) or low (17.8±0.1%) in carbohydrate that replaced the energy expenditure of exercise (~700kcal). In addition, the High-CHO meal replaced all carbohydrate

expenditure (grams) during exercise. Blood samples were collected every fifteen minutes after the meal's completion for one hour to verify the post-exercise meals resulted in different glucose and insulin responses.

4.2.7 Assessment of Insulin Action: Glucose Infusion with Stable Isotopes

Insulin action was assessed 12 hours following the post-exercise meal on the morning of Day 2 using a continuous infusion of 20% glucose containing 2% stable [6,6-^{2}H] isotope (Cambridge Laboratories, Andover MA). Subjects rested quietly in a reclining chair and an indwelling superficial venous forearm catheter was inserted into each arm. One catheter was used for infusion of stable isotopes and the other was used for blood collection. Baseline blood samples were collected to determine naturally occurring isotopic enrichment prior to the infusion. A priming bolus of 200mg [6,6-^{2}H] glucose was given followed by a 90 minute infusion of 2% [6,6-^{2}H] glucose at a rate of 2.5mg/min delivered by a peristaltic infusion pump (Harvard Apparatus Pump 22, Holliston MA). Resting blood samples and respiratory gases were collected at 75 and 90 minutes. At minute 90, the infusate was changed to 20% glucose containing 2% [6,6-^{2}H] glucose, delivered at a rate of 8.45mg/kg FFM/min for 60 minutes. Steady-state blood samples and respiratory gases were collected at 50, 55 and 60 minutes.

Resting and steady-state blood samples were collected to determine the glucose rate of appearance (R_a) and disappearance (R_d) during the glucose infusion. Glucose and insulin concentrations from minutes 50, 55 and 60 were averaged to determine steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations. Respiratory gases were collected and used in calculations of substrate utilization or storage.

4.2.8 Blood Collection and Analytical Procedures

Venous blood was collected in heparinized syringes and immediately transferred to vacutainers (kept on ice) which contained either a glycolytic inhibitor (sodium fluoride and potassium oxalate) for later measurement of plasma glucose concentrations and glucose isotopic enrichment, or an anti-cogulant (K_3-EDTA) for insulin and free fatty acid concentrations. After collection, samples were centrifuged for 10 minutes at 3 000*g*, then plasma aliquots were separated and stored in 2ml cryotubes at -80°C until biochemical analysis.

Plasma glucose concentrations were determined by the glucose oxidase method using a GL5 Analox Analyzer (Analox Instruments, Lunenberg, MA). Insulin concentrations were determined using a radioimmunoassay kit specific for human insulin (Millipore Research Corp., Billerica, MA). Free fatty acid (FFA) concentrations were determined with an NEFA enzymatic colorimetric assay kit (Wako Chemicals USA Inc., Richmond, VA).

Glucose isotopic enrichment was determined by high-performance liquid chromatography mass spectrometry (LCMS) (24). Liquid chromatography was performed with an Agilent 1100 Series, (Agilent Technologies, Santa Clara, CA) running Chemstation 1100 software. A Shodex Asahipak NH2P-50, 4.6 x 250mm column (Showa Denko America Inc., New York NY) was used. Mass spectrometry was performed using an Esquire 6000, and chromatographs were analyzed with Bruker Data Analysis software (Bruker Daltonics Inc., Billerica MA).

To de-proteinate the sample, 0.3mL of plasma was added to 1 mL of ice-cold acetone (CH_3COCH_3). Samples were vortexed and incubated at -20°C for 10 minutes;

after chilling, samples were centrifuged for 2 minutes at 17000*g* and 4°C. Following centrifugation, the sample supernatant was transferred through a polyethersulfone filter (4mm, 0.45µm) into a glass HPLC vial and capped (Fisher Scientific Inc., Pittsburg, PA). Vials were loaded into the autosampler compartment of the LCMS and set to an injection volume of 10µL. The liquid chromatography conditions were: isocratic mobile phase 75:25 - acetonitrile (ACN): water (H2O); flow rate 1.0 mL/min; injection volume 10µL; a column temperature 35°C; 10 minute run time.

After being separated in the HPLC samples were ionized by an electrospray source in the mass spectrometer and detected with the following conditions: capillary -5500V; endplate offset -500V; nebulizer 30.0 psi; dry gas 10.0 L/min; dry temperature 300ºC; scan range of 100 to 210 m/z.

In chromatogram analysis, the glucose peaks were isolated, integrated and the average mass to charge ratio was generated. Isotopic enrichment of the dideuterated glucose (mass to charge ratio = 205) was expressed as a percentage of the total glucose species (mass to charge ratio = 203 + 204 + 205):

$$\text{\% isotopic enrichment} = \frac{205}{(203 + 204 + 205)}$$

4.2.9 Calculations

Fasting insulin action (HOMA-IR) was calculated by: (fasting plasma glucose × fasting plasma insulin) ÷ 22.5 (23). Insulin action during the glucose infusion (C-ISI) was calculated by: 10,000 ÷ √((fasting plasma glucose × fasting plasma insulin) × (SSPG × SSPI)) (22).

Resting and steady-state insulin stimulated glucose turnover was calculated by:

Glucose rate of appearance (R_a) in = $$\frac{F - V[(C_1 + C_2) / 2][(IE_2 - IE_1) / (t_2 - t_1)]}{[(IE_2 + IE_1) / 2]}$$

Glucose rate of disappearance (R_d) = $R_a - V[(C_2 - C_1) / (t_2 - t_1)]$

F represents the isotopic infusion rate. IE_1 and IE_2 are the isotopic enrichments (ratio of labeled [6,6-^{2}H] glucose to total plasma glucose) at time-points t_1 and t_2 respectively. C_1 and C_2 are the concentrations of plasma glucose at t_1 and t_2, and V is the estimated volume of distribution for glucose of 180 mL/kg.

Whole body insulin action was determined by R_d/SSPI (2, 4, 34).

Nonoxidative glucose disposal, usually attributed to glucose storage, was calculated as ($R_d$nox): Glucose R_d – total carbohydrate oxidation rate ($R_d$ox).

Carbohydrate and fat oxidation rates were calculated from the VO_2 and VCO_2 (L/min) using the formulas of Péronnet and Massicotte (26):

Fat oxidation rate = 1.6946 VO_2 – 1.7012 VCO_2

Carbohydrate oxidation rate = 4.5850 VCO_2 – 3.2255 VO_2

Hepatic glucose production (HGP) was calculated as the difference between R_a and the total glucose infusion rate. Hepatic insulin action was defined as the % suppression of resting hepatic glucose production by the glucose infusion during steady-state. % suppression was calculated by: $1 - (HGP_{infusion} \div HGP_{rest}) \times 100$.

4.2.10 Statistics

Data was analyzed using SAS Version 9.1 (SAS Institute, Cary, NC). A paired t-test was used to compare exercise sessions, as well as, the glucose and insulin responses to the post-exercise meals. The caloric value and composition of the meals was evaluated using one-way repeated measures ANOVA, which was also used to evaluate the effect of carbohydrate availability following exercise on: insulin action, R_d, $R_d$ox, $R_d$nox, and % hepatic suppression. A two-way (time × experimental condition) repeated measures ANOVA was used to evaluate whether a significant effect of time (rest to steady-state) and experimental condition existed in: plasma glucose, insulin, FFA concentrations, hepatic glucose production, and rates of fat oxidation. If significance was observed, Tukeys post-hoc analysis was performed.

Regression analysis was used to further evaluate outcome variables. The strength of linear relationships is presented as the Pearson's product moment correlation coefficient (R). For all statistical tests, a p value less than 0.05 was considered significant.

4.3 Results

4.3.1 Exercise Sessions

By design, the two intense exercise sessions (High-CHO and Low-CHO) were similar in exercise intensity (% VO_{2peak}), caloric expenditure (kcal), carbohydrate expenditure (total grams carbohydrate), and duration (minutes), ($p>0.05$, for each).

Respiratory exchange ratios (RER), measured during steady-state exercise were also similar in both exercise sessions (p=0.78), (Table 2).

4.3.2 Post-Exercise Meals

The caloric content of the post-exercise meals was not significantly different between conditions, nor different from BASE, (p=0.52), as shown in Table 3. Meals were significantly different in the amount of carbohydrate (g), % carbohydrate, amount of fat (g), and % fat (p<0.001 for each, respectively). The High-CHO and BASE meals were similar in protein content (p=0.10) and % protein (p=0.14), but both meals were significantly lower in protein content (~ 15g) and % protein (~10%) compared to the Low-CHO meal (p<0.001). By design, the High-CHO condition was in carbohydrate balance; 139.6 ± 9.8g of carbohydrate were expended during exercise and the post-exercise meal contained 138.9 ± 8.4g of carbohydrate. The Low-CHO condition was in carbohydrate deficit; the difference between the carbohydrate consumed (30.8 ± 1.3g) and expended (139.7 ± 9.8g) was approximately 100 grams.

Glucose and insulin concentrations vs. time are shown in Figure 2. Areas under the curve (AUC) for the glucose and insulin responses to the post-exercise meals were calculated using trapezoidal integration. By design, the glucose AUC following the High-CHO meal was significantly greater than the glucose AUC following the Low-CHO meal (p<0.001). As expected, the insulin AUC following the High-CHO meal was also significantly greater than the Low-CHO meal (p<0.001).

4.3.3 Fasting Plasma Glucose and Insulin Concentrations

In response to carbohydrate restriction following exercise (Low-CHO), fasting plasma glucose concentrations the next morning were significantly lower ($p<0.05$) compared to BASE and High-CHO conditions, (Table 3). Fasting insulin concentrations were similar between groups ($p=0.64$). The HOMA – IR was not also not different between conditions ($p=0.38$).

4.3.4 Glucose Metabolism

The insulin stimulated rate of glucose disappearance (R_d) was similar between conditions ($p=0.39$), (Table 4). When the R_d was separated into its non-oxidative ($R_d$nox) and oxidative ($R_d$ox) components (Figure 3), $R_d$nox was higher compared to BASE following the Low-CHO condition ($p<0.05$), but not the High-CHO condition ($p=0.06$), (Table 4). $R_d$ox decreased following both exercise conditions compared to BASE ($p<0.05$). When the exercise conditions were compared, the Low-CHO treatment reduced $R_d$ox 20% more than the High-CHO treatment did ($p<0.05$).

There was no effect of post-exercise carbohydrate availability on insulin action, as defined by glucose uptake (R_d) per steady-state plasma insulin (SSPI), ($p=0.30$). The C-ISI score was also not different between experimental conditions ($p=0.55$). Lastly, hepatic glucose production at rest (HGP_{rest}) and % hepatic suppression during the glucose infusion was not affected by experimental treatment; there was no difference between conditions ($p=0.98$ and $p=0.54$, respectively), (Table 4).

4.3.5 Lipid Metabolism and Fat Oxidation

Resting FFA were elevated following the Low-CHO condition ($p<0.05$) but not the High-CHO condition ($p=0.28$) compared to BASE (Table 5), and were not different between Low-CHO and High-CHO conditions ($p=0.45$). The glucose infusion significantly reduced FFA concentrations ($p<0.01$) by similar magnitudes in all conditions ($p=0.49$). As in the fasted state, FFA concentrations were elevated during the infusion in the Low-CHO condition compared to BASE ($p<0.05$) and were not different between Low-CHO and High-CHO conditions ($p=0.13$).

Resting fat oxidation rates were higher following in Low-CHO compared to BASE but not High-CHO, (Low-CHO condition $p=0.04$, High-CHO condition $p=0.23$), (Figure 4). Fat oxidation was lower during the glucose infusion compared to rest in the BASE condition (Δ-45.6±9.5 mg×min^{-1}, $p<0.01$), (Table 5). In contrast, the glucose infusion did not affect fat oxidation rates in the Low-CHO and the High-CHO conditions: they remained elevated (during the infusion) compared to rest (Δ-19.4 ± 7.2 mg×min^{-1}, $P=0.72$ and Δ-34.0 ± 8.7 mg×min^{-1}, $p=0.32$) and to BASE ($p<0.05$ for both conditions, respectively). Fat oxidation remained elevated to a greater degree in the Low-CHO condition compared to the High-CHO condition (93.4 ± 10.6 and 72.0 ± 7.4 mg × min^{-1}, respectively, $p=0.05$).

4.3.6 Correlations

Changes in insulin action relative to BASE (Δ R_d/SSPI) after the exercise/meal treatments were inversely related to insulin AUC following the post-exercise meals ($r = -0.45$, $p=0.06$), and resting FFA concentrations the next morning ($r = -0.49$, $p=0.03$).

To further explain the differences in ΔR_d/SSPI observed in each condition, the ΔR_d/SSPI data was separated by Low-CHO and High-CHO condition and analyzed using linear regressions.

ΔR_d/SSPI of the High-CHO condition was inversely correlated to insulin AUC following the High-CHO post-exercise meal ($r = -0.79$, $p=0.01$) and resting FFA concentrations ($r = -0.78$, $p=0.007$). ΔR_d/SSPI of the Low-CHO condition was also inversely correlated with insulin AUC and FFA ($r = -0.39$, $r = -0.55$, respectively); however, both associations were weaker and neither was significant ($p=0.30$, $p=0.09$). Interestingly, ΔR_d/SSPI in the Low-CHO condition was strongly correlated with the magnitude of carbohydrate deficit achieved with the post-exercise meal ($r = 0.82$, $p=0.003$), as shown in Figure 5. ΔR_d/SSPI was directly correlated with cardiorespiratory fitness (VO_{2peak}) in the High-CHO condition ($r = 0.68$, $p=0.030$) but not in the Low-CHO condition ($r=0.03$, $p > 0.05$), (Figure 6). Lastly the rate of fat oxidation during the glucose infusion was directly correlated with insulin action ($r = 0.56$, $p=0.01$).

4.4 Discussion

The purpose of this study was to evaluate the effect of post-exercise carbohydrate availability on insulin action in sedentary, overweight adults. It was hypothesized that, relative to baseline, insulin action would be improved when exercise was followed by carbohydrate restriction (Low-CHO) but not when expended carbohydrate was refed (High-CHO). Contrary to our hypothesis, insulin action defined by glucose uptake per unit steady-state insulin was not affected by experimental

condition. However, the Low-CHO condition altered glucose and lipid metabolism at rest and in response to a glucose infusion compared to BASE and the High-CHO condition.

In the Low-CHO condition resting fat oxidation was increased and glucose concentrations were decreased compared to BASE. During the glucose infusion, non-oxidative glucose disposal ($R_d$nox) was increased and glucose oxidation ($R_d$ox) reduced. Lastly, the magnitude of carbohydrate deficit, i.e. the difference between carbohydrate expenditure and intake following exercise, was positively associated with changes in insulin action relative to baseline the next morning. Our results suggest that inducing a large carbohydrate deficit after exercise by refeeding energy (mainly as fat with relatively little carbohydrate) enhances the effects of exercise on glucose and lipid metabolism and may be a useful strategy following exercise to counter impaired insulin action in sedentary, overweight adults.

4.4.1 Glucose Storage and Oxidation

The storage and oxidation of glucose cleared from the blood during the glucose infusion was affected by carbohydrate availability following exercise. Consistent with the results of other studies (3, 9), we found an acute bout of exercise increased $R_d$nox (generally assumed to represent glucose storage), and decreased the rate of $R_d$ox during the glucose infusion. Lean, insulin-sensitive individuals have elevated $R_d$nox and lower $R_d$ox during a glucose infusion compared to their overweight, insulin resistant counterparts (9, 18). In obese individuals, exercise shifts glucose metabolism toward, but not completely to, the profile typically observed in lean individuals (9). We

observed this pattern, i.e. a shift in glucose disposal toward greater storage and away from oxidation following the Low-CHO condition but not the High-CHO condition. Similarly, Bogardus et al. reported increased $R_d$nox and decreased $R_d$ox after a bout of glycogen-depleting exercise when subjects fasted after exercise but not when they consumed 100grams of carbohydrate (3). Our results extend the findings of Bogardus et al. by supporting that the effect of prior exercise to bias glucose disposal toward greater storage is attributable to limited carbohydrate intake rather than energy intake; following exercise in both the High-CHO and Low-CHO conditions, all expended energy was refed.

4.4.2 Insulin Action

Insulin action was not improved after exercise in the High-CHO condition, consistent with prior results from our laboratory (2) and studies done in rodents (5, 12, 41). We hypothesized that carbohydrate restriction (Low-CHO) after exercise would promote greater increases in insulin action than the High-CHO condition but, surprisingly, we observed that, insulin action was not affected by carbohydrate availability. The mean change from baseline was approximately +15% in either condition which was not statistically significant. A 15% increase in insulin action, measured 12-14 hours after a single bout of exercise is consistent with what others have shown (1, 3) but smaller than reported increases following exercise of higher intensity (40). Our data are very similar to a those documented in a study by Ben-Ezra et al., who also carefully matched energy intake with exercise energy expenditure following exercise; they observed a 15% increase in insulin action the next morning (1).

In the Low-CHO condition there was a strong positive relationship between ΔR_d/SSPI and carbohydrate deficit – implying that the greater the carbohydrate deficit following exercise, the bigger the enhancement of insulin action relative to baseline. A similar relationship was not observed in the High-CHO condition because subjects were kept in carbohydrate balance (by design) and carbohydrate expenditure matched intake; therefore, there was no carbohydrate deficit in the High-CHO condition to use as a predictor of insulin action. However, in the High-CHO condition we observed a direct relationship between the ΔR_d/SSPI and cardiorespiratory fitness (VO_{2peak}).

A well-known relationship exists between insulin action and cardiorespiratory fitness: individuals with high cardiorespiratory fitness have higher insulin action than individuals with low cardiorespirtatory fitness (13, 19). Our results suggest that insulin action in sedentary, overweight adults with a higher cardiorespiratory fitness (presumably due to genetic factors since they are sedentary) may respond more robustly to exercise than those with low cardiorespiratory fitness. Indeed, the two subjects with the lowest VO_{2peak} had decreased insulin action following exercise compared to baseline.

The strong positive relationship observed between carbohydrate deficit and ΔR_d/SSPI in the Low-CHO condition may be related to the incomplete replenishment of muscle glycogen that results from restricting carbohydrate intake after exercise (6). Low muscle glycogen concentrations are associated with increased insulin action and glycogen promoting enzymes (8, 12, 16, 17). Since we did not measure glycogen concentrations or enzymatic activity, we have a limited ability to draw conclusions

about how glycogen concentration impacted insulin action but it is plausible that muscle glycogen concentrations were lower the Low-CHO condition compared to High-CHO.

There is evidence that insulin action following exercise may be regulated by mechanisms other than a relationship with low muscle glycogen concentrations. Bogardus et al. demonstrated muscle glycogen concentrations were similar regardless of whether individuals fasted or consumed 100grams of carbohydrate following exercise (3). Despite similar muscle glycogen concentrations, insulin action was different and enhanced only in the fasted condition. An alternate mechanism to glycogen concentrations mediating insulin action is that elevated insulin levels following carbohydrate intake have an effect on metabolic gene expression (27) and on the activity of glycogen promoting enzymes like glycogen-synthase kinase-3 (GSK-3) and protein kinase B (PKB or Akt) (38, 39). Pilegaard et al. (27) recently found, exercise followed by high carbohydrate meals, reversed the elevated expression of genes encoding oxidative transport proteins at the mitochondria; when exercise was followed by low carbohydrate meals, the expression of oxidative genes remained elevated.

At the whole body level, our data support that insulin action is being partially mediated by the insulin response to a post-exercise meal. We measured the glucose and insulin response to the post-exercise meals and, as expected, the insulin response to the High-CHO meal was considerably higher than the response to the Low-CHO meal. The insulin area under the curve (AUC) in response to the High-CHO post-exercise meal had a strong negative relationship to ΔR_d/SSPI in the High-CHO condition; the greatest enhancement in insulin action was following a smallest insulin response to the High-CHO meal. A relationship between insulin AUC and ΔR_d/SSPI the Low-CHO condition

was not observed; plasma insulin concentrations were elevated significantly less by the Low-CHO meal than the High-CHO meal and carbohydrate deficit was the best predictor of ΔR_d/SSPI in the Low-CHO condition. Whether the insulin response to the High-CHO meal is causally related to ΔR_d/SSPI cannot be determined by our study and more research is required to address this mechanistic question.

4.4.3 Lipid Metabolism

In order to reduce carbohydrate intake but still reefed all of the expended energy back following exercise, subjects consumed more fat (+30g) in the Low-CHO condition compared to the High-CHO condition. In agreement with a series of studies from the Horowitz Laboratory, we found that insulin action was not impaired by increased fat availability following exercise. It has been hypothesized that exercise may be protective against fatty acid induced insulin resistance (30) because it increases lipid oxidation (15, 32, 33, 37) as well as increases the synthesis of intramyocellular lipids (IMCL) (31). This theory rests on the idea that by directing muscle fatty acids to oxidation or triacylglycerol synthesis, there is less accumulation of fatty acid moieties (diacylglycerol and ceramide) that may interfere with insulin signaling. We did not measure IMCL synthesis or diacylglceryol and ceramide concentrations, although we did measure whole-body fat oxidation and observed it was elevated in the Low-CHO condition compared to BASE. As Randle et al. (28) first demonstrated, increased fat availability usually impairs insulin action (14, 21) but this effect is not observed when high fat intake (or a lipid infusion) occurs against a background of glycogen-depleting exercise (11, 30, 31, 35). Similarly, we found insulin action following exercise was similar

between the Low-CHO and High-CHO conditions despite the consumption of more dietary fat in the Low-CHO condition.

4.4.4 Future Directions

Maximizing the effectiveness of each exercise bout to improve metabolic health is important to sedentary, overweight individuals beginning an exercise program. In addition, the nutritional environment after exercise likely plays a large role in the accrual of exercise benefits on metabolic health. Recently, our laboratory found six-days of endurance exercise followed by high carbohydrate availability blunted the effect of exercise to increase insulin action in sedentary, overweight individuals (2). At the cellular level, expression of genes related to energy metabolism is sensitive to carbohydrate availability (27). We demonstrated, at the whole body level, that measures of glucose and lipid metabolism were also sensitive to carbohydrate availability when energy expenditure was matched with energy intake following exercise. We propose limiting carbohydrate intake after exercise in sedentary, overweight individuals. This strategy may promote greater gains in metabolic health over time compared with unregulated carbohydrate intake; high carbohydrate availability mutes the effects of exercise on metabolic health, but low carbohydrate availability does not.

4.4.5 Limitations

By design, we did not include an experimental condition that fasted (energy deficit) following exercise. The objective of the study was to evaluate the impact of varying carbohydrate availability without the confounding effects of energy imbalance

thus we refed all of the expended energy back following exercise. A logical future would be to add an energy deficit condition to the existing study design to determine how restricting all expended energy compares with restricting only carbohydrate energy. Then it may be determined which strategy may be the best one to maximize the effectiveness of exercise on insulin action; an important question that remains unanswered.

Also, we did not keep protein intake constant in the three experimental conditions. Matching protein intake in all conditions would have been optimal to minimize any impact of differences in protein content on the results. However, adding protein to carbohydrate exaggerates the insulin response by the pancreas (10) which could have compromised our goal of assessing how the insulin response to low or high-carbohydrate availability following exercise is related to the magnitude of change in insulin action.

4.4.6 Summary

It was demonstrated that whole body glucose and lipid metabolism in sedentary, overweight individuals is shifted toward the metabolic profile of a lean individual (e.g. greater glucose storage and higher fat oxidation) by carbohydrate restriction following exercise. Interestingly, this shift was accomplished independently of an energy deficit and did not require total restriction of daily carbohydrate intake. The Institute of Medicine 2005 Dietary Guidelines for Americans suggests adults consume between 45-65% of total daily caloric intake as dietary carbohydrate. In the Low-CHO condition, total daily carbohydrate intake was 48.2 ± 1.3% of the total daily energy intake.

Further research is necessary to determine the compound effect of carbohydrate restriction on metabolic health outcomes like insulin action. Before recommending that sedentary, overweight individuals choose low-carbohydrate foods when they eat a post-exercise meal, it must also be determined how this strategy may affect future exercise performance or other factors that predict metabolic health, besides insulin action.

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APPENDIX A

TABLES

Table 1. Subject Characteristics

	Mean ± SD
Age (yrs)	22.2 ± 1.0
Height (m)	1.71 ± 0.71
Weight (kg)	76.2 ± 13.0
BMI (kg/m^2)	26.1 ± 3.7
Body Fat %	33.5 ± 7.9
FFM (kg)	47.6 ± 11.0
RMR (kcal/day)	1730 ± 268
TDEE (kcal)	2420 ± 376
VO2peak (L/min)	2.63 ± 0.15
VO2Peak (mL/kg/min)	34.6 ± 3.8

All values are expressed as means ± standard deviation (SD). N=10; n=5, men and n=5, women.

Table 2. Exercise Characteristics

	LOW-CHO	HIGH-CHO	P value
VO_2 (mL/kg/min)	23.89 ± 0.86	23.83 ± 0.81	0.84
% VO_{2peak}	69.03 ± 1.32	69.25 ± 1.86	0.78
Energy Expenditure (kcal)	725 ± 35	726 ± 35	0.34
RER	0.94 ± 0.012	0.94 ± 0.016	0.78
Carbohydrate Expenditure (g)	139.5 ± 11.0	139.7 ± 9.8	0.41
Exercise Duration (min)	83.5 ± 3.5	83.5 ± 3.0	0.57

All values are expressed as means ± SEM. $p > 0.05$ denotes non-significance. VO_2, oxygen consumption; RER, respiratory exchange ratio.

Table 3. Day 1 Food Records

	CONTROL	LOW-CHO	HIGH-CHO	P value
Daily Energy Intake (kcal)	2457.5 ± 105.0	2442.6 ± 96.2	2452.9 ± 107.0	0.950
PM Meal (kcal)	**724.3 ± 35.2**	**689.8 ± 29.5**	**724.2 ± 35.5**	0.520
Meal Carbohydrate (g)	96.5 ± 2.9	30.8 ± 1.3[ab]	138.9 ± 8.4[a]	0.001
Meal Fat (g)	27.7 ± 1.1	43.8 ± 2.[ab]	12.6 ± 1.6[a]	0.001
Meal Protein (g)	16.8 ± 1.5	32.5 ± 1.7[ab]	13.5 ± 1.3	0.001
% Carbohydrate	**54.1 ± 1.6**	**17.8 ± 0.1**	**76.6 ± 2.5**	
% Fat	32.7 ± 0.7	56.9 ± 0.4	15.8 ± 2.4	
% Protein	9.2 ± 1.1	18.7 ± 0.3	7.3 ± 0.7	

Values are means ± SEM. [a] Significantly different from Control, ($p<0.05$).
[b] Significantly different from High-CHO, ($p<0.05$).

Table 4. Plasma Metabolites

	CONTROL	LOW-CHO	HIGH-CHO	P value
$Glucose_{Rest}$ (mM)	5.2 ± 0.2	4.8 ± 0.3[ab]	5.1 ± 0.3	0.001
$Glucose_{Infusion}$ (mM)	9.3 ± 0.5*	9.1 ± 0.5*	9.3 ± 0.7*	0.197
$Insulin_{Rest}$ (ρM)	74.4 ± 9.6	66.6 ± 9.0	67.6 ± 8.1	0.644
$Insulin_{Infusion}$ (ρM)	266.2 ± 34.1*	253.9 ± 43.1*	268.4 ± 33.4*	0.888
HOMA-IR	2.9 ± 0.4	2.4 ± 0.4	2.6 ± 0.4	0.377
C-ISI	4.1 ± 0.6	4.9 ± 0.9	4.4 ± 0.8	0.546

Values are means ± SEM. *Significantly different from Rest ($p<0.01$). [a] Significantly different from Control, ($p<0.05$). [b] Significantly different from High-CHO, ($p<0.05$). Rest, CIG-SIT time points 75, 90 minutes averaged; Infusion, CIG-SIT time points 50, 55, 60 averaged.

Table 5. Glucose Metabolism

	CONTROL	LOW-CHO	HIGH-CHO	P value
Glucose Rd_{tot} (μM/kg/min) Rest	10.2 ± 0.7	10.2 ± 0.9	11.0 ± 1.2	0.980
Glucose Rd_{tot} (μM/kg/min) Infusion	33.2 ± 2.7*	35.7 ± 3.6*	37.1 ± 3.5*	0.389
$R_d$ox (μM/kg/min) Infusion	17.1 ± 2.2	8.6 ± 1.3[ab]	12.2 ± 1.2	0.005
$R_d$nox (μM/kg/min) Infusion	16.9 ± 3.5	27.2 ± 3.2[a]	24.9 ± 3.7	0.042
Insulin Action (μg/kg/min/pM)	26.4 ± 3.8	30.4 ± 4.4	30.2 ± 5.2	0.303
HGP_{Rest} (μM/kg/min)	10.9 ± 0.7	10.8 ± 0.9	11.7 ± 1.2	0.983
$HGP_{Infusion}$ (μM/kg/min)	7.6 ± 2.0	9.3 ± 2.1	10.3 ± 2.9	0.540
% Hepatic Suppression	36 ± 14	37 ± 13	27 ± 11	0.847

Values are means ± SEM. *Significantly different from Rest ($p<0.01$). [a] Significantly different from Control, ($p<0.05$). [b] Significantly different from High-CHO, ($p<0.05$). $R_d$tot, glucose rate of disappearance; $R_d$ox, carbohydrate oxidation; $R_d$nox, non-oxidative glucose disposal. Rest, CIG-SIT time points 75, 90 minutes averaged; Infusion, CIG-SIT time points 50, 55, 60 averaged.

Table 6. Lipid Metabolism

	CONTROL	LOW-CHO	HIGH-CHO	P value
FFA_{Rest} (mM)	0.595 ± 0.042	0.692 ± 0.043[a]	0.651 ± 0.047	0.046
$FFA_{Infusion}$ (mM)	0.396 ± 0.036	0.455 ± 0.038	0.414 ± 0.027	0.430
Δ FFA	0.20 ± 0.03	0.24 ± 0.04	0.24 ± 0.04	0.490
$FatOx_{Rest}$ (mg/min)	80.7 ± 9.6	109.8 ± 10.5[a]	96.7 ± 13.8	0.035
$FatOx_{Infusion}$ (mg/min)	35.8 ± 8.1*	93.4 ± 10.6[ab]	72.0 ± 7.4[a]	0.001
Δ FatOx	45.5 ± 9.5	19.42 ± 7.2	34.0 ± 8.7	0.191
RER_{Rest}	0.83 ± 0.013	0.76 ± 0.010[a]	0.80 ± 0.028	0.002
$RER_{Infusion}$	0.93 ± 0.025*	0.81 ± 0.016*[ab]	0.85 ± 0.011*[a]	0.003
Δ RER	0.082 ± 0.024	0.050 ± 0.016	0.068 ± 0.020	0.621

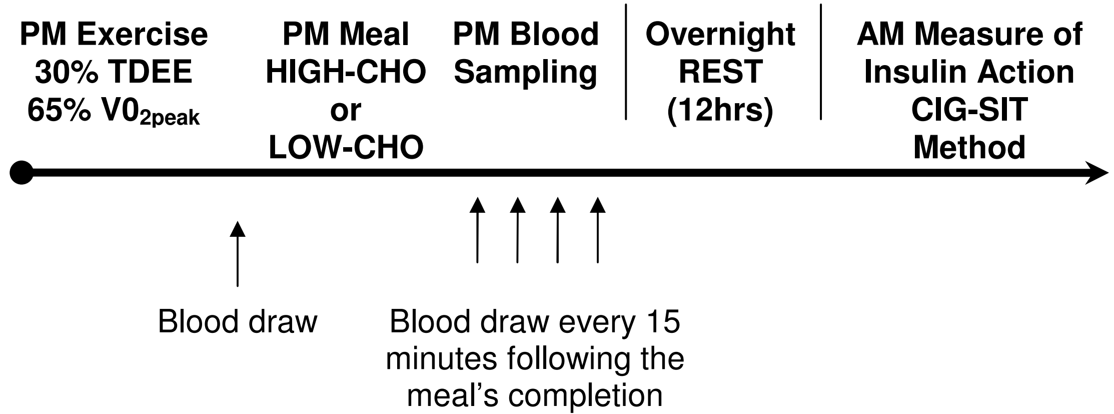
Values are means ± SEM. *Significantly different from Rest (p<0.01). [a] Significantly different from Control, (p<0.05). [b] Significantly different from High-CHO, (p<0.05). FFA, free fatty acids; FatOx, fat oxidation; RER, respiratory exchange ration; Δ from rest to infusion during CIG-SIT. Rest, CIG-SIT time points 75, 90 minutes averaged; Infusion, CIG-SIT time points 50, 55, 60 averaged.

APPENDIX B

FIGURES

Figure 1. Experimental Protocol

A. Timeline



B. Continuous Infusion of Glucose with Stable Isotope Tracers

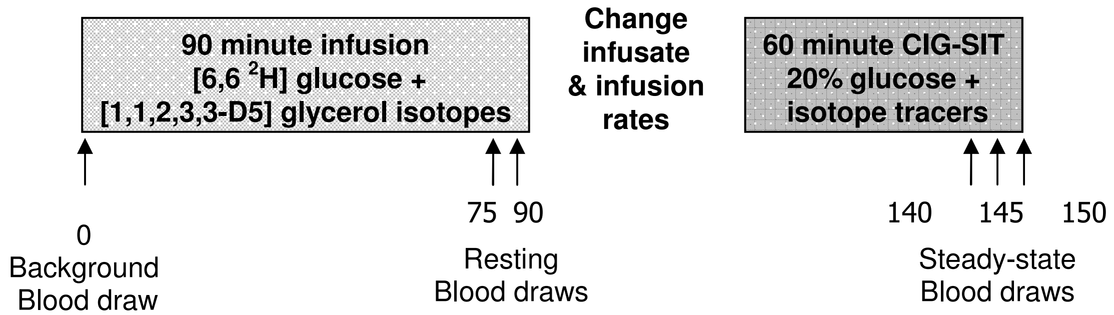
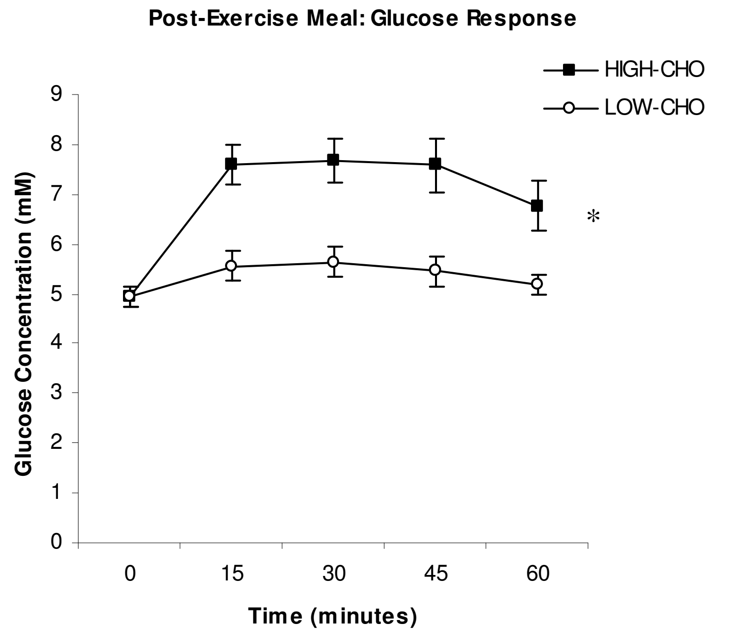
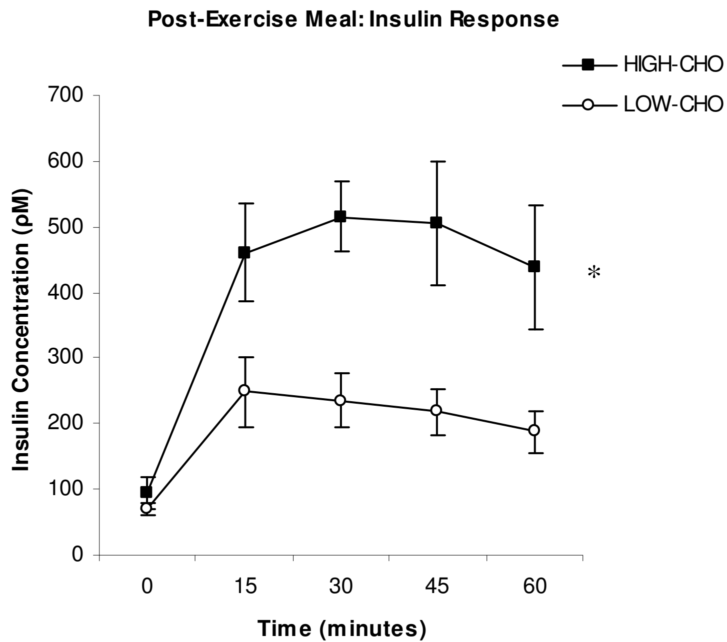


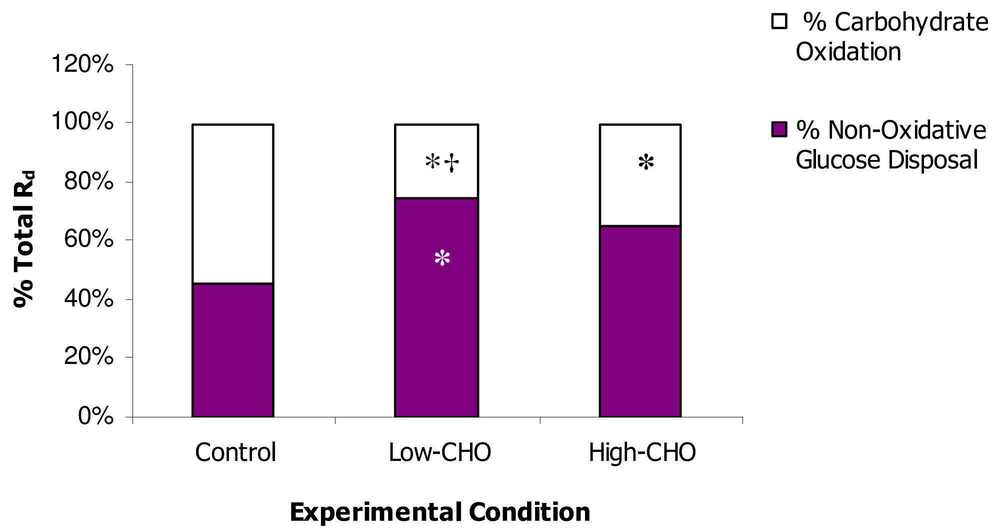
Figure 2. Glucose and Insulin Response to the Post-Exercise Meal





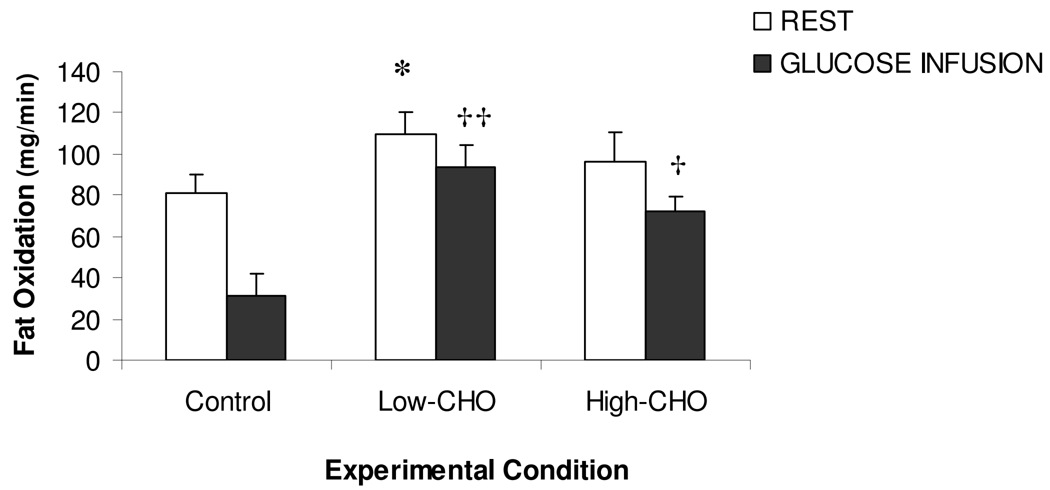
* Glucose and insulin responses following High-CHO meal were significantly greater than Low-CHO meal ($p<0.001$).

Figure 3. Glucose Uptake Separated into Non-Oxidative and Oxidative Components



* Significantly different from CON, $p<0.05$; † Significantly different from High-CHO, $p<0.05$.

Figure 4. Fat Oxidationn



* Significant compared to control, p<0.05 at rest. † Significant increased compared to control, p<0.05; †† Significantly increased compared to control and High-CHO, p<0.05.

Figure 5. **The Relationship between Carbohydrate Deficit and Change in Insulin Action in the Low-CHO Condition.**

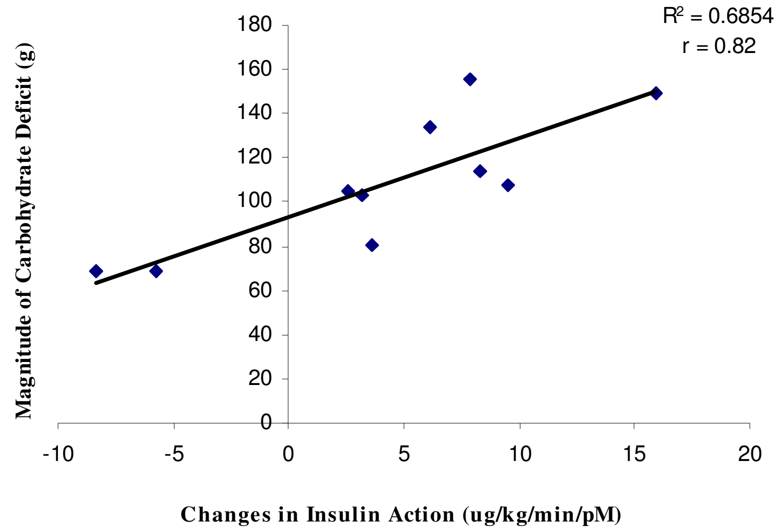
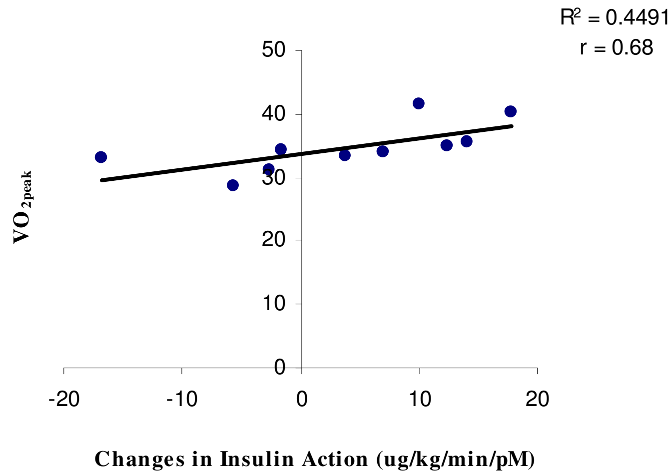


Figure 6. The Relationship between Cardiorespiratory Fitness (VO_{2peak}) and Changes in Insulin Action in the High-CHO Condition.



APPENDIX C
DOCUMENTS

1. Informed Consent Document

The effect of meal composition on insulin action following an acute bout of exercise

Investigators: Barry Braun, Ph.D. (413-577-0146)
Todd Hagobian, M.S. (413-545-0331)
Brooke Stephens, M.S. (413-545-0331)
Carrie Sharoff, B.A. (413-545-0331)
Kaila Holtz, B.S. (413-265-3792)
Stuart Chipkin, M.D. (413-545-0089)

Before participating in this research study, your informed consent is required. Please read each section of the document carefully and initial each page. Sign your name on the last page if you agree to participate in this study. The investigators have read and understand the Assurance of Compliance with the Office of Human Research Protection Regulations for Protection of Human Research Subjects. A copy of this document can be found at http://www.umass.edu/research/humsub.html. The University Human Subjects Review Committee has approved this study and the recruitment of subjects.

Purpose: The prevalence of insulin resistance (impaired uptake of blood sugar following a meal) and type 2 diabetes in adults in modern Western society has increased dramatically over the past 50 years. It is estimated that approximately 17% of adults are insulin resistant and another 5-8% have type 2 diabetes. Studies have suggested that decreased physical activity has contributed significantly to the increased prevalence of insulin resistance and type 2 diabetes. A single bout of exercise, on the other hand, improves the action of insulin (a hormone that lowers blood sugar) up to 48 hours following the exercise bout. The consumption of a meal or caloric beverage(s) after exercise may act to counter the effect of exercise. This may depend upon how much time lapses between the end of exercise and when the meal is consumed and/or the type or composition of the meal (i.e. fat, protein, and carbohydrate content). Our lab is currently analyzing the work done on meal timing. Therefore, the purpose of this study is to determine the effect of meal composition on insulin action following an acute bout of exercise.

PLEASE NOTE: Participation throughout this study is completely voluntary. You may withdraw consent at any time in writing or by telephone (413-545-0331) and discontinue participation in the study without prejudice to you. Please voice any and all concerns that you have regarding any of the procedures, risks and benefits, your rights as a subject, or the study itself. Maximizing your safety and comfort is important. If you have any problems or questions, do not hesitate to contact Dr. Barry Braun (413-577-0146, home: 549-0027), Kaila Holtz (413-265-3792, cell) or Dr. Stuart Chipkin (545-0089). If you would like to discuss your rights as a participant in a research study or wish to speak with someone not directly involved in the study, you may contact the Human Subjects Coordinator at humansubjects@ora.umass.edu or (413) 545-3428.

Requirements: You have been asked to participate in this study because you are 18-55 years old, NOT pregnant, in overall good health, and do not have any risk factors for cardiovascular disease, type 2 diabetes, respiratory disease or any other metabolic disease. In addition, you do not use tobacco products, are not actively trying to lose

weight, and are not taking anything (pharmaceutical or herbal medications or dietary supplements) that could interfere with our measurements (for example; metformin, insulin, ephedra, ginseng, guarana, chromium). If you are uncomfortable having your blood taken you should not participate in the study.

Briefly, you will undergo a maximal oxygen consumption test (a test to measure maximal aerobic capacity), body composition measurement, three bouts of endurance exercise performed on a treadmill or stationary bicycle (whichever you prefer) and four tests to measure your insulin action to determine how effectively your body uses the hormone insulin to take sugar out of your blood. You will be given a specific meal to eat on the day prior to the test to measure insulin action. Most visits will take place in the Energy Metabolism Laboratory (Room 3, Totman Building) at the University of Massachusetts Amherst. The DEXA scan will take place at University Health Services. Parking and directions to the facilities will be made available.

Should you decide to participate in this study, you will be asked to follow the general testing schedule outlined below.

Overview of Testing Schedule:
You will be asked to report to the laboratory for a total of 10 visits

Visit 1: sign inform consent (approximately 1 hour)
Visit 2: subject qualification session, measurement of body composition, resting energy expenditure and maximal oxygen consumption test (approximately 3 hours)
Visit 3, 5, 7, and 9: endurance exercise bout, food intake and blood draws (approximately 3 hours)
Visit 4, 6, 8, and 10: test of insulin action, the morning following the exercise bout or control trial (approximately 3 hours)

Visit 1: Sign inform consent. You will be provided with a full description of the study and have the opportunity to ask any questions. At this time you should inform the investigators of any other studies that you are participating in. Participating in other studies may exclude you from the study. If you are interested in participating in the study, you will be familiarized with the exercise procedures and equipment, and an appointment will be made for a measurement of your maximal aerobic capacity and body composition.

Visit 2: Subject qualification, body composition, resting energy expenditure and physical capacity test. You will also be asked to fill out a Physical Activity Readiness Questionnaire (PAR-Q) and a Health History form that will be interpreted by Dr. Barry Braun or Brooke Stephens. If you have any food allergies please let us know. Your aerobic capacity and body composition will be measured (see below).

Body composition: Body composition will be measured using a whole body X-ray called Dual-Energy X-ray Absorptiometry (DEXA). You will need to wear clothing that contains no metal (zippers, snaps, etc.) and lie quietly on a padded table while a very small amount of x-rays are passed through your body and measured by a moving detector located above you. The procedure takes about 6-11 minutes. The total amount of x-rays you will be exposed to

is relatively small (approximately 2 millirems, less than 1/20 of the radiation received during a single chest-x-ray) and poses no known health risks.

Resting Energy Expenditure: You will rest quietly for 30 minutes either lying down or reclining in a chair in a darkened room. After 30 minutes, breath samples will be collected for 20 minutes. You will be wearing a heart rate monitor and breathe into a mouthpiece and tube. A clip will be placed on your nose so that you can only breathe through your mouth. This test will provide a measurement of your resting metabolic rate and will estimate the number of calories you burn over 24 hours.

Maximal oxygen consumption: Maximal oxygen consumption will be determined by exercising on a stationary bicycle or jogging on a treadmill, whichever you prefer. You will begin pedaling or walking/jogging at a very easy workload as a warm-up. If you are on the bike, you will continue to pedal at the same rate while the tension is increased slightly every 2 minutes. If you are on the treadmill, you will continue to walk/jog at the same pace and the incline of the treadmill will be increased every 2 minutes. It will become more and more difficult to maintain your pedaling or walking/jogging rate as the test progresses. The test will be stopped when you feel you can no longer continue (test usually lasts a total of 8-15 minutes). This test is physically demanding and you may feel fatigued after the test. The results of this standard test will be used to set the appropriate level of physical work during the exercise test. If you are at an increased risk for a cardiac event, are a male over the age of 40, or a female over the age of 50, you will undergo a medical examination prior to enrollment in the study and a physician will be present throughout the test of maximal oxygen consumption.

Visit 3, 5, 7, 9: Endurance exercise, food intake and blood draw: You will report to the Energy Metabolism Laboratory to complete a bout of exercise on a treadmill or stationary bicycle (see below for details) or the no exercise control on four separate occasions. After each exercise bout has ended, you will be provided a meal to eat. The exercise will be the same each trial, but the composition of the meal (i.e. the amount of fat, carbohydrate, or protein) will vary. Approximately one hour after exercise, six blood draws will be taken to measure blood glucose and insulin concentrations in response to the meal.

Exercise: You will either pedal on the stationary bicycle or jog on a treadmill at a comfortable pace. You will be wearing a heart rate monitor and breathing into a mouth piece. The pedaling resistance or treadmill grade will be adjusted during the first 15 minutes of exercise until the rate at which you are working is steady at the desired value. The exercise should feel slightly difficult but not painful; you will probably feel fatigued near the end but will not be giving an all-out effort. You will pedal or jog at this workload for 60-120 minutes. Blood and breath samples will be taken the first 15 minutes during exercise as described above.

Food Intake: All meals following exercise will be provided. The meal will replace all of the expended calories during exercise. The meal will vary in carbohydrate content and will be provided in liquid form.

Blood Draws: One hour following the meal, blood will be drawn for plasma glucose and insulin analysis. A catheter (a small flexible tube) will be inserted into a forearm vein by

a trained professional for blood sampling. Blood collection will be performed under sterile conditions by trained individuals. Blood samples of 3ml each will be taken immediately after the catheter is inserted and at 15, 30, 45, 60 minutes following the post-exercise meal. There is a risk of some immediate discomfort due to the needle insertion, as well as bruising that should subside within a week.

Visit 4, 6, 8, and 10: Test of insulin action:

You will be asked to report to the Energy Metabolism Laboratory in the fasting condition (not having anything to eat or drink (except water) after your exercise bout and meal the night before your visit).

A catheter (a small flexible tube) will be inserted into a forearm vein by a trained professional for blood sampling. A second catheter will be placed in a forearm vein of the opposite arm to allow us to add a glycerol (a part of a fat molecule) and glucose (sugar) stable isotope directly into your bloodstream. A second catheter will be placed in a forearm vein of the opposite arm to allow us to add a precise amount of glucose (sugar) solution in addition to a glycerol and glucose stable isotope solution directly into your bloodstream.

Both the glucose and glycerol stable isotopes are slightly different forms of the glucose and glycerol present in your blood. The isotopes allow us to determine how much fat and carbohydrate (sugar) your muscles are using. They are not radioactive and are used routinely for measurements in human volunteers. The normal glucose (sugar) molecule is changed slightly by adding 2 extra particles (neutrons) to make the glucose used in this study. The glycerol stable isotope is similar to the glucose isotope but provides an indicator as to the amount of fat you are using rather than carbohydrate.

While you are resting comfortably, a blood sample (5ml) will be collected from one of the catheters. Immediately following this blood sample, a small quantity of the glucose (sugar) in a solution of sterile water will be added through the other catheter. A pump will then be connected to the catheter and the glucose solution as well as the glucose and glycerol isotope (a 5th of a teaspoon) will be slowly added through the catheter for period of 150min (2.5hrs). Blood samples will be taken at 75, 90, 140, 145, and 150min. After 150 minutes the catheters will be removed. You will be free to go as soon as we are satisfied that you are completely recovered from the procedures.

Risks and Discomforts:
All possible attempts will be made to minimize the risks involved. Trained individuals will conduct all laboratory procedures with your well being as their first priority. All procedures will be explained and demonstrated until you are comfortable with the proposed study. NOTE: Since the catheterization and blood drawing procedures are a critical element in this study, if you are at all uncomfortable with having your blood taken you should not participate.

X-ray exposure during DEXA: The total amount of radiation you will be exposed to is small (approximately 2 millirems, less than 1/20 of the radiation received during a

single chest-x-ray) and poses no known health risks in non-pregnant women. All female subjects will be provided with a standard pregnancy home-test kit to minimize any potential risk

Exercise: During any type of exercise, especially strenuous exercise, there are slight health risks, along with the possibility of fatigue and muscle soreness. These health risks are small in people with no prior history of cardiovascular, respiratory or musculoskeletal disease or injury. Any ordinary fatigue or muscle soreness is temporary and usually lasts 24-96 hours.

Catheterization: The total amount of blood taken from you during the entire study is relatively small, 180ml taken over the course of 1.5-2 months. Each experimental condition (there are four in the study) involves three catheterizations. The first catheterization is directly after the evening exercise bout and before the post-exercise meal. This catheter will be removed after the five blood draws (3ml each) following the post-exercise meal are taken. The second and third catheters will be inserted one after the other the morning following the exercise bout for the test of insulin action. One catheter is used for the isotope infusion, the other to draw blood from. The test of insulin action requires 6 blood draws of 5ml over two and a half hours and both catheters will be removed immediately following the test. Over the study you will have 12 catheters inserted for blood collection. There are some minor risks involved with catheters. To minimize these risks, catheters will only be inserted by trained individuals, using sterile technique at all times. There may be some pain associated with the needle stick at the moment of insertion, followed by minor discomfort of having the needle withdrawn and the catheter advanced slightly into the vein. There can be local infection if the site is not kept clean following the procedure. Some slight bleeding may also occur following the withdrawal of the catheter. There is the possibility of bruising of the skin in the area around the catheter that poses no health risk and should subside within a week. Fainting also occurs in some persons when they sit or stand following the drawing of blood.

Infusion of isotopes: There is a slight possibility of an anaphylactic reaction (fever, drop in blood pressure, shortness of breath) to the isotope infusion if the solutions contain impurities. All solutions are prepared under sterile conditions from compounds that have been tested for sterility and found to be non-reactive. The risk is very slight. In performing over 300 of these types of studies over 13 years, the principal investigator has never observed such a reaction.

Treatment of research-related injuries: In the unlikely event of an injury resulting directly from participation in this study, investigators will assist you in every way to ensure you get proper medical attention. The University of Massachusetts does not have a program for compensating subjects for injury or complications related to human subjects research but the study personnel will assist you in getting treatment. Dr. Braun can be reached at 413-577-0146 and Ms. Holtz can be reached at 413-265-3792. It also should be understood that by agreeing to participate in this study, you are not waiving any of your legal rights.

Benefits: You will not gain any direct benefit by participating in this study. You will gain some specific information about your personal health, including your glucose tolerance, aerobic capacity, body composition and bone density. If you have any

questions about this information, you are encouraged to contact your own doctor or consult the yellow pages for a qualified physician. You will be directly contributing to our knowledge and assessment of how the combination of exercise and food intake can be used to decrease the risk for developing Type 2 diabetes and cardiovascular disease.

Compensation for your time and effort: There is $100 in financial compensation for your participation in this study. If for any reason you have to withdraw from the study the compensation for your participation will be pro-rated. You will receive $25 for each of the experimental conditions you have completed. At the end of the study, we will also provide information and assist you with any questions you have regarding making beneficial lifestyle changes in terms of diet and exercise.

Confidentiality: The information obtained in this study will be regarded as privileged and confidential. If the results of this study are written in a scientific journal or presented at a scientific meeting your name will not be used. All records will be maintained in a locked file cabinet accessible only to the investigators in this study. We may be required to make records available for review by Food and Drug Administration (FDA) if they request them.

Request for more information: You are encouraged to express any questions, concerns or doubts regarding the study at any time. The investigators will attempt to answer all questions to the best of their ability. The investigators will conduct this study with your best interest, safety, and comfort in mind.

Freedom of Consent: Again, participation throughout this study is completely voluntary. You may withdraw consent at any time in writing or by telephone (413-545-0331) and discontinue participation in the study without prejudice to you or your medical care at UMASS Amherst. Please voice any and all concerns that you have regarding any of the procedures, risks and benefits, your rights as a subject, or the study itself. Maximizing your safety and comfort is important. If you have any problems or questions, do not hesitate to contact. Dr. Barry Braun (413-577-0146, home: 413-549-0027), Dr. Stuart Chipkin (413-545-0089, cell 413-433-7418) or Kaila Holtz (265-3792 cell).

I confirm that ________________________________ has explained to me the purpose of the research, the study procedures that I will undergo and the possible risks and discomforts as well as benefits that I may experience. Alternatives to participation in the study have been discussed. I have read and understood the consent form. I also understand that cells, blood, or other specimens removed from me during the course of the study may be valuable for scientific, research or teaching purposes. I authorize the use of my cells, blood, or other specimens for these purposes. Therefore, I agree to participate as a subject in this study.

____

Subject's Signature   Date

____
Name (printed)

____
Local Address/Phone Number

Witnessed by:

____	________________
Investigator's Signature	Date

____
Investigator's Name (printed)

2. Health and Fitness Questionnaire

HEALTH & FITNESS HISTORY

Name: ____________________ **E-mail:** __________________

Date of Birth: __________

Phone: Home ____________ Work: ______________

Height: _____ **Weight:** _____

1. Have you ever been diagnosed as having any of the following and if yes, how are you currently treating the condition?

Y N High Blood Pressure

Y N High Cholesterol or High Triglycerides

Y N Diabetes

Y N Hypoglycemia (low blood sugar)

Y N Asthma

2. Does anyone in your family (immediate family including your grandparents) have a history of cardiovascular disease? (heart attacks, strokes, etc.) Please explain:

3. Does anyone in your family (immediate family including your grandparents) have a history of type 2 diabetes? Please explain:

4. Have you ever had a glucose tolerance test? Y N
   If yes, what were the results?

5. Have you ever had a fasting blood sugar test? Y N
   If yes, what were the results?

6. For women:

   - Did you have gestational diabetes during any pregnancy?

   - Were you ever tested for gestational diabetes?

   - How much did your children weigh at birth?

- For women: are you on hormonal birth control (pill, patch, etc) or estrogen replacement? Describe in detail:

  - What was the date when your last period started? Is your cycle regular?

7. Do you have any neurological problems including fainting, dizziness, headaches or seizures?

8. Do you have any orthopedic or other health problems that may affect your ability to perform exercise? If yes, please explain.

9. Are you currently taking any medications, including over-the-counter drugs such as aspirin, Tylenol or Ibuprofen? Please list:

10. Do you smoke or use smokeless tobacco?

11. Do you drink coffee or other caffeinated beverages? Y N What kind, how much and how often?

12. Please list all vitamins, minerals and herbs and other nutritional supplements you're taking:

13 Do you have any food allergies or intolerances? Please describe:

14. How would you describe the type of diet you currently eat? Have you recently been on any special diets? What kinds of diets have you used to lose weight or lower cholesterol? Please list and describe:

15. What changes have you made in your diet in the last 6 months?

16. Do you exercise regularly? What kinds of exercise?

How often?

Please describe how much walking you do on a daily basis:

17. How does your current exercise and physical activity compare to 6 months ago? 1 year ago?

18. How much did you weigh at birth (if known)?

19. Have you had a physical exam in the past two years? Y N Please describe your assessment of your overall health:

Participant's Signature: ________________________________
Date: ___________________

APPENDIX D

SUBJECT HANDOUTS

Meal Composition Study
Subject Summary

Name: Jane Doe

Measurement	You	Normal/Healthy
BMI	21	Less than 25 kg/m^2
Body fat %	33.3	Men: Less than 25% Women: Less than 32%
Aerobic Capacity (mL/kg/min)	34.8	Men: Greater than 44 mL/kg/min Women: Greater than 39 mL/kg/min
Fasting Blood Sugar (mg/dL)	93.6	Less than 100 mg/dL
Insulin Sensitivity (C-ISI 0-12 Index)	2.33	Score greater than 3.0
Fasting Triglycerides (mg/dL)	82.5	Less than 150 mg/dL
Energy Expenditure (Daily) in kcals	2128	To lose weight the healthy way, eat 300 to 500kcals less.
Moderate Intensity HR (65% of VO_{2peak})	150-165	When exercising, try and maintain this heart rate for 30 minutes.

Metabolic Syndrome Risk Factors Measured in this study:
- Elevated BMI or Body Fat
- Elevated Fasting Glucose
- Low Insulin Sensitivity
- Elevated Triglycerides

The presence of 3 or more Metabolic Syndrome factors is associated with increased risk for cardiovascular and type 2 diabetes.
For managing both long- and short-term risk, changing your lifestyle can have a big impact. These lifestyle interventions include*:

- Weight loss to achieve a desirable weight (BMI less than 25 kg/m^2)
- Increased physical activity, with a goal of at least 30 minutes of moderate-intensity activity on most days of the week
- Healthy eating habits that include reduced intake of saturated fat, trans fat and cholesterol.

* From the American Heart Association

Attached is some more information about healthy eating and tips to increase your physical activity by the American Dietetics Association.

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