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The Effect of Physical Activity on the Insulin Response to Frequent Meals

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Abstract

Long, uninterrupted bouts of sedentary behavior are thought to negatively influence insulin sensitivity, and may impact metabolic function regardless of adherence to general physical activity guidelines. The purpose of this study was to determine the combined effect of physical activity (1 h continuous exercise v. intermittent exercise throughout the day) and meal consumption on glucose excursions, insulin secretion, and appetite markers in obese individuals with prediabetes. **Methods:** Eleven healthy, obese subjects (>30 kg/m^2) with prediabetes underwent 3, 12 h study days including sedentary behavior (SED), exercise (EX; 1h morning exercise, 60-65% VO_2 max), and physical activity (PA; 12 hourly, intensity-matched 5-minute bouts). Meals were provided every 2 h. Blood samples were taken every 10 min for 12 h. Baseline and area under the curve (AUC) for serum glucose, insulin, c-peptide, total PYY concentrations, and subjective appetite ratings; as well as insulin pulsatility were determined. **Results:** No significant differences in baseline glucose, insulin or c-peptide concentrations across study days were observed ($P>0.05$). Glucose AUC (12 h and 2 h) were significantly different across study days, with AUC attenuated in the PA condition compared to the EX condition ($P<0.05$). The 12 h incremental insulin AUC was reduced by PA compared to SED (173,985±3556.8 v. 227,352±4581.2 pmol/L*min for 12 h, respectively; $P<0.05$). Similarly, a significant main effect of condition in the 2 h insulin AUC was found, with the PA condition being reduced compared to SED condition ($P<0.05$), but no differences between the EX and SED conditions. A significant reduction in 2 h c-peptide AUC was demonstrated with EX and PA compared to the SED condition ($P<0.05$). Deconvolution analysis of insulin secretion revealed no significant differences between experimental conditions. There were no significant differences in total PYY between experimental conditions, though subjective measures of hunger and satiety were reduced with continuous and intermittent exercise. **Conclusions:** Short bouts of physical activity throughout the day attenuate glucose excursions and improve insulin clearance compared to an exercise day with 1 h of morning exercise. Further, both continuous and intermittent exercise mechanisms that improve satiety in obese individuals are not related to changes in concentrations of PYY.

THE EFFECT OF PHYSICAL ACTIVITY ON THE INSULIN RESPONSE TO FREQUENT MEALS

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Chapter I: Introduction

Obesity affects more than 30% of all American adults (91, 192), and has a related annual cost of nearly $150 billion in the U.S. alone (4). The combined effects of chronic conditions associated with obesity at middle age (50 years), including type 2 diabetes (T2D), confer a mortality risk that is 2 to 3 times higher than is found in lean individuals (5). Obesity is closely linked to a series of chronic diseases, one of the most insidious being T2D. One of the early sign of T2D is an impaired fasting blood glucose concentration (108), a common characteristic of the obese individual. Additionally, abnormalities in the action and release of insulin, associated with T2D, are strongly linked to obesity (51, 201). An estimated 40 million (13%) Americans over the age of 20 have type 2 diabetes (T2D) (41, 53), a disease where insulin action, and the resulting physiological response to glucose ingestion is severely impaired. An additional 90 million Americans (30%) are classified with prediabetes (53), a condition which is characterized by impaired glucose tolerance, increased insulin secretion, and a loss of sensitivity of target tissues.

Regardless of the etiology of any particular case of diabetes, the common underlying factor that concerns medical, exercise, and nutritional professionals regarding this condition is hyperglycemia, or the presence of high blood glucose levels in the affected individual. The condition of hyperglycemia initiates damage to the vasculature, nervous and renal systems. This damage leads to common complications of diabetes, at the microvascular (e.g. neuropathy, retinopathy, blindness), and macrovascular (e.g. heart disease, amputation) levels (153). While the precise mechanism behind the damaging effects of hyperglycemia on vasculature is unknown, Shahab (221) reports that impaired endothelial function is a hallmark of the individual with diabetes. Similarly, the neural damage inflicted by hyperglycemia is multi-faceted, with the

upregulation of protein kinase c and inflammatory cytokines likely contributors (218). The complications associated with diabetes are life-altering, and may lead to reductions in physical activity and a further cycle of complications. The normalization of blood glucose levels has been shown to restore adequate endothelial and renal function and prevent further damage (218, 221), which confirms the importance of maintaining healthy glucose uptake.

Proper glucose tolerance is maintained by both pancreatic insulin release and the effective action of insulin at sensitive tissues in the periphery (201). The effectiveness of the glucose/insulin mechanism can be influenced, positively or negatively, by changes in dietary and physical activity habits. These habits may interact to influence the complex relationship between hormones, including insulin and appetite hormones, and obesity (22, 157, 211).

An important contributor to glucose tolerance and insulin sensitivity is an individual's total dietary intake, though the pattern of meal consumption has also been examined as a means of enhancing health (175). It has been demonstrated that there are differential glucose, hormonal, and appetite responses to alterations in the frequency of meals consumed daily, even when calorie content is identical (21, 86, 137, 259). However, the potential health benefits of these alterations has not been consistent, as some studies demonstrate improved fasting blood glucose and glucose tolerance outcomes (86, 259), while others do not (137). While many concerns remain regarding the efficacy of frequent meal consumption on health, overall trends show that 75 to 85% of Americans have adopted a snacking (or grazing) eating pattern (56, 275). According to a recent study, approximately one-third (32%) of selected adult samples adhering to a 3 meal, 2 snack pattern daily (150). A recent study in our lab has demonstrated that an isocaloric increase in the frequency of high-carbohydrate meals led to an augmented 12 h glucose concentrations (119). In light of an increased prevalence of frequent meal consumption

and the current obesity epidemic, it is important to consider how frequent meal consumption may affect the daily glucose excursions and pattern of insulin secretion in individuals at risk for glucose intolerance. Similarly, an increase in isocaloric meal frequency has also been shown to disrupt the relationship between appetite hormones and insulin, and may indicate the potential for a negative effect of increasing meal frequency (226).

An additional, though highly variable, component of everyday life that affects glucose tolerance and the progression towards T2D is participation in physical activity. Exercise is known to positively influence variables related to glucose tolerance and blunt the rise in blood glucose subsequent to nutrient consumption (121, 191), presumably through an increase in the short-term non-insulin dependent glucose uptake into active muscle tissue, or an increase in the sensitivity of muscle tissue to insulin stimulation (27, 97). Due to this alternate means of stimulating glucose uptake, the pancreas does not release as much insulin in order to ensure postprandial systemic glucose uptake (243) when exercise is performed. Exercise has also been shown to influence appetite perception, and may alter the release and action of appetite hormones (84, 105).

While exercise is an excellent means of improving glycemic control and appetite, recent reports on physical inactivity indicate that as many as 25% of Americans partake in no form of leisure time exercise or physical activity (44), and this may be an underrepresented estimation. A long-term study of individuals in Australia reveals that physical inactivity is strongly associated with an increased incidence of diabetes (170). This increasing trend of inactivity may be a major contributor to a variety of health problems found in developed nations, particularly related to glucose-tolerance and T2D (24, 74, 170).

Reductions in glucose uptake due to a lack of physical activity may be very important when considering experimental outcomes associated with glucose tolerance. In support of this theory, the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab) has yielded strong epidemiological relationships between increased sedentary behaviors (e.g. TV viewing time, low activity levels on a pedometer) and characteristics of metabolic disease risk, including the metabolic syndrome (24, 74), and abnormal glucose tolerance (75). These findings are frequently shown to be independent of the effects of adherence to a regular exercise program (24, 74, 75), and point to the importance of frequent 'lifestyle activity' interspersed throughout the day. Lifestyle activity is known to compose a much larger portion of the total daily energy expenditure than a typical continuous exercise bout (106), and consideration of the possible effects of lessening, or even excluding this important component of daily life remains very important.

Due to the adaptability of the body to changes in physical activity and dietary patterns, a better understanding of how to properly align dietary and physical activity guidelines for the maintenance of proper glucose tolerance is important. Dividing an individual's sedentary time throughout the day with short, purposeful exercise bouts may offset the potential negative effects of increased meal frequency on the hormonal response to nutrient intake. This is particularly important in the obese individual with prediabetes, as the progression from normal to impaired glucose tolerance occurs over the long-term (82, 205), and provides an important window of opportunity for intervention in the months or years before the probable onset of T2D. Whether the potential protective effects of intermittent exercise differ from those found with intensity and duration matched continuous physical activity remain to be established in this cohort.

Specific Aims and Hypothesis

The primary purpose of this study was to examine the effects of continuous vs. intermittent physical activity participation on glucose excursions, insulin secretion, and appetite regulation in response to frequent meal consumption in obese individuals with prediabetes. The specific aims of this project were:

1) To determine the effect of continuous versus intermittent physical activity on glucose excursions and insulin secretion in response to frequent meal consumption over 12 h in obese individuals with prediabetes.

H_o: It was hypothesized that both continuous and intermittent physical activity would significantly attenuate the glucose, insulin, and c-peptide response to frequent meals as compared to a control day with no physical activity. In addition, it was hypothesized that intermittent activity may further enhance the reduction of these values through a continuous stimulation of the GLUT-4 pathway.

2) To determine the effect of continuous versus intermittent physical activity on total PYY and subjective measures of appetite in response to frequent meal consumption over 12 h in obese individuals with prediabetes.

H_o: It was hypothesized that total PYY (+) and subjective measures of hunger (+) and satiety (-) would be altered with continuous and intermittent physical activity during frequent meal consumption.

3) To determine the relationship between total PYY and insulin concentrations; and total PYY and insulin concentrations with self-perceived measures of hunger and satiety

response (VAS; visual analogue scales) to continuous versus intermittent physical activity during frequent meal consumption over 12 h in obese individuals with prediabetes.

H_o: It was hypothesized that there would be a significant inverse correlation between the change in PYY and insulin concentration, and that this correlation would remain with continuous and intermittent exercise. In addition, measures of hunger (-) and satiety (+) would correspond with changes in PYY during frequent meal consumption, and that this would persist during continuous and intermittent exercise.

Chapter II: Review of Literature

This literature review is divided into four parts. Part I examines current obesity trends and their implications, and explores how the glucose regulating pathways can malfunction with the condition of obesity. Part II examines theories that link obesity with the progression of impaired glucose tolerance, insulin resistance, and type 2 diabetes. In particular, this section examines defects in glucose tolerance and insulin secretion. Part III details the research on dietary contributions to obesity and impaired glucose tolerance (IGT), particularly the impact of variation in daily meal frequency. Part IV examines the literature on physical inactivity, as well as the impact of using multiple, short sessions of physical activity to break up long bouts of daily sedentary behavior breaking up periods of daily inactivity, on indices of obesity and IGT.

Part I: Obesity & Type 2 Diabetes- Prevalence and Health Impact

Obesity Trends

Obesity is one of the five largest risk factors associated with chronic disease in developed nations, including the United States, and is cited as the leading preventable cause of chronic disease in lieu of the gradually decreasing cigarette smoking rates (93). The obesity epidemic is not confined to the United States with over one billion people worldwide considered obese (185). Excess body weight (measured as a high BMI) now falls within the top ten risk factors in

traditionally undeveloped nations; where hunger and under-nutrition are traditionally considered to be the leading nutrition-related health risks factors (93).

Over the past several decades, the prevalence of obesity in developed nations has grown at an alarming rate (91, 192). This growing trend is highlighted by a US Department of Health and Human Services document, which reports an 8% increase in obesity (body mass index >30 kg/m^2 (BMI)) among adult males between a 1988-1994 study and a 1999-2000 study (3). According to recent population studies, over two-thirds of adults in the United States are now considered overweight (BMI$\geq$25 kg/m^2), while obesity affects almost one-third of the adult population (117). According to these estimates, the persistent growth of excess body weight in the American public now places the prevalence of obesity at more than double the *Healthy People 2010* initiative's ideal obesity range of 15%.

The accumulation of excess body weight almost inevitably has a severe impact on health and quality of life. Approximately 300,000 U.S. citizens die each year from chronic diseases associated with obesity, including type 2 diabetes mellitus (T2D), hypertension, hyperlipidaemia, and coronary artery disease. Due to T2D, and other chronic diseases, there is an additional mortality risk associated with excess body weight compared to normal weight status. Using data from members of the American Association of Retired Persons (AARP) aged 50-71y at enrollment; Adams et al (5) examined health risks in relation to BMI status over a 10-year period. Mortality rates (11.63%; n=527,265) were 20-40% higher in overweight individuals and 2-3 times higher in obese individuals, even when the data were adjusted for age, race or ethnicity, level of education, smoking and alcohol usage, and physical activity rates (5). A recent study by Crawford and colleagues (54) using newly updated electronic medical record data reported strong, direct correlations between BMI and disease burden, with rates of T2D,

hyperlipidaemia, and hypertension rising significantly with increased BMI across both gender and ethnic divides (54). Type 2 diabetes is a disease of particular interest with regards to obesity, with approximately forty million Americans (13%) currently diagnosed (41, 42, 53).

While multiple social and lifestyle factors are affected by obesity, the medical complications associated with excess body weight and body fat can be financially devastating. A recent metanalysis has estimated that the additional average yearly medical cost for an overweight individual is $266, while an obese individual spends an additional $1723 per year on average (248). The combined burden of these costs on the American health care system is estimated at greater than $100 billion annually, or 5-10% of the current U.S. health care budget (248).

Type 2 Diabetes Trends

For the past twenty-five years or more, T2D mellitus has comprised approximately 90-95% of diabetes cases (42, 109), and has attracted a lot of attention with respect to how negative lifestyle habits can affect health over time. Approximately 13% of American adults are *classified* with T2D, with higher prevalence rates seen in minority communities including Hispanic- and African-Americans (171, 273). These statistics are even more damning when considering that previous research using the 2005 NHANES data predicted that the true diabetes burden is likely to be an additional 30% higher when incorporating undiagnosed cases (70). The addition of these undiagnosed cases brings the NHANES estimate of T2D prevalence to approximately 17%, which is close to the American Diabetes Association's (ADA) current rate of 17.9% (7). Aside

from diagnosed cases, many individuals are also classified with prediabetes, where the advancement of the disease has not reached fruition.

Prediabetes consists of either high fasting plasma glucose (≥100 mg/dL and <126 mg/dL) or a high 2-hour post glucose ingestion value (plasma glucose ≥140 mg/dL and <200 mg/dL) (261). According to these criteria, an estimated 57 million Americans fit the pattern of prediabetes (7), an 'insidious condition' according to the Strong Heart Study (261). Individuals who fit the 2003 American Diabetes Association criteria for prediabetes had increased unadjusted hazard ratios of 2.77 for males and 2.88 for females towards the progression to T2D (261). The rates of T2D prevalence are expected to continue growing at an exponential rate, due in part to the 57 million individuals diagnosed with prediabetes, as well as improved treatment options, and thus longevity, for individuals with acute diabetes. Researchers from the Centers for Disease Control and Prevention (186) used 2004 estimates of T2D prevalence and evidence of a decreasing relative risk of death among persons with diabetes, to project that approximately 48.3 million individuals will be living with T2D by 2050 (186).

As the occurrence of diabetes continues to escalate, the financial burden of this disease in the United States will have a tremendous cost. In 2007, the cost of medical treatment, missed work days, and other costs related to T2D was estimated at $174 billion dollars (233). Diabetes rates and their associated cost are expected to parallel the rising rates of obesity (54).

Part II: The Obesity-Diabetes Link: How Obesity May Influence the Progression to T2D

Obesity and T2D

The progression of an individual towards type 2 diabetes closely shadows the progression of obesity (51, 201), and involves several steps including an initial impairment in glucose metabolism, subsequent compensatory increase in pancreatic insulin secretion, decrease in the sensitivity of insulin receptors in response to high insulin levels, and eventual pancreatic failure as the need for insulin production outweighs the functional capacity of the pancreas. Systemic inflammation and oxidative stress, hallmarks of the obese phenotype, are thought to be key mediators in the progression from obesity to T2D, by contributing to declining insulin sensitivity (155).

The close relationship between obesity and T2D progression was highlighted by Zhang et al (273) who reported a 33.3% increase in disease incidence between normal and overweight Caucasian individuals, with a disproportional increase found between normal and overweight individuals in minority populations (e.g., 60% increase in non-Hispanic black; 227.3% increase in Hispanic). Racial disparities by BMI category (273) were severely diminished with the crossover from overweight to obese status, with differences between groups narrowing. This finding implies that there may be a threshold of body weight across which potential protective mechanisms may be diminished (273). This highlights the importance of early intervention when attempting to address T2D progression before the transition to acute disease status.

Prediabetes is an important condition to study within the health and exercise sciences, as at-risk individuals may avert diabetes onset when they participate in interventions designed to

reduce body fat and weight (2, 251). In prediabetes, obesity has been coined “absolutely modifiable” in relation to increased T2D risk (261). Sullivan and Ratner (234) recently reviewed several large, intensive lifestyle intervention trials targeted towards individuals with prediabetes, including the Diabetes Prevention Program (DPP; United States), Finnish Diabetes Prevention Study, and Chinese Da Qing IGT and Diabetes Study, and found that lifestyle interventions provided a robust reduction in the incidence of T2D progression in these cohorts. In the DPP trial (154), 3234 individuals with prediabetes (stratified into control, intensive lifestyle intervention, and pharmacological intervention groups) were followed across three years to assess the efficacy of various interventions (e.g., pharmacological, lifestyle) on the progression from prediabetes to T2D (154). Intensive lifestyle changes, including the loss of 7% of baseline body weight, adherence to a low-fat low-calorie diet, and 150 minutes a week of moderate-intensity physical activity resulted in a 58% decrease in the progression to T2D compared to the control group (154). Interestingly, this reduction in diabetes progression was also greater than pharmacological treatment (Metformin 850 mg, twice daily) which reduced the three-year incidence of diabetes diagnosis by an average of 31% (154). Similarly, the Finnish Diabetes Prevention Study supports the theory of diabetes prevention with lifestyle intervention in individuals with poor glucose tolerance (251). Toumilehto et al (251) reported that subjects with impaired glucose tolerance who remained weight stable over the course of a year had an increased incidence of diabetes diagnosis compared to those who lost an average of 4.2 kilograms (23 vs. 11% change). This trend has been established through numerous well-controlled and intensive randomized control trials over the past decade (234).

While early lifestyle intervention is a promising avenue in T2D prevention, factors related to weight loss and weight management are often complex and difficult to modify.

Additionally, as individuals progress *further* into T2D disease progression, it is not clear if a reversal of symptoms and complications is possible. It is important, therefore, that lifestyle modifications, including those related to dietary and physical activity habits, be initiated early in the onset of obesity or diagnosis of impaired glucose uptake, as the preservation of glucose tolerance and insulin production/function is critical for healthy endocrine function.

The Glucose/Insulin Mechanism

The uptake of glucose into tissues is a delicate process, which is carefully monitored to maintain the narrow range of blood glucose concentrations necessary for a homeostatic environment (32). This balance is primarily mediated by the metabolic hormones, insulin and glucagon, which respond to changes in blood glucose levels with a positive feedback mechanism. For the purpose of this document, a review of insulin secretion and action will be presented, followed by an evaluation of the pathological consequences imposed upon the glucose/insulin mechanism by obesity.

Insulin and Insulin Measurement

Insulin is a peptide hormone that is responsible for the control of glucose uptake, utilization, and storage in the tissues and adipose tissue. Insulin is produced in the beta-cells (β-cell) of the pancreas continually, and is stored in vacuoles for subsequent release in response to elevations in blood glucose levels (99, 168). Insulin is often measured through different means, which each reflect different aspects of how insulin acts in the body. For example, a high fasting

concentration of insulin is often used as a simple means of diagnosing insulin resistance in an individual. Glucose tolerance testing, where insulin may be measured in a specific time series (often several blood draws over a 2 h period) can reflect how insulin responds to either an oral glucose tolerance test (OGTT) or intravenous glucose tolerance test (IVGTT). The gold standard means of assessing insulin resistance is known as the hyperinsulinemic euglycemic clamp. This method is accomplished through the steady administration of a glucose load (designed to keep blood glucose levels between 5 and 5.5 mmol/L), and increasing titration of insulin into the system in order to mimic an increasing insulin response. This technique is more reflective of the sensitivity of the individual's insulin receptors, and is a more precise means of diagnosing insulin sensitivity. The various methods used to characterize insulin sensitivity and action often make the interpretation of data difficult, particularly as these methods have different levels of sensitivity and address different aspects of the insulin mechanism.

Insulin Secretion

The stimulation of insulin production in response to glucose is initiated by the products of glucose metabolism, a process quite distinct from other such processes in the endocrine system, which typically rely on ligand-receptor interactions (67).When glucose molecules are transported into the β-cells of the pancreas via GLUT-2 receptors, glucokinase begins metabolizing glucose into glucose-6 phosphate, a precursor of glycolysis and eventual adenosine tri-phosphate (ATP) production (67, 99). A subsequent signaling cascade involving ATP production, ATP-sensitive potassium channels, and oscillatory increases in free calcium due to the activation of these K^+ channels, gives rise to the release of insulin across the β-cell membrane (67, 99). The magnitude

of change in the flux of calcium levels crossing into the β-cell give rise to the pulsatility, or continual titration, of insulin release (67, 219). The pulsatile characteristic of insulin release is critical, as it comprises the majority of total insulin release (approximately 75%), and allows for small fluctuations in the release of insulin to closely match blood glucose concentrations (219).

Pulsatile insulin release is characterized by meal-induced secretory bursts, sometimes referred to as insulin spikes, superimposed upon a chronic, basal secretion (219). The overall insulin secretory response is augmented due to either high glucose levels or prolonged exposure of β-cells to glucose stimulation (187). In addition to the pulsatile characteristic, meal-induced insulin secretion has a biphasic pattern of response (Fig 1); with a transient first-phase response, followed by a gradual and sustained second-phase response (187). Figure 1 depicts a blood glucose stimulus (500 mg intravenous injection of glucose at time point 0) which causes a spike, or first-phase response of insulin release (187). There are typically two theories that relate the nature of this initial response, which is thought to take place within the first few minutes (4-10min) of stimulus (187). One theory of the first phase of insulin release states that the magnitude of the glucose dose induces insulin release, with each β-cell releasing insulin in a dose-response relationship to the glucose concentration (187).

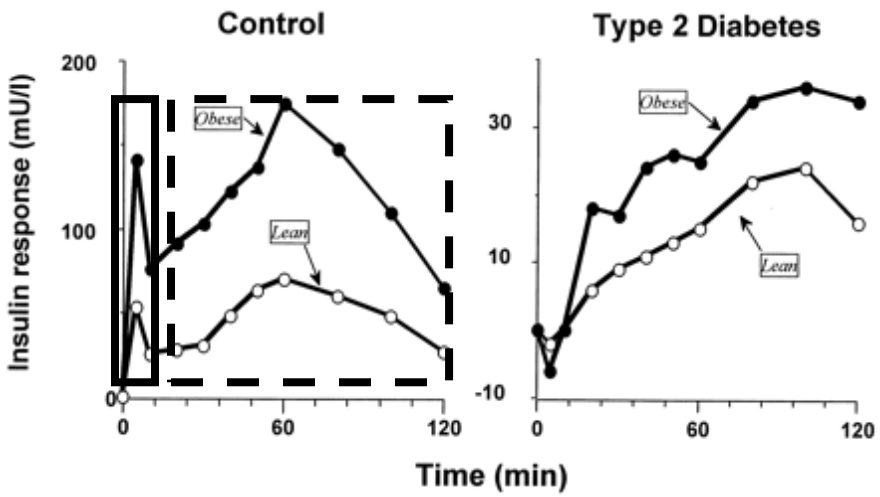

Figure 1: Plasma insulin response to glucose infusion (500 mg/0 m; followed by 20 mg/m until 60 m), note- x-axis on healthy controls is four-fold that of T2D (187). First-phase (representing first phase and time dependent inhibition (TDI)) highlighted by black box in control condition. Time-dependent potentiation is characterized by the dashed box.

The second theory proposes that β-cells respond in an all-or-nothing manner, releasing all of their available insulin in response to an individual threshold of glucose sensitivity (197, 198). Interestingly, individuals with T2D do not demonstrate a standard first-phase insulin response (Fig 1), partially explaining their loss of sensitivity to glucose stimulation (68, 69, 143).

DeFronzo and colleagues (68) reported a varied insulin response to a glucose tolerance test in individuals (n=32) with T2D; where reduced insulin secretion is present in only half of the participants, and the other half responded with increased insulin secretion. Further analysis of this differential trend revealed that the first-phase insulin response was universally absent in these individuals with diabetes (68).

The first-phase insulin response is an important moderator of the total insulin response to glucose, with small changes in insulin magnitude having a major impact on glucose disappearance (69, 143). As evidenced in Figure 1, the first-phase release of insulin is absent in both lean and obese individuals with T2D. Davies and colleagues (60) studied three groups of individuals classified based on an OGTT (healthy n=7, persistently IGT n=7, transiently IGT n=6) and administered an IVGTT, drawing arterial blood samples at one-minute intervals. Though fasting insulin concentration was consistent among the study groups, insulin values during the 3, 4, and 5-minute samples, characteristic of the first phase response, were significantly lower in the persistent IGT group ($p<0.02$) than in normal controls, and those with transient IGT (60). In agreement with findings in individuals with T2D (144), the 30-minute insulin to glucose ratio or insulinogenic index, which is indicative of the first-phase insulin response, (60) was also significantly lower in individuals with persistent IGT ($p<0.03$). Impaired first-phase insulin release in obese individuals and/or those with prediabetes, as well as the absence of the first-phase insulin response in individuals with persistent IGT and T2D can have an important influence on the subsequent second-phase and overall insulin response.

The second phase characteristic of the biphasic pattern of insulin release is highlighted by time-dependent inhibition (TDI) and time-dependent potentiation (TDP) phases (187). The TDI, visible in Figure 1 (*decrease* in the control group insulin response following minute 5; inside black box) is characteristic of intravenous glucose administration, and not generally seen with oral glucose administration (187). Curiously, the TDI response, which normally acts as an inhibitor of insulin release does not diminish the ability to secrete insulin during the TDP period of second-phase insulin release (187). Time-dependent potentiation, which is characterized by an enhanced and prolonged response under the influence of the duration and magnitude of glucose

exposure (Fig 1; dashed box), provides the stimulatory signal for subsequent insulin exocytosis as needed (187, 188). The TDI and TDP are likely redundant mechanisms to help fine-tune the normal second-phase response of insulin release. The second-phase insulin response is reliant upon current physiological glucose concentrations, and can vary in direction and magnitude depending on obesity, and the progression of T2D in an individual.

Previous studies (196, 263) have demonstrated that under glucose-matched conditions, the second-phase insulin response to a glucose challenge may be exaggerated in obesity, but diminished in T2D. Perley and Kipnis (196) divided a cohort of lean and obese individuals with and without T2D into four groups (lean, without diabetes; lean, with diabetes; obese, without diabetes; obese, with diabetes) in order to characterize the second-phase of insulin response in response to different physiological levels of blood glucose (196). Four separate glucose infusions were administered to these groups in order to determine the magnitude of the insulin response under different conditions (196). Each group received two glucose loads of differing magnitude (measured to match the original OGTT insulin profile of both the healthy and impaired response), administered both orally and intravenously (196). The authors (196) reported that the obese participants (with and without T2D) secreted 3-5 times the amount of insulin during each glucose infusion (oral and intravenous) when compared to their lean counterparts ($P<0.001$). Further the obese individuals with T2D secreted significantly greater amounts of insulin ($P<0.01$) in response to the glucose loads than their lean peers with T2D (196). Within the obese cohort, however, the group with T2D had an attenuated insulin response to the glucose dose (40 and 18% reduction, respectively) when compared to the lean individuals with diabetes (196). These results demonstrate that obesity and diabetes status, separately or in concert, are key modulators of the second-phase insulin response to glucose ingestion seen with obesity (196).

The biphasic stimulation of insulin release from the pancreatic β-cells in response to increasing glucose concentration occurs in a concentration-dependent manner in a healthy individual. Once released from storage vacuoles, insulin acts as a ligand in binding to a specific α-subunit of the insulin receptor, thus activating the β-unit of the receptor's tyrosine kinase activity (99). The activated insulin receptor proceeds to phosphorylate tyrosine residues on target proteins including those of the insulin receptor substrate (IRS1-6) family (32). The downstream signaling from IRS proteins put several pathways into motion, of which the most important for glucose uptake is the phosphatidylinostol 3-kinase (PI3K) pathway (32). This PI3K pathway is responsible for the translocation of glucose transporter type 4 (GLUT-4) receptors to the surface of skeletal muscle and adipose tissue cells, allowing glucose uptake (32).

Insulin regulates the process of glucose uptake from the bloodstream, and partitions glucose into cellular compartments including those of skeletal myocytes and adipocytes (168). In addition, insulin acts as a primary signal for the storage of glucose as glycogen in both the liver and skeletal muscle cells (168). As insulin is the signal hormone responsible for appropriate glucose metabolism and storage, the proper release and functioning of this hormone is imperative for proper endocrine function associated with the consumption of food.

Ward and colleagues (264) demonstrated that the insulin response in individuals with T2D often mimics a healthy response when prevailing blood glucose levels are matched between normal and irregular profiles (143). Regardless, individuals with diabetes often have exceedingly high blood glucose concentrations, and the loss of an appropriate insulin response in these individuals can lead to chronic hyperglycemia and a need for an excessive compensatory insulin response. A chronic elevation of insulin can subsequently lead to a down regulation of the insulin receptor (95), resulting in insulin resistance and eventual β-cell impairment and failure.

Defects in Insulin Secretion

Type 2 diabetes is a complex metabolic disease, comprised of one or many conditions that alter insulin receptor sensitivity, and eventually the secretion of insulin; leading to a hyperglycemic state (69, 168). In addition to acknowledgement of alterations in the secretory phases of the insulin response to meal ingestion, physiological characteristics of the obese, glucose-intolerant individual can also alter normal insulin receptor sensitivity and patterns of insulin secretion (57). According to a recent review (57) elevated levels of free fatty acid (FFA) release common in obesity can lead to impaired insulin secretory capacity and β-cell function, particularly in those with a family history of T2D. Kashyap and colleagues have shown that individuals with a family history of T2D have lower levels of insulin secretory release in response to a simultaneous glucose/lipid infusion (ISR; corrected for insulin resistance; -25% of first phase response; -42% second-phase) compared to those without a family history (146). An *in vitro* study has also revealed similar trends in the circulating fatty acid influence on insulin action. Muscle biopsy, and subsequent microarray analysis has shown that an experimental increase in circulating free fatty acid levels (0.48±0.02 vs. 1.73±0.43 mmol/L; to similar levels seen in obese individuals) caused a significant decrease in insulin-stimulated glucose disposal (8.82±0.69 vs. 6.67±0.66 mg/kg/min; $P<0.05$) in healthy volunteers (207). The combination of these findings highlights the role of increased free fatty acid turnover characteristic of obesity, as a physiological modulator of the progression from a healthy insulin response and insulin action to the condition of insulin resistance. Further work by DeFronzo has potentially defined an inflammatory pathway, characteristic of T2D, that can influence insulin action (Fig 2). The possible role of inflammation, a hallmark of obesity and T2D, as a moderator of the normal response of insulin will be discussed further.

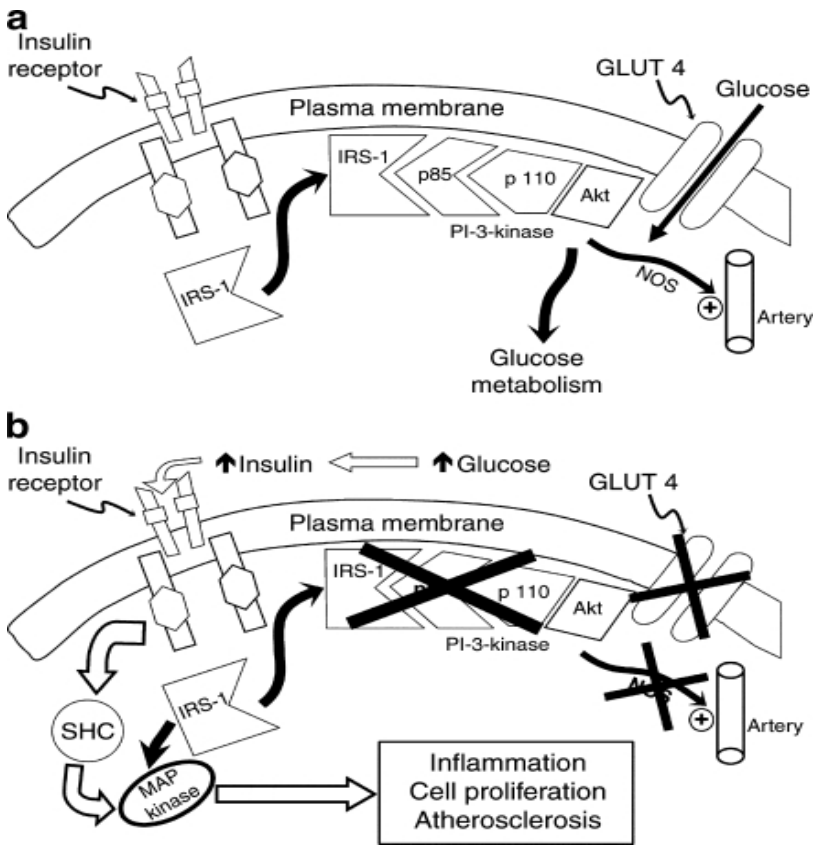

Figure 2: Insulin signaling pathway at the tissue level of a) healthy and b) T2D individuals

Defects in Insulin Action: Insulin Resistance

Insulin resistance (IR) is one of the hallmarks of T2D, and it is present in the vast majority of individuals affected with T2D (68). Insulin resistance is a condition where physiological responses to insulin are attenuated; or the normal chronological insulin response is delayed (168). Insulin resistance occurs downstream of the insulin receptor, where insulin initiates its biological effects via the activation of post-receptor hormonal cascades (e.g., the IRS-

1 pathway; Fig 2). Insulin resistance is located mainly at the level of skeletal muscle (68, 168). As a consequence of the gross size of the skeletal muscle pool, it would come as no surprise to find that skeletal muscle accounts for approximately 75% of insulin-stimulated glucose uptake, with adipose and liver tissues making smaller contributions to glucose uptake. The loss of sensitivity associated with IR is almost always associated with a positive energy balance, or an excess of caloric intake, and this positive energy balance can markedly alter normal endocrine function (57). Obesity is generally implicated in the progression of insulin resistance (57, 207).

The intracellular signaling pathway plays an important role in insulin resistance, not the specific ligand/receptor binding of insulin per se, but in the series of reactions that occur *following* the binding of the insulin molecule to its specific receptor. With insulin binding to its receptor, there is a phosphorylation of protein kinases through pathways including those of IRS-1 (Figure 2) and IRS-4. The IRS-1 pathway is thought to initiate a downstream cascade which stimulates the P13K/Akt and Erk MAP kinase pathways and results in glucose uptake through a series of phosphorylation reactions (32, 168). Insulin resistance is typically regarded as a defect in cell signaling, as the IRS-4 pathway initiates cellular communication leading to glucose uptake (32, 168). 'Post-receptor' defects, including those that inhibit IRS-1 and IRS-4 phosphorylation, may disrupt the normal downstream signaling cascade allowing for normal glucose uptake to occur (168, 196).

As a hormone, insulin is not limited to the control of glucose uptake, and may have effects on other pathways, including those related to energy balance, appetite and satiety. In addition, the influence of other hormones (29, 241) may interact to influence insulin action, and insulin resistance (257). In fact, up to 60% of the meal-induced insulin response to an oral glucose load may be accounted for by the direct influence of incretin-producing cells of the

gastrointestinal tract (241). Similarly, the interaction between orexigenic, or appetite-stimulating, homones including ghrelin (29), and satiety hormones with insulin may comprise part of a vicious cycle of obesity, overeating, and insulin resistance.

Neurohormonal Regulation of Insulin Secretion and Action: The Role of Incretins

Insulin concentration and kinetics are affected by obesity. Additionally, insulin is a key regulator of appetite, communicating via both orexigenic (184, 216), and anorexigenic or appetite-inhibiting (103) pathways. It has also been postulated that these pathways may be redundant, as members of the incretin family have been shown to be insulinotropic (258). In order to assess the interplay between insulin and incretin horomones, Edwards and colleagues (79) administered either extendin 9-39, a known inhibitor of the potent anorexigenic hormone glucagon-like peptide 1 (GLP-1), or normal saline to healthy individuals (n=9) in concert with 150g OGTT testing. The extendin 9-39 infusion resulted in a 35% decrease in the postprandial area under the curve for glucose concentration (exendin 9-39, 152±19 vs. saline, 113±16 mmol/L*min, $P<0.05$), along with a significant increase in peak postprandial glucose levels ($P<0.005$) compared to saline infusion. Similarly, Reinehr et al (206) examined the incretin profiles 42 obese children in a one-year weight loss program to determine the effect of varying levels of weight loss on appetite measures. These authors (206) reported that significantly decreased levels of GLP-1, insulin, and HOMA IR assessment ($P<0.05$) were present in children who successfully lost a significant amount of weight (an average of 2 points on the BMI scale) when compared to those who maintained their body mass. These findings confirm the important role of the incretin family in contributing to healthy insulin function, and provide evidence for

the communication between the insulin and incretin pathways. Despite these findings, countless unexplored avenues in examining the interplay between insulin and appetite mechanisms remain.

Summary

In summary, it is important to note that healthy insulin levels and action may be impaired due to obesity (57, 168, 207). However, it is also essential to recognize that in obese individuals the progression from normal to impaired glucose tolerance occurs over the long-term (82, 205), and thus provides an important window of opportunity for intervention in the months or years before the probable onset of T2D. Therefore it is imperative to examine how changes in dietary and physical activity behaviors commonly associated with weight loss may affect glucose and insulin concentrations during normal physiological conditions, including meal consumption and physical activity participation. Additionally, establishing more links between insulin and measures of satiety (physiological and objective), and the influence of acute lifestyle modifications on these relationships warrants attention. In the continuum ranging from the glucose-tolerant lean individual to the obese individual with T2D, the obese individual with prediabetes occupies a unique position along this spectrum, and provides an essential opportunity to examine the beneficial effects of acute and chronic lifestyle patterns on health outcomes and appetite measures.

Part III: **Dietary mediation of obesity and glucose tolerance: Meal frequency**

Considering the increasing prevalence rates of obesity outlined in Part I, along with the significant consequences of obesity, it comes as no surprise that much research has focused on the underlying causes of weight gain and weight maintenance. Body weight maintenance exists as a delicate balance between energy intake and energy output. According to Schoeller (220), the 'energy balance equation' reflects this relationship using a mathematical model as follows:

Energy intake (EI) = Energy expenditure (EE) + change in body stores

The next two parts of this literature review will detail dietary and physical activity/inactivity modulation of the energy balance equation and obesity. In addition, how these dietary and physical activity/inactivity modulators influence the glucose and insulin responses to meal ingestion, and some measures of appetite control will be examined.

An imbalance on either side of the energy balance equation will result in either weight gain or weight loss. The excess body weight that most people accumulate is due to a shift towards the EI side of the equation, and resulting positive energy balance (116), which may manifest due to an excess of calorie intake, reduction in physical activity, or combination thereof. As little as one hundred kilocalories per day of positive energy balance can quickly accumulate, causing weight gain and eventually result in overweight or obese status (117). Assuming an uncompensated surplus of one hundred kilocalories per day, the average individual could potentially gain 10 pounds in a year. By this same logic, as few as 10-20 surplus

kilocalories a day could result in a relatively modest 1-2 pound weight gain per year, negligible in the short-term, but likely impactful on health outcomes over the course of years and decades. Attention to energy intake, or 'ingestive behavior', is a practical means of addressing energy balance.

Ingestive behavior encompasses variables including the number of total calories consumed, the daily macronutrient profile, and periodicity of eating occasions; and has been identified as a key link between food intake and indices of health (173). Epidemiological trends point to the fact that recent population-wide changes in ingestive behavior may coincide with a progressive increase in caloric intake, paralleling the rise in obesity rates. For example, recent analyses of USDA and NHANES surveys by Popkin and Duffey (199) demonstrate that individuals (greater than two years of age) in the United States (2003-2006) consume an average of 2533 kcal/day compared to 2090 kcal/day consumed in a study from 1977-1978.

This increase in food consumption can be partially attributed to increased financial prosperity in the United States between the early twentieth century and the present day, and a subsequent increase in the availability of cheap, nutrient-dense food (17); including oils, meats, cheese, frozen dairy, soft drinks and fruit juices. Fruit and vegetable consumption over this time period has also increased (17), highlighting improvements in overall food accessibility. This increase in the total available number and variety of foodstuffs that an individual is able to consume has helped contribute towards the growing obesity epidemic. In support of these findings, a recent analysis of the NHANES dietary records (1971-75 through 1999-2002) revealed that the increase in BMI over the past forty year period is closely associated with an increase in the total *quantity* and *energy density* of foods consumed (145). While past generations were often concerned with a lack of calorie-dense foods being obtainable, the obesity

epidemic has led to an increased awareness of the desirability of nutrient-dense foods (lean meats, fruits, vegetables, and whole grains), as opposed to popular energy-dense foods.

Factors which contribute to alterations in energy intake are interesting in that they affect individuals on both ends of the economic spectrum similarly. For example, it may be difficult for low-income individuals in large, urban environments to consume a balanced diet consisting of nutritionally high-quality foods. According to Schier (217), while *overall* food availability has increased, reducing the likeliness of starvation or malnutrition, the healthfulness of the foods available to low-income individuals is often skewed towards high calorie, low nutrient foods that are more likely to contribute to weight gain. In support of this, Sheldon et al (224) evaluated the cost of fulfilling the United States Department of Agriculture 'Thrifty Food Plan' recommendations for an individual or household (a composite 'grocery list' to stock a healthy kitchen) across 21 different retail food stores in Central Falls, Rhode Island during 2007 and 2008. These authors (224) revealed that the cost of stocking this average grocery list cost up to 40% more in stores located in poorer, urban neighborhoods of the city where large chain grocery stores are scarce and individuals must rely on discount and convenience markets. These types of increased costs, or a complete lack of access to high-quality foods in these communities often causes low-income individuals to purchase and consume affordable, low-quality, energy-dense foods (high in calories, sugars, and fat) which may contribute to obesity (13, 217, 224).

At the opposite end of the economic spectrum, when individuals find they have an increased disposable income or prosperity, it may result in a shift from home-cooked meals towards commercially-prepared restaurant and fast food meals (17). Further, the portion sizes of packaged meals and meals served outside of the home, and correspondingly the amount of calories consumed during these meals, has greatly increased over the past few decades (81, 159,

174, 270). For example, Young and Nestle (174) obtained the portion size information of commonly available packaged food items, and reported that almost universally portions had increased beginning in the 1970's, continued to increase sharply in the 1980's and throughout the 1990's. An example of this excess is demonstrated by the increased size of the average chocolate chip cookie, which now exceeds the USDA standard portion size by 700% (174).

In addition to the known increases in the average American's daily caloric intake, the frequency of eating occasions (both foods and calorie-containing beverages) has also increased. For example, just as total calorie consumption had increased by ~19% between the aforementioned 1977-1978 and 2003-2006 NHANES studies (199), the frequency of consumption of energy-containing items over the course of the average day during these time periods also increased.

Current Meal Frequency Trends

While it is well known that an increase in total energy intake is a major contributing factor to the current obesity epidemic, there are also other important variables related to energy intake that affect metabolic and endocrine responses to nutrient ingestion. Eating styles including the pattern, frequency, and size of daily meals and snacks (151, 210) may influence dietary factors and obesity.

Meal frequency, or the number of times an individual ingests calorie-containing items daily, is an ingrained pattern of eating in the daily life of an individual. These patterns are modulated by genetic (65), psychological (271), socio-cultural (47, 64), and chronological factors (63, 265), and have even been shown to be influenced by alterations in human biological

rhythms due to changes in the lunar cycle (66). Simply stated, the factors regulating meal frequency as well as the outcomes of these various patterns of consumption are complex and varied. An important consideration in the classification of meal frequency is the definition of a meal, as this is often a term that is used loosely and interchangeably even within scientific studies. For example, Crawley and Summerbell (55) defined eating occasions as the consumption of energy containing items in bouts separated by at least 30 minutes. In comparison, Holmstrup et al (180) defined a meal as 'the consumption of 300 kilocalories worth of energy-containing foods separated by at least 15 minutes'. An individual consuming their daily meals in the same pattern (for example a typical 3-meal consumer who has a 400 calorie dessert 20 minutes after their dinner) could easily be classified as a four-meal consumer in one study (55), and a three-meal consumer in the other (180). In the late 1990's, a comprehensive review of previous meal frequency studies (96) recommended a standardized definition for an eating occasion for use in epidemiological work, though a particular definition has not been adopted.

Many individuals consider the three-square meal regimen to be the cornerstone of daily intake patterns, and it has definitely been established as a standard dietary practice. Like many of the processed foods that have become staples of the American meal, the three-meal pattern of meal consumption may be a remnant of the Industrial Revolution (104). According to this theory (104), as individuals began to leave the farm for the factory, and thus close quarters with home and hearth, the three- meal pattern began to emerge as common practice. A small breakfast meal, opportunity for one break (thus the 'lunch break' at work) and a large supper meal upon returning home from the factory; gradually replaced the traditional large breakfast, large noon meal, smaller supper meal, and ample opportunity for grazing in between characteristic of farm life (104). In lieu of potential positives and negatives regarding either pattern, an understanding

how the three-meal pattern evolved may provide some insight into the changes that have taken place in these norms over the past seventy-five years.

Despite the fact that major gaps in knowledge regarding the health benefit of various meal frequency patterns, increasing the number of daily eating episodes from three per day has become common. In addition to confirming the overall increase in total energy intake over the past thirty years (199), an overall increase in the number of daily eating occasions is also reported. Popkin and Duffey (199) found that the average number of meals per day in the upper 50^{th} percentile of individuals in the National Health and Nutritional Education Survey (NHANES) database changed from 4.9 eating occasions per day in 1977 to 6.6 occasions in 2003-2006. Similarly, Kerver et al (150) reported that 24-hour dietary records collected during NHANES III showed that ~32% of selected adult samples adhere to a three-meal, two-snack pattern daily. In further support of these findings, the World Health Organization (87) reported in 1996 that most individuals consume between five and six eating occasions daily.

This recognized increase in the pattern of eating, sometimes referred to as grazing, is likely due to increased accessibility to food, and more available opportunity to consume snacks and meals during work/school. Increased food availability, as well as media recognition of the potential benefits of adopting the grazing lifestyle, have resulted in up to 75-85% of Americans adopting some sort of 'frequent' pattern of daily meal consumption (56, 275).

In many ways, eating patterns have become more chronological than biological, wherein they follow the outside environment (e.g., the clock) as opposed to our internal appetite mechanisms. Lennernäs and Andersson (163) reported that meal/snack consumption in diverse populations including shift workers, obese and healthy controls, and elderly individuals occurs around the clock as opposed to naturally in response to hunger cues. The opportunity to consume

calorie-containing foods across the day in a grazing pattern could potentially lead to individual's achieving positive energy balance. Regardless of the *potential* for increased energy intake, a frequent meal pattern, especially when daily caloric intake is kept the same, has been encouraged by health-care professionals and the lay media (94, 175).

Despite recommendations to maintain the same total intake when increasing meal frequency, intake is not always maintained when individuals consume meals outside of a three-meal per day pattern, and may be augmented. Classic studies in populations including children (189) and adults (58) have demonstrated that total calorie intake is increased with an increase in meal frequency. For example, one study (58) using United States Department of Health and Human Services (USDHHS) data demonstrated that the average total calorie consumption increase between 1977 and 1996 (male- +268, female- +143 kcals/day) was due to an increase in the daily frequency of eating (male- +.61, female- +.58 occasions/day), and not the total energy content of any given meal or snack.

For the past seventy-five years, even studies demonstrating beneficial effects of an increase in meal frequency include a caveat for individuals predisposed to an increase in total caloric intake with increased meal frequency (104). For example, most recommendations speak to the efficacy of a *decreased* number of meals when an individual has the tendency to overeat on a frequent plan (9, 227). In order to understand the reasons that frequent meal consumption is recommended, it is important to examine the progress of the literature in this area since its earliest inception. It is imperative to utilize these previous studies in order to continue to examine why under certain conditions and within certain populations, an increase in meal frequency may, or may not be beneficial.

Research in Meal Frequency

When attempting to make a cohesive story out of the decades of meal frequency research that have been undertaken, it is important to note that the emphasis of studies has changed over the years. The significance of meal frequency has not always been directed towards the problem of obesity. In fact at different times during this line of research, emphasis has shifted from work productivity outcomes, to protein retention and interventions to combat malnutrition, and more recently towards the prevention of risk factors associated with cardiovascular disease. The current focus on the problem ranges from the control of adult and childhood obesity, to the regulation of endocrine mechanisms and potential effects on appetite. However, despite these shifts in research aims, a thorough examination of the literature yields several important facts that transcend differences in populations, research methods, and study rationale.

In the late 1920's and early 1930's pioneering research concerning the physiological outcomes of spreading the daily caloric load from three to five eating occasions was performed at Yale University, and may represent one of the earliest examples of a recommendation to consume meals more frequently than the standard three-square meal regimen (104). Haggard and Greenberg (104) studied 213 children, college-students, factory workers, and professionals to determine the potential relationship between the distribution of daily calories and surrogate measures of work capacity (e.g., respiratory quotient (RQ) values above fasting baseline) and work productivity. Their initial findings (104) determined that 63.3% of these individuals (135 out of 213) consumed their daily calories in a three-meal pattern, quite the opposite of the patterns seen in the modern day. These authors (104) also found that those who consumed meals more frequently (five vs. two occasions per day) spent a longer period of the day with an elevated RQ above baseline (seven vs. two hours; significance not reported). These findings

were interpreted as an advantage in work capacity for the individuals consuming meals more frequently (104).

Further, these authors (104) attempted to substantiate their findings by comparing the actual work productivity of a subset in their sample (shoe factory operatives; n=103) with characteristics of their meal consumption pattern. An examination of the worker's productivity rates revealed that individuals consuming meals more frequently produced more shoes per hour than those consuming their daily calories in fewer meals (2 meals/day- 172 shoes/h; 3 meals/day- 183 shoes/h; 5 meals/day- 191 shoes/h). When the investigators (104) attempted to control for variations in worker skill by dividing another subset of the cohort (three-meal/day consumers; n=40) into a control (n=20) and experimental group (n=20). The control group was allowed to continue their normal pattern of three-meal consumption for ten weeks, while the experimental group was cycled through five 2-week variations on their meal patterns (3 meals/day; 5 meals/day; 3 meals/day; 5 meals/day; 3 meals/day). Work productivity among these groups was averaged for each 2-week period of the intervention (104). The average production rate for the control group (3 meals) was between 183 and 184 shoes per hour for the duration of the 10-week intervention, with very little deviation found in any single day of production (-5.4% lower to 2.2% higher), however, the experimental group demonstrated considerable changes across the different meal pattern conditions (104). During three-meal per day consumption, the average 2-week production rate for this group was 176 units per hour, with similar one-day maximum variations in production seen in the control group (-4.1 lower to 1.8% higher). Upon the addition of two meals per day, which were supplied as additional calories thus not calorically-matched with the three-meal days, the subjects improved their production to an average of 193 shoes per hour, demonstrating a 9.7% increase in work rate (104). Aside from a recommendation to

increase to five daily meals based on their findings, Haggard and Greenberg (104) cautioned that 'the food eaten at the 2 extra meals is not to be added to that of the regular meals, but subtracted from them; it is merely eaten at another time'. Therefore, despite the addition of calories to their 5 meal experimental condition, one of the earliest known studies in meal frequency already considered the potential ramifications of increased total calorie consumption with an increase in eating periodicity.

During the late 1940's and early 1950's, more important foundational work in the area of meal frequency using controlled research methods was performed. The recent memory of starvation in World War II concentration camps, and the very real problem of malnutrition in a growing global community resulted in some early work by Dr. Ansell Keyes on proper methods of refeeding and nourishing individuals who were victims of starvation. In the spirit of Dr. Keyes' work, some of the early studies in meal frequency focused on the human body's ability to maintain muscle mass on different dietary regimens. Though this initial work did not necessarily define 'frequent meal consumption' in the same way that the modern day 'grazer' might, investigators demonstrated that breaking an individual's daily protein requirement into more frequent bouts resulted in improvements in lean muscle mass retention. Wu and Wu (125) showed that nitrogen utilization (measured as 2 h urinary nitrogen output) is improved when an individual consumed an identical amount of protein (78 g/per day; 2500 kcal total intake) over a three-month period as four meals compared to two meals. Similarly, Leverton and Gram (165) administered controlled diets to a small group of relatively sedentary female college students (n=14) over the course of a 36-day intervention. The dietary intervention was divided into two 18-day intervals during which the participants consumed four meal, isocaloric diets (designed to maintain body weight; ranging from 1,950-2,200 kcal/day; 60-63 g high quality protein/day)

where the protein sources were consumed either during two, or all four meals (165). The authors (165) concluded that nitrogen retention, measured by urinary nitrogen output, was decreased (9.02±0.41 vs. 8.33±0.29 g, $P<0.05$), and therefore utilization was improved, when protein consumption was spread from two meals to four meals. These early studies in the human response to increased meal frequency demonstrated promising, but fairly isolated, indications of the beneficial effects of increased meal frequency consumption. While these early studies were limited to examinations of work productivity and protein retention, they helped to set a framework for continued, varied studies of the physiological responses to meal frequency alteration. Subsequently, a shift towards animal studies, with only a small number of ongoing human studies, became more prevalent in the meal frequency literature during the early 1960's; along with a shift in emphasis towards the growing problem of cardiovascular disease.

Meal frequency, blood lipids and cholesterol

As high blood lipids and cholesterol levels became increasingly recognized as potent risk factors for the development of cardiovascular disease during the late 50's and early 60's, research in both animals and humans began to explore the possibility of meal frequency playing a role in this interaction. Groundbreaking work by Cohn and Joseph (48, 49) reported that rats fed using scheduled meal times had an increased incidence of atherosclerotic lesions and higher serum cholesterol levels than those allowed to freely graze all day, despite 'scheduled eaters' consuming fewer total calories than 'grazers'. Likewise, Cohn and Joseph (49, 183) demonstrated that chickens placed on a five-week meal-feeding (1 hour morning feeding, 1 hour evening feeding) regimen had seven times the coronary artery disease risk compared to free-grazing controls. These studies (49, 183) showed as an increased incidence of atherosclerotic

lesions in the coronary (89 vs. 44%) and thoracic (100 vs. 44%) arteries; increases in fatty acid synthesis rates, and serum cholesterol levels (584 vs. 294 mg%) in the meal-fed animals. Similar calorie-matched studies (98) with monkeys (n=12) placed on either intermittent (meal-fed) or continuous (nibbling) regimens demonstrated that serum cholesterol was higher (41.3±8.36 vs. 11.0±6.83 mg/100ml; $P<0.02$), and plasma fibrinolytic activity retarded (significant increases in clotting time) in meal-fed compared to nibbling animals. Work in this area branched out further, with Rosmos et al (213) using IVGTT and OGTT testing to examine the efficiency of glucose clearance in a cohort of adult dogs. The authors reported lower plasma cholesterol levels (235±13 vs. 299±18 mg/100 ml; $P<0.05$), but no significant differences in glucose clearance in adult dogs who ate *less* frequently over the course of approximately eight months (1 vs. 4 isocaloric meals/d).

A prevailing notion at this time was that outcomes in animal studies accurately reflected those found using humans, as reported during the early 1960's by Cohn as 'indistinguishable' (50). Subsequent work by Paik and Yearick (194) in the mid-1970's found increased concentrations of lipoprotein lipase (19.42±4.83 vs. 13.09±2.97 nmol/mg protein; $P<0.01$) and hormone sensitive lipase (8.80±2.11 vs. 6.82±1.59 nmol/mg protein; $P<0.01$) in the adipose tissue of meal-fed rats versus *ad libitum* fed rats, despite an ~50% increase in the daily calories consumed in the *ad libitum* group. These findings (194) strengthened the conclusions of previous studies that suggested a greater capacity for fat storage in infrequent versus frequent meal consumers (49, 98, 183).

Numerous human studies have demonstrated positive physiological outcomes related to plasma cholesterol and blood lipid concentrations with increased meal frequency. Early work in the 1960's showed that serum lipids (102) and plasma cholesterol levels (131), both strong

predictors of cardiovascular disease, were decreased upon adoption of a nibbling program. Gwinup et al (102) conducted a very small study with individuals confined to a metabolic ward (n=5), designed to assess the influence of extreme changes in meal periodicity over a 2-week intervention (1 meal/day vs. 10 isocaloric meals/day) on serum lipid concentrations. In each case, the gorging pattern of meal consumption was associated with a rise in plasma cholesterol and esterified fatty acid concentrations, with little to no change in body weight indices (102). While nibbling (10 meals daily) and gorging (1 meal daily) represented truly extreme measures of meal periodicity in this study (102), these findings helped to reestablish the importance of variable meal intake patterns on influencing physiological outcomes.

Similar work in the late 1960's by Fabry and Tepperman (86) confirmed some of the early evidence of a change in the inherent properties of serum lipids, with particular regard to the direct development of cardiovascular disease with meal frequency changes. They (86) reported that older men (n=1,359; 60-64 y) who consumed three or fewer meals per day had a significantly augmented incidence of ischemic artery lesions (as diagnosed by ECG examination), than their peers who consumed five or more meals per day (30.4 vs. 19.9%; $P<0.05$).

Jagganthan et al (131) similarly reported that serum lipid and cholesterol levels of 51 healthy male university students divided into four groups consuming two different eight-day, high-fat liquid diets (fat- butterfat *or* corn oil) 45%; protein- 14.7%; carbohydrate- 41.3%) on either a 'meal-eating' (1/6 of total calories for breakfast, 1/6 lunch, 2/3 dinner) or 'nibbling' (1/8 of total calories every 2 hours) protocol. Findings from the butterfat diet demonstrated a moderate change in serum cholesterol levels (-2.1±3.75 vs. -7.6±3.41 mg/100ml; P=0.05) among meal-eaters (n=9) and nibblers (n=11), respectively (131). Similarly, individuals consuming the

corn oil liquid meals had a greater change in serum cholesterol concentration (-14.5±5.58 vs. -6.6±6.04 mg/100ml; $P<0.05$) when consuming meals in a nibbling fashion (131). There were no significant differences in plasma triglyceride concentrations following eight days of meal eating or nibbling using either the butterfat or corn oil diets, potentially due to the short nature of the intervention.

The effect of meal frequency was also brought into question by Irwin and Feeley (128), who examined the impact of meal frequency on plasma cholesterol levels in young women (n=15). Each individual acted as their own control as they adhered to a series of three separate twenty-day, isocaloric (2,000 kcal/day, 58 g PRO, 87 g FAT) regimens (3 meals/day- equal portions, 3 meals/day- ¼, ¼, ½ of daily calories per meal; and 6 meals/day- equal portions). The greatest increases in serum cholesterol levels were seen following the three-meal regimen where two small and one large meal were consumed ($P<0.05$), however, they did not report any statistically or clinically significant reduction in cholesterol levels when moving from three meals to six isoenergetic meals (128). Later work by Tepestra et al (239) also confirmed the work of Jagganthan (131) and Irwin (128) in demonstrating that there were no significant differences in diurnal triglyceride levels between traditional and higher end meal frequencies (3 vs. 8 meals) in healthy volunteers. Similarly, Holmback et al (118) demonstrated a lack of significant differences between cholesterol levels in seven, healthy males who participated in two counterbalanced (four- and six-meal) isoenergetic meal regimens. This lack of difference was attributed to the ability of the human body to 'buffer small differences in meal size and timing provided energy balance is maintained' (118).

Wadhwa (259) studied the effects of dividing the daily caloric load (fat- 40% as either butter oil (n=6) or corn oil (n=7); protein 13%; carbohydrate 47%) into a 'gorging' (1/8 total

calories for breakfast 8 am, 7/8 dinner 5 pm) or 'nibbling' (1/8 of total calories every 2 h) pattern of meal consumption on changes in plasma triglycerides in healthy, young males (n=13). Subjects had plasma triglyceride levels and an OGTT (100g) measured (259). While there were no significant differences reported between the plasma triglyceride levels in the butter oil or corn oil group in relation to meal periodicity (115±12.7 vs. 101.0±3.8 mg/100ml; $P>0.05$), there was a significant augmentation of the fasting blood glucose concentration (99.0±3.5 vs. 85.7±0.6 mg/100ml; $P<0.001$) when gorging was compared to nibbling, which was more pronounced in the corn oil vs. the butter oil group (259). This study supports earlier work establishing physiological differences between groups that divide their isoenergetic daily calorie loads into smaller meals, it also questions the role of macronutrient *composition*, and more importantly the composition of *fat* consumed during frequent meal protocols.

After receiving little attention during the 1980's, meal frequency work in general, and particularly in relation to blood lipids, experienced a resurgence of popularity with several research studies in the 1990's (9, 77, 140, 172, 178, 185). A 1997 review article dedicated to the topic of meal frequency (172) indicated that the overall consensus of previous work expressed the positive benefit of an increased, isoenergetic meal frequency on lipid parameters in healthy individuals, a legitimate conclusion based on the history of meal frequency research.

Subsequent studies by both Arnold (9) and Edelstein et al (77) confirmed that more frequent meal consumption led to decreased plasma cholesterol, and total cholesterol and LDL, respectively. Arnold et al (9) examined the effect of alternating 4-week isoenergetic diets consumed as either three or nine daily meals on plasma lipid indices and the glucose/insulin response to a meal test. While there were no significant differences in the glucose/insulin responses following the three-meal and nine-meal interventions ($P>0.05$), there were

improvements in plasma cholesterol levels both from baseline, and in comparison to three-meal consumption, following the frequent, nine-meal per day isocaloric consumption (9). Adherence to the nine-meal regimen resulted in significantly lower levels of total cholesterol compared to baseline and three-meal values (9-meal-4.05±0.75 vs. baseline-4.49±0.87 mmol/L, $P<0.0005$: and 9-meal vs. 3-meal- 4.33±0.85 mmol/L; $P<0.005$), and a similar reduction in low-density lipoprotein (LDL) concentrations (9-meal- 2.48±0.60 vs. baseline- 2.89±0.71 mmol/L, $P<0.0005$) between the nine-meal and baseline measures (9).

Likewise, the Rancho-Bernardo study, a large comprehensive survey (n=2034 Caucasian men and women, (50-89 y) designed to interpret the relationship between meal frequency and plasma cholesterol levels in a free-living environment (77), found that 72% of individuals reported a three-meal pattern, while 9% and 14% reported a 1-2 and >4 meal per day plan, respectively. The findings (77) of this study revealed that both total cholesterol (0.23 mmol/L average decrease; $P=0.01$) and LDL cholesterol (0.16 mmol/L average decrease; $P=0.06$) concentrations were lower in the individuals consuming four or more meals per day than those consuming 1-2 meals. The authors in both of these studies (9, 77) hypothesized an attenuation of the enzyme HMG-CoA reductase as a moderator of these decreases in plasma cholesterol, and subsequent cardiovascular risk.

Changes in cholesterol production were further examined by Jones et al (140), who administered deuterium oxide to characterize plasma cholesterol synthesis in response to an increase in the periodicity (three vs. six isocaloric meals) of a standard liquid diet (53% carbohydrate; 32% fat; 15% protein; Ensure Plus) over the course of a 48 h intervention. A reduction in the frequency of daily meals was associated with augmented cholesterol synthesis (measured as the percent of total body deuterium enrichment plateau attained; 6.08±0.45 vs.

4.00±0.25%; P<0.01) in the 3 traditional versus 6 evenly-spaced isocaloric meals, respectively (140). Similarly, McGrath and Gibney (178) found a reduction in cholesterol levels when they altered the dietary patterns of infrequent consumers ('non-snacking'; n=11; 3.1±0.1 meals/day) to a frequent meal pattern ('snacking'; 6.0±0.8 meals/day). Infrequent consumers demonstrated significantly decreased measures of total cholesterol (P=0.03) as well as increased HDL/LDL cholesterol ratios in response to a switch from three to six daily meals, though their total nutrient intakes and dietary composition during the intervention did not change.

In order to further assess the potential chronic effects of altering meal periodicity, Murphy et al (185) examined the effect of two weeks of nibbling on plasma cholesterol in a cohort of young, female (n=11) college students. During two randomized two-week interventions, participants consumed isoenergetic diets varying only in the number of daily eating occasions (three-meal vs. twelve-meal). The authors reported no differences between total cholesterol, LDL cholesterol, or lipoprotein lipase (LPL) measures (fasting and in response to a high-fat (80 g) meal test) between three- and twelve-meal regimens (185). The gorging pattern of meal consumption (three-meals per day) resulted in significantly higher plasma HDL levels (P<0.05) than the nibbling profile (185).

The findings of these studies concerning meal periodicity imply that an increase in the frequency of daily meal consumption, with an isocaloric diet, was beneficial in relation to plasma cholesterol and triglyceride levels, and the overall ratio of healthy to unhealthy plasma cholesterol. This research had a strong influence on the recommendation of frequent, isocaloric meal consumption, particularly in promoting cardiovascular health.

More recent studies have confirmed the benefits of frequent meal consumption on measures of blood lipids and cholesterol. The EPIC-Norfolk study (245) provided convincing

epidemiological findings of the consistent, inverse relationship between meal frequency and both total and LDL concentrations in a population-wide investigation of the residents of Norfolk, United Kingdom (n=~25,000, 45-75 y). There was a decrease in total cholesterol levels (male- -0.29; female- -0.22 mmol/L) and LDL cholesterol (male- -0.26; female- -0.17 mmol/L) when individuals consumed six or more meals compared to one-two daily meals across this large cohort (245), although HDL concentrations and the LDL/HDL ratio were decreased. Adjusting the findings for BMI, total energy intake, physical activity, etc. caused the relationship between increased meal frequency and decreases in the HDL and LDL/HDL ratio to disappear; though the relationship between increased meal frequency and reductions in total and LDL cholesterol remained significant (245). An increase in meal frequency is not always shown to improve lipids (118); and the relatively low number of daily meals in the control group (1-2 meals per day) in this study (245) points to the contradictory claim that meal restriction and fasting may improve blood lipids, particularly HDL (232).

In contrast to the established trend of an improvement in blood lipid measures with increased meal frequency, a small number of studies have demonstrated no significant differences between lipid measures when meal periodicity is altered. Holmback et al (118) studied the effect of an increase from four to six isocaloric, evenly-spaced, high-fat meals (45% fat, 45% carbohydrate, 15% protein) on 24 h measures of fat storage (triacylglycerol, non-esterified fatty acids), energy expenditure, substrate utilization, and plasma insulin concentrations in healthy individuals. These authors (118) reported no differences ($P>0.05$) in any of these measures with an increase in meal frequency. Additionally, a *decrease* in meal frequency was also shown to have positive effects on health indices in the fasting samples of healthy, middle-aged subjects (118). Stote et al (232) further examined the effect of meal

reduction on cholesterol levels by comparing two-randomized 8-week protocols of three vs. one meal per day. These authors (232) found both positive (increased HDL- +8.4%% increase, reduced triacylglycerols) and negative effects (increased TC- +11.7, increased LDL- +16.8%) of meal reduction, in lieu of a small (~65 kcal) calorie reduction reported in the one-meal condition. Therefore, the effect of meal frequency reduction and intermittent fasting on cholesterol levels remains equivocal.

Although the overarching consensus reveals that an increase in meal frequency confers a positive benefit on an individual's plasma lipid and cholesterol levels (9, 77, 245) several studies contradict these reports (118, 259) indicating both individual and population wide variance in responses. While studies examining lipid and cholesterol values under various meal frequency conditions were predominantly conducted in the short-term (118, 178, 185), it has been suggested that an increase in meal frequency, particularly if an increase in appetite control is achieved, could lead to changes in body composition over the long-term.

Meal frequency and body composition/obesity

While a majority of early meal frequency studies focused on blood lipids and cholesterol, research has also described body composition outcomes under various habitual and experimental meal frequency patterns (85, 90, 179). For example, Finkelstein and Fryer (90) examined the potential for weight loss and protein retention between young (20-22 y) overweight (20-40% greater than height-weight table recommendations) women consuming a low-fat diet (1400-1700 kcal, 105-115 g protein, 40-50 g fat; based on the stage of the intervention) as either three (n=4) or six (n=4) meals per day over the course of sixty days. No significant differences ($P>0.05$) across measures of body weight, skinfold thickness, hemoglobin, protein-bound iodine, or

nitrogen balance were found as the result of the two different dietary protocols; leading to the conclusion that meal frequency increases do not affect protein retention or weight loss (37).

The meal frequency and body composition literature is not limited to examinations of the effects of various meal patterns on adiposity. Several studies have highlighted the benefit of frequent meal consumption in individuals over the age of 65 years, where frailty starts to become as great of a concern as adiposity. Shahar et al (222) promoted between meal snacking on energy dense foods in the elderly, following an examination of the nutritional intake of a group of older Israeli men and women (>65 y; n=377). When compared to younger Israeli individuals (average age of 35 y), older men and women consumed a lower percentage of their daily calories as snacks (17.4 v. 22.9% of daily calories; P=0.001). Older individuals in this study (222) who increased between meal caloric intake to greater than 17.4% of their daily total caloric load demonstrated a greater capacity to meet daily nutritional recommendations than those who consumed a lower percentage of their total calories from snacks. While these findings (222) seemingly point to an increased caloric load in the elderly consuming more in-between meal snacks, and an increased potential for extra stored fat, a study on the traditional Greek eating pattern (a greater number of meals and snacks daily) demonstrated reduced adiposity in older Greeks (260). The number of daily eating occasions was inversely correlated with body fat percentage (r_s= 0.15; P<0.03; n=186), as calculated by body circumferences and BMI (r_s= 0.01; P<0.01; n=256), as well as an increase in daily food variety (260). Wahlqvist et al (260) concluded that the maintenance of BMI with increased meal frequency may contribute to quality lean body mass (as indices of body fat percentage were lower with an increase in meal frequency), in older adults. Under this rationale, an increase in meal frequency may also be beneficial in those experiencing cachexia or other wasting conditions. It must be noted that

differences in population, age-range, dietary composition, and many other variables related to data collection often influence the application of these findings across disparate population groups.

The influence of an increase in meal frequency in healthy animals, children, and adults compared to frail elderly or otherwise cachexic individuals, reinforces the importance of reevaluating population-wide recommendations for increasing meal frequency. Additionally, the impact of meal frequency on body weight status may be altered depending on the weight-stability, or weight-change that a subject may be experiencing, as well as the length of chronological adaptation to the given meal regimen.

Several studies have examined the influence of different feeding patterns on weight changes in the rat, with conflicting findings. In a study of increased food accessibility, researchers allowed rats, who are normally adapted to a single daily meal, to have either *ad libitum* access to food for 24 h a day, or two, timed meal periods per day in order to assess potential changes in body weight (181). Despite the similar amount of food consumed during these two conditions, rats who were allowed to nibble gained 18% less weight than those exposed to two, timed daily meals (181). Likewise, Drewnowski (72) established that rats who gorged (infrequent, timed meals) on a high-fat, high-sugar diet gained significantly greater adipose tissue mass than those who nibbled freely. Interestingly though, when fed a 'lab chow' with a healthier macronutrient profile (i.e., lower fat), there were no differences noted in weight gain (72). Shahraki et al (223) also found no significant differences in body weight between rats fed for sixty days on either a nibbling or gorging regimen when consuming standard lab chow.

In contrast, a series of studies by Leveille et al (62, 164, 182, 193) concluded that there were significant differences in the adipose tissue gain of rats fed under either a meal or *ad*

libitum regimen, compared to the normal one-meal daily regimen. One study (182) examined the changes in adipose tissue mass in a cohort of male Sprague-Dawley rats who were accustomed to one two-hour long meal interval per day, when they were changed to three weeks of either an identical diet (n=20), a diet containing two one-hour meal sessions (n=5), or *ad libitum* food access (n=20). Both groups of rats who were subject to changes in their dietary patterns (i.e. *ad libitum* 'nibblers', and two-meal gorgers) gained a similar amount of weight ($P>0.05$). Interestingly, the rats who were changed to two meals gained 38% of their weight as fat, while the *ad libitum* fed animals gained only 20% of their weight as fat (182).

Previous reports by Cohn (50) have classified the animal and human response to alterations in meal frequency as 'indistinguishable', however, inherent differences between the dietary habits of humans and animals, have dictated that comparable human studies are imperative. For example, researchers have reported disparate findings between the adipose tissue accumulation outcomes of rats consuming a high-fat diet (72) versus a standard macronutrient profile diet (72, 223). Free-living humans are exposed to a wide variety of psychological and social cues, as well as a tremendous amount of food accessibility and variety that makes the direct comparison of human and animal studies difficult.

One of the early studies on meal frequency and body composition in humans by Fabry et al (85) manipulated the daily meal frequency of schoolchildren (n=226) in three different boarding schools over the course of one year. This study (85) assessed the potential changes in weight status with variations in the daily pattern of dietary intake, while maintaining consistent levels of caloric intake and macronutrient composition. The meal patterns utilized over the course of the year consisted of three, five, or seven meals per day. The authors (85) reported that in children aged 11-16 y, an increase in meal frequency from three to seven isocaloric daily

meals led to a decrease in weight-height proportionality (‘growth-adjusted BMI; $P<0.05$ in males and $P<0.01$ in females) and 3-site skinfold thickness ($P<0.05$) (85). A subsequent study by Fábry examined the amount of weight accrued during a period of positive calorie balance among individuals who consumed their calories in different meal patterns (86). It was demonstrated that an increase in excess weight (58.6 vs. 28.8%) in older men who consumed meals in an infrequent (3 or fewer meals/day) compared with a frequent manner (5 or more meals/day). Taken together, these early findings highlight the importance of alterations in meal periodicity on body mass and composition, even under calorie matched regimens (85, 86).

In the late 1970’s findings from the Tecumseh Community Health Study (179) demonstrated that adiposity was inversely related to the number of daily eating occasions recorded. In this study, Metzner et al (179) demonstrated that the adiposity index (calculated as an aggregate of body mass index, and subscapular/triceps skinfolds) was attenuated in men and women as they increased meal frequency over the course of a day ($\leq$2 vs. 6 or more meals/day; $P<0.01$). In the 1990’s and 2000’s, epidemiological studies of meal frequency and body composition became increasingly popular as the obesity epidemic became more evident, despite methodological and accuracy concerns. Ma et al (169) reported that adults in the U.S. demonstrated a decreased incidence of obesity with an increase in the number of daily eating occasions (odds ratio of obesity; >4 meals=0.55, compared to <3=1.00; 95% confidence interval; 0.33 and 0.91, respectively) even when accounting for an individual’s total calorie intake and physical activity levels. Similarly, Rudiavets et al (215) examined the impact of a change in the number of daily meals, while maintaining total daily calorie load on the BMI and waist-to-hip circumference in a large cohort of men (45-64 year-old; n=330). Correcting the data for confounding variables (including dieting, low-energy recording, physical activity, and total

calorie consumption), the authors (215) discovered a significant inverse linear trend ($P<0.05$) between the number of daily meals consumed and indices of BMI and waist-to-hip ratio. Epidemiological findings on the benefits of increased meal frequency on body composition are not limited to adults, as more recent work has focused on eating patterns in relation to childhood obesity.

Following the trend for epidemiological meal frequency research, Barba and colleagues (15) examined childhood obesity rates in relation to daily meal frequency in a large cohort of children (n=3669 boys and girls; 6-11y) in Southern Italy during 2003-2004. This study (15) confirmed previous findings of decreased obesity measures with an increase in daily meal frequency as seen in adults. When these children were divided into categories based on the number of daily eating occasions (as reported by a parent/guardian; <3 meals (n=332), 4 meals (n=1534), or >5 meals/day (n=2002)), average indices of BMI (<3- 20.5±0.4; 4- 19.7±0.2; >5- 18.8±0.2; $P<0.001$) and waist circumference (<3- 68.0±1.2; 4- 66.6±0.6; >5- 63.9±0.4; $P<0.001$) were lower in children classified as more frequent eaters.

Toschke et al (246) also reported the 'protective' quality of frequent meal consumption against obesity in large sample of German children (n=4370 boys and girls; 5-6 y), by determining odds ratios for obesity based on the frequency of daily meals. These authors (246) reported that a high BMI was less likely in children consuming four (0.73%, 95% CI 0.44-1.21) or five or more (0.51%, 95% CI 0.29-0.89) meals per day, compared to those who ate three or fewer despite an increase in total calorie consumption in the nibblers.

In contrast, Drummond et al (73) discovered gender disparity in the relationship between meal frequency and adiposity when they compared body composition measures (body weight, BMI, 4-site skinfold analysis) and meal frequency (average eating occasions over a 7-day dietary

record) in free-living men and women (n=48 and 47, respectively; 20-55 y). Male participants had a negative correlation between eating frequency and body weight (r=-0.3436; P=0.03) with increased daily meal frequency, while females demonstrated no such relationship (r=0.1396; P=0.41).

Yannakoulia et al (269) also found that menopausal status was an important determinant of the relationship between meal frequency and adiposity, when they compared measures of body composition (BMI, waist circumference, dual-energy x-ray absorpiometry (DEXA)) with eating frequency indices garnered from three-day dietary records in a sample of women from various stages of menopausal status (n=220; age=48.2±12.2 y). Correcting for valid dietary reporting among the larger study sample (valid reporting= EI/BMR ratio$\geq$1.04; BMR derived from Schofield equation), one hundred and nineteen individuals were used in the final analysis. Using a stepwise multiple regression model, Yannakoulia et al (269) reported that eating frequency did not influence body weight or composition (P>0.05) in pre-menopausal women, but played a significant role in the body composition (r=0.30; P=0.03) of post-menopausal women, resulting in a 2.5% increase in body fat percentage with each extra daily meal consumed (269). Again, a major contributing trend towards this positive relationship between meal frequency and adiposity was the fact that post-menopausal women seemed predisposed to greater energy intake when compared to their pre-menopausal controls (269).

This possible effect of an increase in total calorie consumption was reported by Howarth et al (124) who examined data from the Continuing Survey of Food Intake by Individuals 1994-1996 in order to determine relationships between eating behaviors (patterns of ingestion, dietary composition) and BMI in younger (20-59 y) and older (60-90 y) free-living, food-secure individuals. This study demonstrated that total energy intake (younger: β=1.23±0.07, P<0.001;

older: β=1.21±0.11, P<0.001) was positively associated with BMI, while the pattern of meal ingestion was not important. These conflicting findings stress the importance of consistent definitions and methods in epidemiological meal frequency research, and highlight the fact that different populations may experience different outcomes in response to increased meal ingestion. This is only one of several concerns with epidemiological studies related to ingestion behavior.

The accuracy of dietary reporting in epidemiological studies of meal frequency was questioned by Crawley and Summerbell (55), who reported correlations between feeding frequency (averaged from 4 d dietary records; defined as each daily energy-containing intake separated by ≥30 minutes) and BMI in a sample of British teenagers aged 16-17 y (1970 Birth Cohort Study). A significant inverse correlation was reported between feeding frequency and BMI in males (P<0.005) and females (P<0.05), which remained after dietary under-reporters were removed (defined as individuals who reported an energy intake less than 1.35 times their estimated daily energy requirements (BMR)). Holmstrup et al (180) also reported that middle-aged males (n=44) recording an increase in daily meal frequency had a decreased sum skinfolds (7-site skinfold examination; r=-0.402; P<0.05) and body fat percentage (r=-0.401), though this relationship disappeared with the exclusion of under-reporters (n=16; defined as individuals who reported an energy intake less than 1.2 times their daily estimated energy requirements (BMR)). Combined these findings (55, 180) highlight the concerns with dietary recording bias as a potential confounding variable in establishing relationships between dietary intake behavior and body composition outcomes (246). Establishing a consistent definition of dietary underreporting may also help to clarify the findings of epidemiological meal frequency findings.

Several studies point to concerns with the accuracy of study participants recording various dietary recall protocols. The accuracy of dietary recall used in epidemiological studies of

meal frequency is questionable (10, 16, 28), often affected by the weight status of the individual reporter (61, 159, 167), the size of meals reported (262), and the extent of an individual's dietary recall training (30, 139). Another major consideration when examining epidemiological findings related to food intake behavior and health outcomes (obesity, heart disease, etc.) is known as reverse causality (246). In the context of meal frequency study, reverse causality refers to the fact that a given behavioral pattern (in this case dietary frequency) does not necessarily precede, and may in fact be a consequence of a related health outcome. Concerns with the applicability of epidemiological meal frequency research, despite lay and popular opinion, have increased the need for randomized, controlled studies to understand and implement effective meal frequency strategies for various populations (175).

The lay media commonly portrays an increase in meal frequency, or the consumption of small, frequent meals in a positive light. The demand for material regarding proper nutrition is tremendous, and individuals have become increasingly capable of accessing this information (88). The overuse of the term 'expert' in the presentation of this information is alarming, and it is well-established that many individuals will base their daily nutritional decisions on messages that they receive from the media, regardless of the source (88). In order to examine the lay message regarding meal frequency, a Google search of the term 'small meals throughout the day' was conducted. This search yielded approximately 3,640,000 results, with the first page featuring links to various online sources including for-profit websites like 'allexperts' and 'askmen.com', and more reputable sites like 'myclevelandclinic.org'. Material from the first six results of this search was summarized in order to convey the lay message regarding meal frequency. The outcomes of increasing the daily frequency of eating, while maintaining daily caloric intake, is often reported as one of three major claims. 1) An increase in meal frequency is proposed as a

means of enhancing the daily thermic effect of feeding, and therefore increasing the metabolic rate of an individual (6, 11, 203, 268), 2) An increase in isocaloric meal frequency is purported to maintain steadier blood glucose levels throughout the day, resulting in a smaller insulin demand, and improvement in glucose tolerance (240), and 3) An increase in daily meal frequency is supposed to augment the satiety response to meal ingestion, curb cravings and prevent subsequent overeating (6, 11, 203, 268). Additionally, one of the websites accessed contradicted the reports of the first statement regarding an increase in the thermic effect of feeding with increased meal frequency (237), adding an additional layer of complexity to the decision making of casual nutrition information consumer (88).

These three claims to the effectiveness of increasing daily meal frequency have been embraced by the popular media, despite potentially confounding claims to their truth reported in the lay and scientific literature. A difficult realization is that blanket recommendations to the effectiveness of increased meal frequency are often directed towards individuals (e.g. obese, hypertensive, individuals with diabetes) to whom the advice may be the most detrimental (134). There are still a large number of hypotheses that need to be addressed before proper, population-specific meal frequency guidelines can be implemented. Scientific research pertaining to the three common lay claims attached to an increase in meal frequency will be discussed further.

Meal frequency and energy expenditure

An often misunderstood view on energy expenditure regulation is that there is a defect in the thermic effect of feeding with obesity (50), and an increase in the daily thermic effect of feeding when an individual eats smaller amounts more frequently. In a comprehensive analysis of meal frequency studies related to energy expenditure (EE), Bellisle et al (21) reported that there are no inherent differences in 24-hour EE when individuals either nibble or gorge. The

aggregate of these findings (21) concerning energy expenditure and meal frequency were determined using closed-circuit chamber calorimetry.

As early as 1982, prior to the widespread recommendation of an increase in meal frequency, Dalosso et al (59) reported that there were no significant differences ($P>0.05$) between 24-hour EE values when an individual spent 31 hours in a chamber calorimeter following two weeks of isoenergetic two- or six-meal per day regimens. Verboeket-van de Venne and Westerterp (255) examined the acute effects of changes in isocaloric meal patterning (2 meals- gorging vs. 7 meals- nibbling) in a sample of thirteen individuals (2 male, 11 female) on EE measures in a calorimetry chamber. The authors (255) concluded that despite 'stronger diurnal periodicity of nutrient utilization', gorging resulted in no significant differences in 24-hour EE compared to nibbling (5.57 ± 0.16 vs. 5.44 ± 0.18 kJ/min; $P>0.05$). This lab (256) was also the first to report that meal frequency has no effect on total daily EE. Subsequently, Smeets and Westerterp-Plantenga (225) examined the effect of a one-meal difference at the lower end of the meal frequency spectrum (2 vs. 3 meals) on markers of EE in normal-weight women (n=14). The findings of this study (225) verified that there was no significant difference between the ingestion of two or three isocaloric daily meals on 24-hour EE (8.2 ± 0.8 vs. 8.5 ± 0.6 MJ/day; $P>0.05$), diet-induced thermogenesis (0.86 ± 0.23 vs. 0.90 ± 0.30 MJ/day; $P>0.05$), or sleeping metabolic rate (5.93 ± 0.56 vs. 5.98 ± 0.47 MJ/day; $P>0.05$). Independently, Taylor and Garrow (238) reported that overweight women (n=10) in a calorimetry chamber fed 4.2 MJ per day as either two or six meals demonstrated no difference in total EE (9.96 vs. 10.0 MJ/day; $P=0.88$).

In contrast, Westerterp-Plantenga et al (266) examined a cohort of normal-weight, individuals sequestered in a chamber calorimeter to determine the interaction of fat-free mass and meal frequency on daily resting EE. In individuals with a high fat-free mass (n=19; young

meals), meal frequency was inversely related with resting EE (r^2= 0.80, P<0.0001) and residuals of EE as a function of fat-free mass (P<0.03). According to these findings (266), meal frequency explained 85% of the variation in EE. The influence of fat-free mass in young men (63.9±7.5 kg), was greater than the fat-free mass of older women (n=10; 42.0±6.3 kg; P<0.001), younger women (n=15; 45.5±5.2 kg; P<0.001), and older men (n=12; 56.8±5.9 kg; P<0.001) on EE, indicating that a higher amount of fat-free mass was integral to the inverse relationship between habitual meal frequency and energy expenditure (266).

In another contradictory study using open-circuit spirometry, a negative relationship was demonstrated between meal frequency and the thermic effect of feeding. Tai et al (236) reported that a mixed calorie load consumed as a large meal generates a higher thermic effect of feeding (0.849 vs. 0.611 kJ/min) than the same meal broken into five equal portions, measured over a six-hour period.

Despite these few reports to the contrary, the gold-standard measurement of energy expenditure (closed-circuit spirometry) has proven that increased meal frequency does not mediate differences in energy expenditure (59, 238, 255). The lack of a relationship between meal frequency and diet-induced thermogenesis, energy expenditure, and metabolic rate was recently reiterated in the International Society for Sports Nutrition's Position Stand on Meal Frequency (156). This statement (156), however, indicates the potential for an increase in meal frequency to positively impact blood markers of health, including insulin levels.

Meal Frequency, Glucose Homeostasis, and the Insulin Response

Alterations in the frequency (and size) of energy-containing meals and beverages are known to alter subsequent metabolic and hormonal responses to calorie intake (101, 135, 137).

Reduced glucose excursions and a subsequent decrease in insulin load in response to small, spaced nutrient loads have been hypothesized for nearly eight decades, and were first studied as a potential means of correcting adverse health conditions related to diabetes.

In the 1930's Ellis (80) studied individuals with severe diabetes and demonstrated that the partitioning of daily carbohydrate intake into smaller portions, in concert with insulin administration, favorably attenuated their blood glucose levels. In order to further examine the potential glucose-lowering effects of spreading the nutrient load, Jenkins et al (136) examined the effects of a 'continuous sipping' model, where glucose-tolerant individuals (n=9) consumed fifty grams of a glucose solution either as a bolus or in small amounts at evenly spaced intervals (sipping) over a 210-minute test period. In this study (136), postprandial glucose and insulin AUC, and FFA levels were compared across 240 minutes, with an IVGTT performed at the 240-minute mark to assess potential differences in subsequent glucose disposal rate. The sipping condition resulted in a 'minimal perturbation' of plasma glucose and insulin concentrations, greater than 50% reduction in insulin area under the curve (Figure 3), and significantly enhanced rate of glucose disposal (K_G; $P<0.05$) compared to the bolus condition. This economy of insulin secretion was hypothesized to be related to a suppression of the inhibitory effect of FFA on glucose disposal as a result of the smaller, more frequent introduction of glucose across the test period (134, 136).

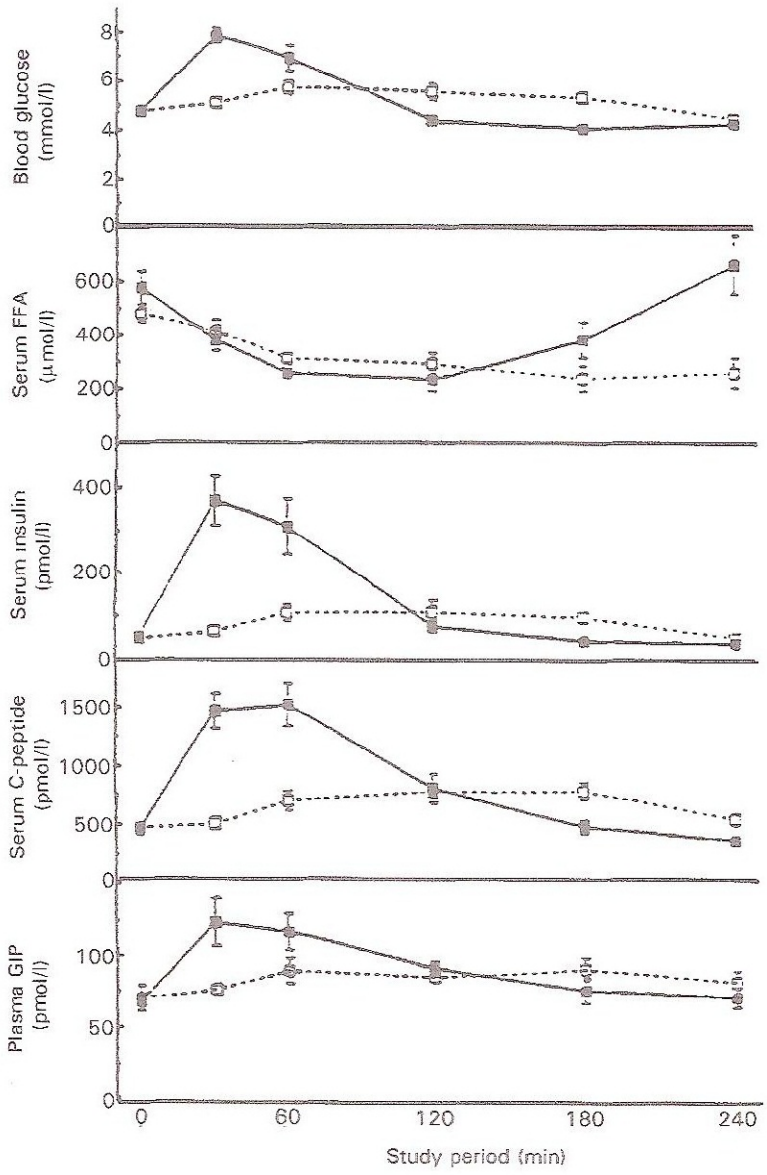

Figure 3: Mean blood glucose, serum free fatty acid, insulin, c-peptide, and plasma gastric inhibitory polypeptide response to 50g glucose solution consumed as either a bolus (■; at baseline, consumed within 5-minutes) or continually sipped (□; evenly spaced consumption over 210-minutes). (136)

Wolever (267) also demonstrated the beneficial effect of 'continuous sipping' when compared to a three-meal protocol over the course of a twelve-hour test period in a cohort of

healthy men (n=7). Similar to the findings of Jenkins (136), who compared continuous sipping to one large glucose ingestion, Wolever (267) reported a 32% reduction in total integrated insulin AUC over a twelve-hour period, with no significant differences in blood glucose, in response to sipping.

While a model of continuous sipping may not be an easily translatable, practical means of nutrient consumption for an individual, the outcomes of these studies were used in order to shape protocols addressing the concept of 'grazing', or consuming several small, whole-food meals over the course of a day; and their potential relevance of increasing meal frequency on enhancing insulin economy, particularly in individuals with T2D. Experimental models that compare the effect of various patterns of meal consumption using standardized diets build upon the knowledge base generated from continuous sipping protocols. These findings will be discussed in relation to weight status (lean, obese) and metabolic health (glucose tolerant, T2D).

Lean, Healthy Individuals

An acute, controlled study of the effects of meal periodicity in healthy, young men (n=15; 27.2±6.4 y) was undertaken by Rashidi et al (204), who examined the effect of 2-week intervals of either a nibbling (9-meal) or isocaloric gorging (3-meal) protocol on subsequent fasting glucose, insulin and plasma lipid concentrations. This study was undertaken in order to clarify controversial findings, often confused between subject populations, regarding the impact of an increase in meal frequency on glucose and insulin concentrations and the lipid profile. The findings of this study (204) revealed a robust reduction in insulin secretion ($P<0.05$) and plasma glucose ($P<0.01$) concentrations, a significant reduction in lipoprotein a ($P=0.02$) and non-

significant increase in HDL cholesterol levels ($P>0.05$) with nibbling. These favorable alterations in the glucose/insulin and lipid profiles in healthy, young men further strengthened the increased meal frequency message.

Using a similar, acute protocol, Jones et al (141) compared 4 h insulin, GIP, and FFA concentrations in healthy, male individuals (n=12) and noted that time periods coinciding with meal ingestion, resulted in higher mean insulin values ($P<0.05$) when meals were consumed as three compared to six daily meals.

Not all acute studies demonstrate favorable reductions in glucose concentrations and insulin secretion in response to an increase in the frequency of meal consumption. Solomon et al (226) examined the effect of feeding frequency on the insulin responses in humans. Lean male participants (n=5; BMI=23.8±0.8 kg/m^2) remained sedentary in the lab for eight-hour periods, during which they consumed one of three randomized, counterbalanced meal regimens. Participants either fasted (FAST) for eight hours, or consumed 1667 kilocalories (64% CHO, 23% FAT, 13% PRO) as two large (LOFREQ), or eight small (HIFREQ) meals (226). The authors (226) reported that the total insulin area under the curve did not differ between the LOFREQ and HIFREQ conditions ($17.9\pm2.6*10^3$ vs. $15.9*3.3x10^3$ µU/ml*8 h; $P>0.05$). Thus, controversy remains in the scientific literature regarding the efficacy of frequent meal consumption, in both obese and healthy individuals.

Using this information from previous research, recent work in our lab (119) evaluated the glucose and insulin response to acute alterations in the frequency (three vs. six meals) of high-carbohydrate (15% PRO, 65% CHO, 20% FAT) isocaloric meal consumption in healthy, young individuals. This study demonstrated increased blood glucose levels over the course of twelve

hours during the 6CHO compared to 3CHO meal condition (Figure 4; P=0.029) when assessed as area under the curve (AUC), however, despite the increased blood glucose levels, plasma insulin concentrations were not significantly different between meal conditions (119).

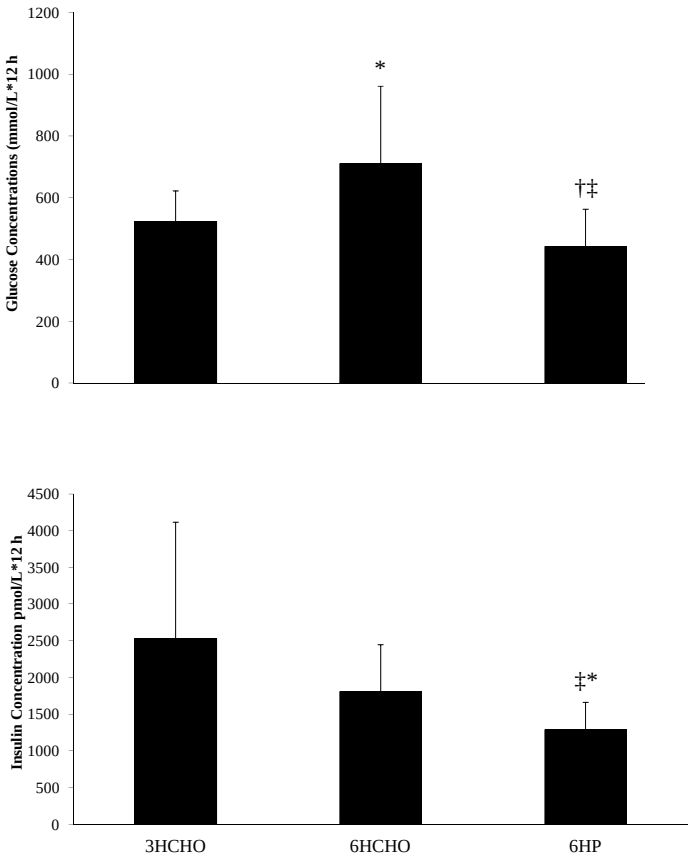

Figure 4: Integrated 12h area under the curve for glucose and plasma insulin concentrations; *P<0.05 vs. 3CHO; † P<0.01 vs. 6CHO; ‡ P<0.05 vs. 3CHO (119)

When divided into four hour blocks, the net glucose AUC revealed that blood glucose levels were highest in the 6CHO condition (p=0.026 vs. 3CHO), though this was not reflected in the insulin values. Interestingly, the study demonstrated a smaller insulin response (Figure 5; 0700-1100>1100-1500 p=0.018; 0700-1100>1500-1900 p=0.018) as the day progressed (119).

Therefore, it appears that healthy young individuals show higher glucose concentrations over the course of a day with frequent meals, yet the insulin levels do not increase accordingly (119). The longer term implications of this phenomenon are unknown.

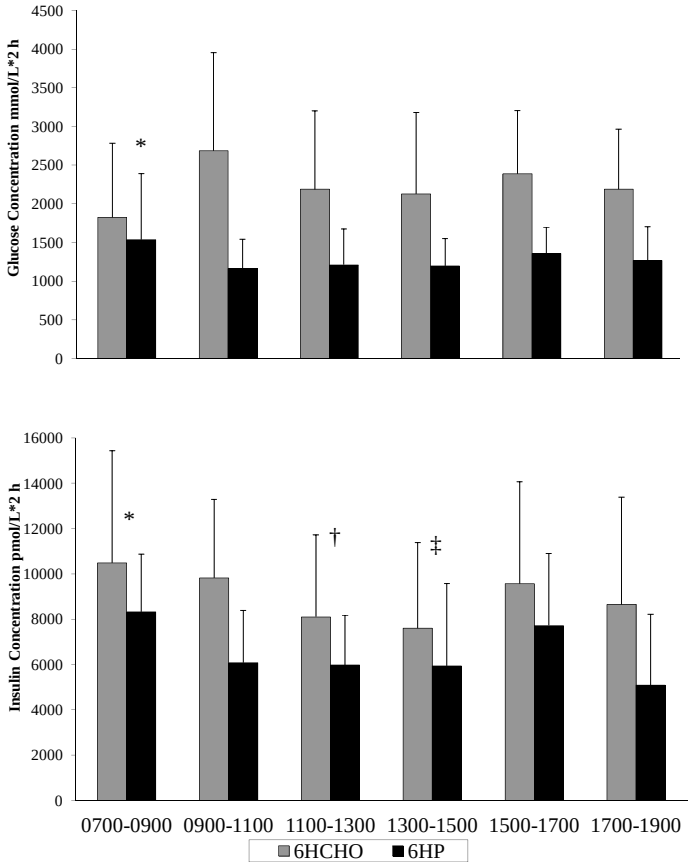

Figure 5: Integrated 2 h area under the curve for glucose and insulin levels for the 6 meal/day. * P<0.001 6CHO vs. 6HP; †P<0.01 vs. 0700-0900>1100-1300; ‡ P<0.05 vs. 0700-0900 (119)

In addition to acute studies of the physiological effects of increasing meal frequency, the chronic effects of spreading the nutrient load have also been examined to determine if adaptation to meal periodicity extends beyond the short term. Jenkins et al (137) assessed healthy, glucose tolerant males (n=7) following two counterbalanced, identical diets consisting of a seventeen-

meal and isocaloric three meal regimen followed for two weeks each. Fasting values and the responses to a standardized breakfast meal were measured for blood glucose, serum insulin, c-peptide, and blood lipids following each 2-week interval (137). While there were no significant differences reported in fasting glucose ($P>0.05$) and lipid concentrations (FFA, TG; $P>0.05$); a 28% reduction in serum insulin levels ($P<0.01$) and 20% decrease in urinary c-peptide output ($P<0.02$) were reported when an individual consumed their meals in a frequent manner throughout the day (137). There were no differences demonstrated in the glucose, insulin or c-peptide responses to the standardized breakfast meal. Therefore, in this healthy cohort, an economy of insulin secretion was reported when frequent consumption was utilized, though the impracticality of consuming seventeen small meals may somewhat overshadow this benefit.

Using a relatively high-fat diet (40% FAT, 13% PRO, 47% CHO), Wadwha et al (259) placed healthy, young male subjects on 4-week gorging (2 meals; 1/8 and 7/8 of daily calories), nibbling (8 equal meals), or three-meal (3 meals; 2/8, 3/8, and 3/8 of daily calories) eating regimens in order to compare the ramifications of these commonly consumed dietary patterns on insulin production. Oral glucose tolerance testing at the conclusion of each dietary protocol revealed that the gorging regimen resulted in higher mean blood glucose concentrations (99.0 ± 3.5 vs. 85.7 ± 1.6 mg/100ml; $P<0.001$) and increased 2 h insulinogenic indices (0.71 ± 0.14 vs. 0.21 ± 0.053 mg/100ml; $P<0.05$) when compared to nibbling (259). Therefore, it was concluded that an increased contribution of fat to the diet did not alter the expected increases in glucose and insulin concentrations during a gorging compared to a nibbling regimen.

Similarly, Murphy et al (185) examined the effect of two-week, counterbalanced nibbling (12 meals per day) and gorging (three, isocaloric meals per day) protocols on the fasting and postprandial glucose and hormonal response (insulin, GIP, GLP-1) to a standard high-fat test

meal in eleven young, healthy women (22±0.9 y). They noted (185), that 24-h insulin, GLP-1, GIP and glucose responses to the test meal did not differ following a two-week protocol of either twelve or three daily meals ($P>0.05$).

In summary, despite widespread acceptance, the effects of an increase in meal frequency in lean, healthy individuals remain controversial. Six of the nine studies in lean, healthy individuals reviewed demonstrated a beneficial hormonal response with nibbling (136, 137, 141, 204, 259, 267) compared to three which demonstrate either no benefit, or controversial findings (119, 185, 226). Even in lean, healthy individuals, further evidence of the safety and efficacy of frequent meal protocols is warranted.

Obese, Healthy Individuals

A small number of studies in obese individuals have assessed the efficacy of increasing meal frequency. For example, Fogeltoo et al (92) examined 24 h leptin, glucose and insulin profiles of a cohort of obese women (n=5), in order to assess the efficacy of increasing meal frequency from three to eight daily meals on hormones related to energy balance. These authors reported a decrease in the overall secretion of insulin ($P<0.05$) with an increase from three to eight isocaloric meals, and correlated these reductions to a significant, beneficial change in each individual's twenty-four hour leptin rhythm (amplitude decreased amplitude ($P=0.0089$) and a phase delay ($P=0.021$) of 168 minutes) with nibbling (92). The eight small insulin peaks observed with nibbling comprised a smaller total insulin release than the three large peaks found with gorging, resulting in an improved insulin economy.

Subsequent study of the chronic response to changes in meal frequency by Gwinup et al

(101) examined the glucose response of a small group (n=4) of glucose-tolerant, hyperlipidemic individuals to an OGTT (75g) after completing two, randomized 14-day periods where they consumed their daily calories as either one large bolus or ten small isocaloric loads. The authors (101) demonstrated that the glucose tolerance curves in response to an OGTT were higher for each subject following two weeks on the 1 meal per day regime versus the 10 meals per day regime. This data supports the previous findings that there is a benefit of increasing meal frequency, although the conditions chosen in the study are extremes and would not normally be recommended.

Speechly and Buffenstein (230) examined the influence of alterations in meal frequency on the magnitude of the insulin response through the use of an isocaloric pre-load, consumed by glucose-tolerant, obese (BMI=40.02±10.93 kg/m^2) men (n=7) as either one large bolus or five small meals. Interestingly, these authors also observed an attenuation in peak insulin concentrations (171.2±127.8 vs. 133.7±70.2 $\mu U/ml^{-1}$, respectively; $P<0.01$), but a prolonged duration in the insulin response to an isocaloric nutrient load when obese individuals consumed five, equal portions as compared to one large bolus (230). Despite higher overall peaks, the increased duration of the insulin response (230) resulted in no differences in the total AUC of the insulin response to the caloric loads ($P>0.05$). Therefore, while it appears that an increase in isocaloric meal frequency may benefit obese individuals with a reduction in the peak height of insulin spikes (92, 230), the potential for an increased duration of these smaller responses remains (230). Similar controversial findings have been encountered when examining the effects of an increase in meal frequency in healthy individuals (119).

One of three studies performed in healthy obese individuals found an improvement in insulin economy with nibbling (92), while two found questionable results (101, 230). While the

recommendation to increase meal frequency in obese individuals, like their lean healthy counterparts, seems favorable; questions as to the efficacy of these interventions, particularly in the long-term remain.

Individuals with Type 2 Diabetes

Jenkins et al (135) examined the acute metabolic effects of consuming an identical 1600 kcal diet as either 3 or 12 evenly-spaced intervals in a group of 11 older (64.1±3.7 y) individuals with T2D. Each of the two experimental conditions also included a snack (20% of daily calories) to be consumed at 10:30 p.m. The model of 'slow absorption', or increased meal frequency, reduced the subject's mean blood glucose (9.6±0.9 vs. 11.1±1.0 mmol/L; -12.7%; P=0.026), insulin (276±52 vs. 336±49 pmol/L; -20%; P=0.063), c-peptide (1490±196 vs. 1621±191 pmol/L; -9%; P=0.029) concentrations over the course of the study period. Similarly. Bertelsen et al reported that the consumption of two meals lead to an 84% increase in the peak amplitude of glucose concentrations (6.1±0.5 vs. 3.3±0.5 mmol/L, P<0.005), and a greater overall insulin response (P<0.03) as compared to a six-meal regimen in older (>65 y), individuals with T2D (23).

While most studies of individuals with T2D have found an improvement in the glucose and insulin responses to an increased frequency of nutrient ingestion (23, 80, 135), this is not a uncontested outcome. Arnold et al (8) examined both indices of nutrient intake and fasting measures of carbohydrate metabolism, in free-living healthy individuals with T2D following the acclimatization to a 4-week, crossover protocol where equicaloric, evenly-spaced diets consisting of either three- or nine-meals per day were consumed. Dietary intakes across the entire 4-week

conditions, as well as the outcomes of 75g OGTTs at the 2- and 4-week time points of each condition were reported. Plasma glucose (baseline- 7.3±1.7, 3/meal- 7.5±1.8, 9/meal- 7.6±1.6 mmol/L; P>0.05) and insulin concentrations (baseline- 21.4±14.9, 3/meal- 21.5±12.1, 9/meal- 21.0±9.8 μU/ml; P>0.05) were not significantly different following 4-weeks of a three- or nine-meal per day regimen, despite no differences in total nutrient intakes in either condition. Thus, these researchers (8) recommended that individuals choose their preferred meal periodicity.

Similarly, Thomsen et al (242) examined the glucose and insulin concentrations following a test meal in response to chronic exposure to a 3- or 8-meal protocol in free-living, older (60±3 y), overweight (28.1±1.4 kg/m^2) individuals with T2D (n=10). The authors found no differences between three- and eight-meal consumption in relation to the glucose peaks (13.7±0.8 vs. 13.2±0.8 mmol/L; P=0.58) glucose AUC (765±120 vs. 715±113mmol/L*240 m; P=0.99) or insulin AUC (30,408±10,104 vs. 34,464±11,748 pmol/L*240 m; P=0.55) following two weeks of increasing meal frequency from three to eight isoenergetic meals daily (242).

A beneficial insulin response was found in studies of increasing meal frequency (and continual sipping) in individuals with T2D. Three studies (23, 80, 135) showed a positive outcome related to frequent meal consumption, while two (8, 242) showed no significant changes in either direction. Importantly, none of the studies in individuals with T2D demonstrated a detrimental effect of increasing meal frequency (8, 242).

Importance of Examining Frequent Meal Consumption

Despite these controversies, overall trends show that 75 to 85% of Americans have adopted a snacking (or grazing) eating pattern (56, 275), with approximately one-third (32%) of

selected adult samples adhering to a three-meal, two-snack pattern daily (150). An increase in meal frequency (or presence of snacking behavior) may be related to shift in macronutrient consumption, favoring carbohydrate (76), at the expense of protein and fat; therefore it is important to consider the potential outcomes of a high-carbohydrate, frequent-eating regimen. In support of this theory, Drummond et al (73) reported that free-living men and women who consumed more daily meals had a higher percentage of carbohydrate in their total daily caloric load ($P<0.05$) than those who ate less frequently.

The understanding of how the glucose/insulin response to alterations in meal frequency remains a contested area of research. As demonstrated in this literature (147, 149, 226) further study may illuminate the potential role of insulin in mediating metabolic signaling and processes, particularly those related to appetite, addressing the third common claim to the effectiveness of increasing meal frequency.

Meal Frequency and Appetite

According to non-scientific sources, an increase in isocaloric meal frequency may affect appetite control through a reduction in hunger, and subsequent control of appetite over the course of the day (6, 11, 203, 268). Currently however, only a small number of studies have examined the relationship between meal frequency and appetite hormones or subjective measures of appetite.

Solomon and colleagues (226), who examined the influence of insulin on appetite hormone responses following an increase in meal frequency during an eight-hour period in 5 lean, young men, did not report differences in the total area under the curve for insulin or a change in the orexigenic hormone ghrelin ($P>0.05$). They did, however, conclude that the

increase in meal frequency disrupted the relationship between insulin and ghrelin by altering the normal time delay seen between these hormones (226). Under low frequency meal conditions (3 meals/8 h) insulin preceded the ghrelin response by 20-50 minutes, whereas this did not occur with frequent consumption (8 meals/8 h' time delays; $P>0.05$). Likewise, a reduction in meal frequency over the course of the month of Ramadan (baseline, 2-, 4- and 6-week samples) was examined by Kassab et al (148) who collected fasting insulin, leptin, and neuropeptide-Y (a potent orexigenic hormone) in a cohort of healthy female volunteers (n=46; BMI, 25.3+/-0.7 kg/m^2). The reduction in meal frequency associated with this holiday (one large meal consumed after sunset) was shown to decrease concentrations of neuropeptide-Y by 30.4% during the month of Ramadan, and to disrupt the leptin/insulin relationship, albeit at the lower end of the meal frequency spectrum (148).

The finding that a reduced meal frequency is associated with changes in appetite-regulating hormones, specifically tending towards a reduction in appetite stimulation (148, 226), may be partially attributed to the known delay in gastric emptying present during frequent feeding, though this does not seem to affect subjective measures of appetite per se (129). The amount of food consumed, along with the macronutrient profile of that food, and potential timing of a preload (extremely important when considering frequent meal consumption), is also thought to have a strong effect on subsequent nutrient consumption (200). In an effort to examine the potential effects of increased meal frequency on gastric emptying (using 13C-octanoic acid breath test), and subjective measures of hunger and satiety (using visual analogue scales (VAS)), Jackson et al (130) compared two, counterbalanced breakfast-eating regimens in healthy men (n=16). Participants consumed 43% of their average daily energy requirement; ADER) as either two, large breakfast meals (separated by 3 h) or six, hourly isocaloric loads. In contrast to prior

findings, the low-frequency consumption led to decreased 3 h AUC values for perceived hunger ($P<0.05$) and increases in perceived satiety ($P<0.05$) compared to the frequent meal condition (130). Also, there were no significant differences between either condition in relation to the rate of gastric emptying $P>0.05$).

Speechly and Buffenstein (228) anticipated an inherent difference in the regulation of appetite, particularly satiety, in response to an isoenergetic pre-load (20% of an individual's ADER; either high-, or low-fat) consumed either as a bolus or frequently (5 small, hourly intervals) between lean and obese individuals. These authors (40, 230) further proposed a 'sustained release of appetite hormones' in response to increased meal frequency. Indeed, fluctuations in insulin concentrations associated with infrequent, large meal consumption during their trials were negatively correlated with subjective appetite, and mirrored the consumption of 26.2% more food at a test meal 5 ½ hours following diet administration in both lean and overweight individuals (229, 230). These findings (228-230) are similar to those found in some lean individuals (130) and highlight the fact that obese individuals consuming a frequent meal plan may not have a structured insulin/appetite mechanism, and therefore may overcompensate at a subsequent meal. In agreement with these findings, Leidy and colleagues (160) demonstrated that there were no significant differences in ghrelin ($P>0.05$) and decreases in subjective fullness and PYY ($P<0.05$) responses in overweight and obese men (habitual 3-meal consumers) who randomly consumed ~2100 kcals as either 3 large meals (divided by 4 h) or 6 small meals (divided by 2 h).

In summary, a recent review of literature by Leidy and colleagues (162) reports that above the standard three-meal pattern, there appears to be no additional benefit of increased meal frequency on appetite measures. However, given the limited number of studies in this area, the

short duration of these studies, and the limited range of techniques adopted from the broad arsenal of available techniques for appetite measurement (VAS, appetite hormones, gastric emptying, neurological scans of the CNS), further research into this area is warranted (162).

Summary

A further understanding of the physiological effects of meal frequency through the use of randomized, controlled protocols (under experimentally-manipulated and free-living conditions in disparate populations) remains necessary (175). The current evidence regarding the potential benefits of increased meal frequency on glucose and insulin responses and appetite regulation is equivocal and given its wide-spread adoption, further information is needed prior to implementing blanket dietary recommendations. In particular, how individuals with different body composition (lean, overweight, obese), health status (T2D, prediabetes), and physical activity levels (sedentary, athlete) respond to alterations in meal frequency, remains to be established. While findings related to the effect of increased meal frequency on appetite regulation seem to be generally negative, PYY, which responds to short-term alterations in meal patterning and composition, remains a promising avenue for further research in the appetite response to meal frequency (160).

Part IV: Physical Activity Mediation of Obesity and Glucose Tolerance

Physical activity is an integral component of a healthy lifestyle. Participation in physical activity has been shown to influence glucose and insulin responses to nutrient ingestion, and over the longer term affect energy balance, weight status (22, 157, 211), and insulin resistance (114, 252). Reduced physical activity levels are strongly correlated with obesity, as well as obesity-related disorders including T2D and the metabolic syndrome (24, 45). According to Bertrais and colleagues (24) there is a reduced relative risk in the age-adjusted rate of T2D diagnosis in relation to the number of kilocalories per week burned through physical activity (>500kcal= 1.00; 500-999kcal= 0.94, 1000-1499kcal- 0.79, 1500-1999kcal= 0.78, 2000-2499kcal= 0.68; 2500-2999kcal= 0.90; 3000-3499kcal= 0.86, >3500kcal= 0.52). Similarly, Swartz et al (235) found that a 4-week increase in accumulated walking participation (9213 v. 4972 steps/day) in overweight women (n=18; BMI=35.0±5.1 kg/m^2 resulted in decreased indices of systolic and diastolic blood pressure (P=0.001, and P=0.002, respectively), as well as 2 h post OGTT glucose concentration (75g; P=0.001) and 2h glucose AUC (P=0.025). Reductions in postprandial glucose excursions, improved insulin economy, and improvements in body composition are likely contributors to these reductions in T2D risk with increased activity participation. In line with these, and other findings, individuals who experience elevated postprandial blood glucose levels (overweight or obese individuals, glucose-intolerant individuals, individuals with T2D, etc.) are recommended to increase physical activity to gain control over their glucose levels.

Physical activity and glucose uptake

It is well-established that participation in physical activity and exercise acutely enhances the uptake of glucose into working cells, and reduces insulin demand. This beneficial reduction

in the secretion of insulin with exercise has been reported for at least 30 years (110, 158), and is a key reason that exercise bouts are recommended for obese individuals, and those with insulin resistance and type 2 diabetes.

The reduction in insulin demand with exercise is most likely related to the direct stimulation of the insulin-independent (glucose transporter type 4; GLUT-4) mechanism (71, 120), enhanced sensitivity of muscle tissue to insulin stimulation (27, 97), or increased insulin ligand-receptor binding and post-receptor signaling (249). Supporting this, Thorell and colleagues performed muscle biopsies before and after a euglycemic-hyperinsulinemic clamp in nine, healthy individuals. Subjects were tested following both a resting condition and a 60-minute exercise bout (70% VO_{2max}) in order to evaluate the potential effect of physical activity on glucose uptake mechanics. Their findings revealed that plasma membrane GLUT-4 was significantly increased in response to hyperinsulinemia (following by the insulin infusion during the resting condition; 32%; $P<0.05$ vs. rest), exercise (following exercise, prior to the insulin infusion during the exercise condition; 35%; $P<0.05$), and insulin plus exercise (following exercise and the insulin infusion during the exercise condition; 44%; $P<0.05$ vs. rest), indicating an additional potential for glucose uptake (12% increase between insulin and exercise+insulin) following exercise in the hyperinsulinemic state (243). An increase in GLUT-4 translocation to the plasma membrane in response to exercise may in turn reduce the requirement of insulin-stimulated GLUT-4 translocation, ultimately resulting in attenuated insulin levels required for blood glucose regulation.

In order to assess this potential benefit of exercise on glucose uptake, O'Connor et al (191) compared the effect of a two-hour endurance bout of physical activity (60% VO_{2max}) vs. a matched rest period on the 2 h glucose and insulin response to a subsequent OGTT in six, trained

endurance runners (average 52.5±8.3 miles per week). They found that, despite higher glucose levels during the two hour period following the exercise condition OGTT ($P<0.03$), the insulin response to glucose ingestion following exercise was significantly lower ($P<0.04$) than the matched rest period, indicating a greater amount of insulin-independent glucose uptake with exercise (191). Due to this enhanced glucose uptake demonstrated with exercise, the pancreas may not need to produce as much insulin in order to ensure adequate postprandial systemic glucose uptake (243). In a similar vein, Burstein et al (38) studied the effects of one-hour of steady state, moderate exercise on the metabolic clearance rate (assessed by a three-stage euglycemic clamp) of glucose in six obese individuals with T2D (150-200% ideal body weight), seven normoglycemic obese individuals, and six lean controls. In both of the obese groups, the exercise bout significantly ($P<0.05$) attenuated the amount of insulin necessary to achieve 50% of the maximal clearance rate of glucose (obese, T2D- 200 to 130, obese, normoglycemic- 160 to 95 µU/ml) compared to a sedentary control condition, indicating an improvement in insulin action (38).

Though responsible for the increased glucose uptake following physical activity, the up regulation of GLUT-4 receptors to the surface of active muscle cells may be relatively transient (43, 120, 158). The transient nature of this increased glucose uptake is most likely due the need for glycogen repletion in the working muscle following exercise (110, 208, 209). Paralleling the acute phase of increased glucose uptake after physical activity is a shift towards increased insulin sensitivity which may last from several hours (97) up to 48 hours (43, 120, 158). Supporting this, Cartee et al (43) demonstrated a 25% increase in glucose uptake, and two-fold increase in the effect of insulin on glucose uptake in the excised trochlearis muscles in a group of exercising rats compared to sedentary controls. These increased indices of glucose uptake were diminished by

18 h, as the rats were allowed to consume carbohydrate (43), allowing for glycogen repletion (110, 208, 209).

Aside from the benefits of an acute exercise bout on glucose uptake, a chronic response to exercise has also been demonstrated. Houmard et al (123) examined the effect of 14 weeks of moderate-intensity aerobic training (~75% max heart rate) on the GLUT-4 concentration of biopsied gastrocnemius muscle and insulin sensitivity (insulin sensitivity index; ISI) of thirteen previously sedentary middle-aged men (47±1.3 y). They demonstrated (123) that chronic exposure to exercise resulted in a 1.8-fold increase in GLUT- 4 concentration (2,629±331 to 4,140±391 absorbance units/100mcg protein; $P<0.001$), and 2-fold increase in ISI (2.1±0.5 to 3.4±0.7 SI 10^5 min/pM; $P<0.05$). Additionally, they demonstrated (122) that seven young (25.0±1.1 y) healthy men with limited physical activity participation increased muscle GLUT-4 content by 2.8-fold ($P<0.05$) in response to one hour of cycle ergometer training (76±2% max heart rate) for only seven days. In summary, it has been demonstrated that exercise training can increase total GLUT-4 receptor levels in skeletal muscle (122, 123), and that an acute bout of exercise is known to increase the translocation of GLUT-4 receptors to the plasma membrane in an insulin independent process in healthy (38, 122, 123, 243), endurance-trained (191), obese individuals, and individuals with type 2 diabetes (38). Despite this population-wide beneficial response to exercise, it is well known that a large percentage of individuals do not participate in physical activity on a regular basis.

Sedentary Behavior

Regular or extended sedentary behavior is an increasingly common trend in our society. Sedentary behavior negatively impacts energy metabolism, and greatly increases the risk for T2D and premature mortality (45, 107). The negative consequences of sedentary behavior may be far greater than a simple reversal of the positive benefits experienced by regularly active individuals (45, 107). An investigation of the NHANES 2005-2006 database (250) revealed that Americans not only have limited physical activity participation (3.2% meeting physical activity guidelines), but that weight status has an effect on activity levels as well (or vice-versa). Normal weight individuals (BMI<24.9 kg/m^2) take approximately 7,190±157 steps per day, while this decreases by more than 1,400 steps/day to 5,784±124 in obese individuals (BMI>30 kg/m^2) (250). Following this example, it is likely that obese individuals may spend a greater portion of their day practicing sedentary behaviors.

Sedentary behavior, often categorized as participation in activities that involve energy expenditure of 1.0-1.5 metabolic equivalent units (METs; e.g. sitting at computer, watching television, or driving) is very common, with an average of 56.8% of the day spent in these types of sedentary pursuits. Sedentary behaviors have been shown to influence physiological mechanisms, including glucose tolerance, even in individuals who meet physical activity guidelines (106, 107, 127).

While physical activity initiatives call for at least 150 minutes per week of moderate to vigorous physical activity in order to prevent the onset of metabolic disorders including cardiovascular disease and T2D (195), many individuals do not even come close to meeting these basic guidelines. According to the National Cancer Society, between 24 and 28% of Americans are sedentary (12), or do not participate in any leisure-time physical activity. As new

definitions of sedentary behavior emerge, and the overestimation of physical activity patterns is considered, even higher rates of sedentary behavior in the United States and other developed nations may be discovered. During days when individuals are at work, it has been demonstrated that an average of nearly two additional hours per day of sedentary behavior (110±99 min/day) are accumulated than on leisure days (176). Hamilton et al. (106, 107) proposed that even individuals who meet the criteria for physical activity defined by the Centers for Disease Control and Prevention and the American College of Sports Medicine may spend exceedingly long periods of their day in activities (Figure 6), depending on their activity habits during the other twenty-three or so hours per day.

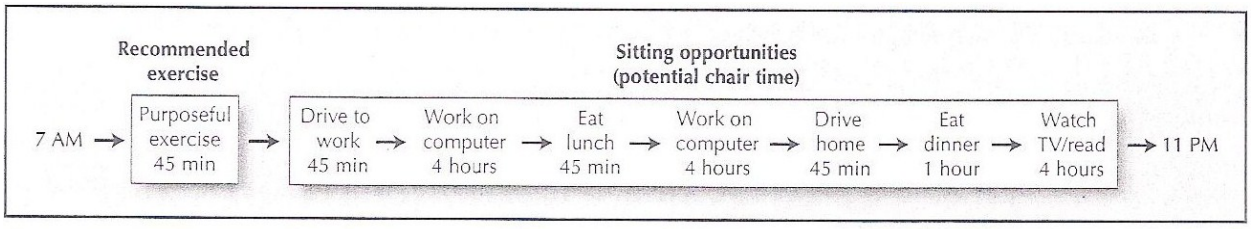

Figure 6: Example of accumulated sedentary behavior in a physically active individual

Figure 6 represents a possible scenario for an individual who performs a 45-minute exercise bout in the morning (which meets or exceeds guidelines), but then spends the rest of their waking hours in the sedentary activities of daily living including working at a desk job, driving to and from work, and watching television in the evening. According to this example, up to 95% of an individual's waking hours, assuming eight hours of sleep can be spent in a sedentary manner even when they closely follow guidelines for physical activity. While daily

exercise may be beneficial, long periods of sedentary time may be more detrimental to health. The ramifications of increased duration of sedentary time, and how these long blocks of sedentary time may relate to negative metabolic outcomes have been reported in several, recent animal (25, 272) and human studies (74, 75, 126, 127).

Recent work by Hamilton and colleagues (25, 26, 272) examined the effect of prolonged sedentary behavior on gene expression and enzyme activity in rats. In particular, the enzyme lipoprotein lipase (LPL), a rate-limiting enzyme for triglyceride breakdown and effector of lipid deposits in vascular beds (272) was targeted as being a strong mediator of metabolic syndrome and cardiovascular disease risk. In one study, the authors (26) examined the effect of various lengths (two to eighteen hours) of sedentary behavior (controlled by hindlimb unweighing) on LPL gene expression and activity in a large group (n=178) of Sprague-Dawley rats. Animals were sacrificed at two-hour intervals, and muscle from the soleus and red quadriceps assayed for LPL activity to assess changes with sedentary behavior (26). In addition, two small subgroups of animals (n=8 each) were used to determine the effects of chronic exposure (11 days) of hindlimb unloading versus a control 'regular activity' condition on LPL expression and activity (26). Significant decreases in LPL activity occurred during the progression from two to eighteen hours of sedentary behavior ($P<0.01$) and, though LPL activity was reduced to less than 20% of control activity following 10 h of hindlimb unweighing (statistically insignificant), there were no further reductions in LPL activity with an 11-day sedentary bout (26). This study emphasizes the short-term impact of sedentary behavior, and perhaps more importantly that regular, light ambulatory behavior may offset these decreases in LPL activity (26). These authors (25) also showed that as compared to ambulatory controls, rats subject to twelve hours of hindlimb unweighing, with or without a subsequent four-hour reloading period, showed significant alterations in gene

expression. In particular, twenty-five genes involved with protein synthesis and metabolism, and energy regulation were down-regulated following 12h of unloading (25), though reloading the animals reversed the changes in most of these genes (21 of 25). The sheer magnitude of genetic and metabolic changes found with physical inactivity in these initial investigations, as well as the transient nature of these changes and the ability to reverse them with light, ambulatory activity provides profound evidence for trying to minimize sedentary behaviors (112, 126, 127).

Subsequent work in humans has demonstrated similar negative effects of accumulated sedentary behavior as those found in animal models. Hu et al. measured the impact of varying levels of activity on the ten-year incidence of type 2 diabetes diagnosis in a large sample of 37,918 men aged 40-75y (126). Upon follow-up, it was reported that 1,058 individuals had progressed to type 2 diabetes. There were significant differences across quintiles of increasing MET hours, with participants accumulating the most physical activity having a lower relative risk of type 2 diabetes diagnosis (1.00, 0.78, 0.65, 0.58, 0.51; $P<0.001$ for trend) than their less active peers, after adjusting for age, smoking behavior, and alcohol-use (126). The authors (126) also measured television time, and discovered that a greater amount of weekly TV viewing was associated with an increased risk of type 2 diabetes ($P<0.001$). The measurement of TV viewing time is a common, nonintrusive means of assessing sedentary behavior and has been compared to various indices of metabolic health (74, 126, 127). Using television viewing behavior as a surrogate for the reporting of sedentary behavior, Hu et al (127) conducted a longitudinal examination of the relationship between sedentary behavior patterns, obesity, and glucose tolerance in 50,277 women with a BMI<30 kg/m^2. Six years after baseline measurements, 3,757 women had progressed to a BMI>30 kg/m^2, and 1,515 women had been diagnosed with type 2 diabetes (127). The amount of TV viewing (organized into quintiles by hours viewed per week;

0-1, 2-5, 6-20, 21-40, and >40 hours) was positively associated with an increased age-adjusted relative risk for obesity (1.00, 1.23, 1.42, 1.68, 2.00; P<0.001 for trend) and type 2 diabetes diagnosis (1.0, 1.10, 1.30, 1.53, 1.98; P<0.001 for trend). Thus, sedentary behaviors such as television viewing, were associated with a significant increase in obesity and diabetes risk, independent of exercise levels and age (127).

In agreement with these animal and human studies in the United States, considerable work relating sedentary behaviors to health risk, especially that of type 2 diabetes risk, has been performed on Australian adults (74, 75, 111, 112, 170). The Australian Diabetes, Obesity, and Lifestyle Study (AusDiab; 1999-2000) recruited 11,247 adults aged 25 years and older from 42 randomly selected census districts across the Australian continent and measured lifestyle factors including smoking, family history, and physical activity levels, as well as baseline health indicators including blood pressure and rate of T2D diagnosis (170). A follow-up study was performed on 5,842 of these individuals after 5 years, where changes in these health indicators and lifestyle were analyzed using multivariate regression analysis (170)}. This study found that individuals who took part in >150 minutes per week of physical activity (self-reported questionnaire; 54.4%) had a significantly decreased risk of T2D diagnosis by the 5-year follow-up (P<0.0001) than those who were active <150 minutes per week (40.7%) (170).

Further analysis of a subset of the AusDiab cohort (glucose-tolerant; 67 men, 106 women) was performed in order to compare sedentary time (measured as accelerometer counts/minute <100) with a two-hour post OGTT glucose concentration in order to assess sedentary time independent of regular physical activity recommendations (112). After adjusting for waist circumference, sedentary time was positively associated with a higher 2h post OGTT plasma glucose concentration (P=0.002). Thus, sedentary time was associated with increased

blood glucose response to an OGTT, and led to recommendations for replacing sedentary time (including TV time) with light-intensity physical activity for the prevention of T2D (112). Further studies by these researchers have associated an increase in TV time with indices of abnormal glucose metabolism (75) and metabolic syndrome diagnosis (74). In concert with these findings, Healy et al. examined the effects of sedentary behavior on a population of 4,757 participants of the NHANES 2003-2004 and 2005-2006 cohort (113). Investigation of the relationship between total sedentary time and indices of cardiovascular and metabolic health revealed that individuals who spent more time in a sedentary state were more likely to have high levels of fasting insulin ($P<0.05$) and insulin resistance ($P<0.05$). While a blanket recommendation to increase physical activity participation seems logical in relation to the findings of abnormal glucose and insulin metabolism in relation to an increase in sedentary behavior, physical activity participation itself does not ensure that an individual is not taking part in extended periods of sedentary behavior (Figure 6). As demonstrated in previous work (25, 26) the rapid onset of deleterious effects of inactivity and the transient nature of these negative changes in the presence of even light ambulatory activity, maintaining a series of low-intensity ambulatory activity sessions throughout the day may help to maintain metabolic benefits for a longer portion of the day (106).

In support of this growing theory from the 'inactivity physiology' literature, a small number of studies have observed the effects of breaking up sedentary behavior. Healy et al. examined accelerometer data from a small cohort (n=162) of the AusDiab study (111). The authors (111) used cutoffs of <100 counts per minute as sedentary, and >100 counts per minute as light active in order to examine the number of 'breaks' in the sedentary habits of the individuals taking part in the study. While accounting for total sedentary time and activity

intensity, it was reported that an increased number of breaks in sedentary behavior was inversely related to 2 h plasma glucose (β- -0.18, -0.34 to -0.02, P=0.025) following an OGTT (111).

Bankoski et al (14) examined the effects of sedentary behavior on the metabolic syndrome using NHANES data on accelerometry counts to quantify physical activity participation. These authors confirmed that individuals with metabolic syndrome diagnosis spent more time in sedentary pursuits (metabolic syndrome 67.3% vs. healthy controls 62.2%; P<0.01), had longer uninterrupted sedentary times (metabolic syndrome 17.7 min vs. healthy controls 16.7 min; P<0.01), and fewer breaks in these sedentary times per day (metabolic syndrome 82.3 breaks vs. healthy controls 86.7 breaks; P<0.01) (14). Breaking up longer periods of sedentary time with light, ambulatory activity, regardless of intensity may be beneficial and could be a 'stand-alone' or complementary component of a physical activity program (14, 111).

In this growing area of interest, only one other study in humans has experimentally modified physical activity conditions to replicate conditions of prolonged versus limited sedentary behavior (231). In this study, Stephens and colleagues (231) measured insulin action in 14 young (26.1±4.5 y), lean individuals during three conditions (24 h sitting, energy surplus; 24 h sitting, energy balance; 24 h no-sitting, energy balance). When compared to the sitting condition (with energy surplus), there was a significant increase in insulin action (39%; P<0.001) when consistent, low-intensity physical activity was performed. While this particular study demonstrates the effectiveness of light-intensity physical activity on markers of metabolic risk, remaining on one's feet for 24h a day may not translate well into practice. Additionally, the examination of consistent patterns of nutrient ingestion during these physical activity interventions may help to tease out potential changes due to the sedentary behavior and activity participation alone.

It remains important to determine if practical means of breaking up long sedentary periods have the same beneficial metabolic effects as demonstrated in epidemiological (14, 111) and controlled studies (231), however, research has also questioned the convenience and plausibility of undertaking frequent, short bouts of activity. Barr-Anderson et al (18) reported that short, frequent exercise bouts may be appealing to individuals who are overweight or lead busy lives (18). While it may prove physically and mentally difficult to motivate oneself to perform a suggested single, prolonged bout of physical activity (30 or more minutes per day); smaller bouts may be more manageable and enjoyable (18). In a systematic literature review of the integration of such activity bouts into the daily lives of individuals over 40 schools and business organizations, these authors (18) reported that in addition to increasing individual's daily physical activity participation, breaking up long sedentary bouts improved indices of performance (e.g. academic achievement) in addition to those of health (e.g. BMI, blood pressure).

Summary

Whether or not the effects of imposing short, ambulatory bouts throughout the day on control of glucose and insulin responses to meal consumption are as pronounced and beneficial as those associated with standard physical activity guidelines remain to be elucidated. A systematic review of the literature in this area has demonstrated both epidemiological (14, 111) and cross-sectional (231) evidence of the metabolic benefit of reducing the duration of sedentary periods. Further research on the metabolic effects of breaking up sedentary behavior is necessary, both to tap into unexamined populations (e.g. obese, T2D, elderly, etc.) and study

potential interactions with common nutrition patterns (e.g. frequent meal consumption, high-fat diet, intermittent fasting, etc.). As information related to the use of physical activity in the remediation of long bouts of sedentary behavior becomes more pronounced, clearer and more precise physical activity recommendations designed to improve metabolic risk factors, and optimize physical activity participation may evolve.

Chapter III: Effect of continuous v. intermittent physical activity on glucose excursions and insulin secretion in response to frequent meal consumption in obese individuals with prediabetes

Long, uninterrupted bouts of sedentary behavior are thought to negatively influence insulin sensitivity, and may impact metabolic function regardless of adherence to general physical activity guidelines. Thus, the purpose of this study was to determine the combined effect of physical activity (1 h continuous exercise v. intermittent exercise throughout the day) and meal consumption on glucose excursions and insulin secretion in obese individuals with prediabetes. **Methods:** Eleven obese subjects (>30 kg/m^2) with prediabetes underwent 3, 12-hour study days including sedentary behavior (SED), exercise ((EX) 1h morning exercise, 60-65% VO_2 max), and physical activity ((PA) 12 hourly, intensity-matched 5-minute bouts). Meals were provided every 2 h. Blood samples were taken every 10 min for 12 h. Baseline and total area under the curve (tAUC) and area under the curve above baseline (iAUC) for serum glucose, insulin, c-peptide concentrations, as well as insulin pulsatility were determined. **Results:** No significant differences in baseline glucose, insulin or c-peptide concentrations across study days were observed ($P>0.05$). Glucose tAUC (12 h and 2 h) were significantly different across study days, with tAUC attenuated in the PA condition compared to the EX condition ($P<0.05$). The 12 h insulin iAUC was reduced by PA compared to SED (173,985±3556.8 v. 227,352±4581.2 pmol/L*min for 12 h, respectively; $P<0.05$). Similarly, a significant main effect of condition in the 2 h insulin iAUC was found, with the PA condition being reduced compared to SED condition ($P<0.05$), but no differences between the EX and SED conditions. A significant reduction in 2 h c-peptide tAUC was demonstrated with EX and PA compared to the SED condition ($P<0.05$). Deconvolution analysis of insulin secretion revealed no significant

differences between experimental conditions. **Conclusions:** Short bouts of physical activity throughout the day attenuate glucose excursions and the insulin response compared to an exercise day with 1 h of morning exercise. This reduction is likely due to an increase in insulin clearance, as secretion was not affected.

Introduction

Type 2 diabetes is characterized by a series of hyperglycemic complications which impact health and reduce the quality of life. Individuals with type 2 diabetes are at an increased risk for cardiovascular damage, blindness, (153), peripheral neuropathy and amputations (218). Changes in lifestyle habits designed to control blood glucose levels have been shown to remediate and reverse these complications (221). Lifestyle changes including adherence to low-fat low-calorie diet, and participation in 150 minutes/week of moderate-intensity physical activity which result in weight loss have been shown to reduce the three-year incidence of T2D diagnosis in individuals with prediabetes by 58% (154, 251).

Physical activity enhances blood glucose uptake and reduces insulin demand in a wide variety of populations, including obese individuals and individuals with prediabetes (110, 158). The reduction in insulin concentrations with physical activity is closely associated with a GLUT-4-mediated increase in glucose uptake during and following exercise which is stimulated by muscle contraction. One study has shown insulin concentrations to decrease by 51%, corresponding with a 48% reduction in the secretory rate of insulin release following an hour-long bout of low-intensity (40% VO_{2peak}) exercise (166).

In addition to exercise participation, recent investigations point to the negative aspects of accumulating long periods of sedentary behavior regardless of adherence to physical activity guidelines, and recommend the use of short, bouts of activity to break up sedentary periods throughout the day (126, 170). An increased number of breaks in sedentary behavior, corresponding with short active bursts, are associated with reduced 2 h plasma glucose concentrations in middle-aged, healthy individuals following an oral glucose tolerance test (111).

These recent findings (111) raise the question of whether short, frequent bouts of physical activity may be more beneficial than one large bolus of exercise in modulating insulin secretion, and reducing blood glucose levels.

Thus the purpose of this study was to determine the effect of physical activity (continuous v. intermittent) on glucose excursions and insulin secretion in response to meal consumption in obese individuals with prediabetes. It was hypothesized that both a morning exercise bout and short, frequent physical activity bouts would attenuate glucose excursions and insulin secretion over the course of a day as compared to the sedentary condition. In addition, we hypothesized that short, frequent activity may further enhance this beneficial response as compared to the single long exercise bout due to a repeated stimulation of underlying hormonal mechanisms.

Methodology

Study subjects

All subjects completed an informed consent document, approved by the Syracuse University Institutional Review Board prior to participation in this study. Subjects in this study included young (18-35 years old), obese (BMI >30 kg/m^2) individuals. Exclusion criteria included weight loss or gain in the prior 3 months, gastrointestinal problems, or orthopedic limitations to normal walking activity. In addition, an impaired level of glucose tolerance as measured by a 12 h fasting blood glucose concentration (>5.55 mmol/L) was required for participation. Subjects could not be using any glucose-lowering medications or other medications that may affect glucose metabolism (e.g. antidepressants, oral contraceptives, steroid hormones).

Indices of resting blood pressure (measured with Omron HEM automatic blood pressure monitor (Omron, Kyoto, Japan); values <140/90 mm/Hg), cardiovascular disease (medical questionnaire), and lipid profile (measured with Cholestech LDX (Cholestech Corporation, Hayward, CA**)**; total cholesterol value>200mg/dL, low-density lipoprotein cholesterol value>160 mg/dL), uncontrolled by medication were evaluated in order to ensure a healthy subject cohort. All subjects engaged in light to moderate physical activity no more than five times per week, ensuring no major differences in training status within the cohort. Female subjects did not use oral contraceptive agents, and were consistently tested within the first eight days of their menstrual cycle to minimize the effect of estrogen on glucose tolerance.

Experimental Design

Each subject reported to the Human Performance Lab on three separate occasions for twelve hours of meal testing, beginning at 0700 h. Each subject randomly completed all three conditions, including sedentary (SED), exercise (EX; 60-65% VO_2 peak; 1 h continuous bout from 0705-0805 h), and physical activity protocols (PA; 12 5 min intermittent bouts, hourly). The exercise duration and intensity were matched between the EX and PA study days.

Study Design

On an introductory visit, each subject's habitual dietary intake and meal frequency, as well as general health, physical activity, and physical inactivity levels were recorded through the use of questionnaires. Individuals were measured for height and weight. Body composition was

assessed using air-displacement plethysmography (BODPOD system, Life Measurement, Inc. Concorde, CA) according to manufacturer's specifications (89).

Aerobic capacity was assessed with a continuous treadmill exercise stress test. Expired gases and heart rate were analyzed during the test and VO_2 peak was calculated. Subjects initially walked at a pace of 2.5 miles per hour (mph) and a 0% grade, with speed increased by increments of 0.5 mph per stage until a speed of 3.5 mph was achieved at minute six (20). Starting at minute eight, the workload increased by a grade of 2% per stage until the subject reached volitional fatigue (20). Criteria for a successful test were determined in accordance with ACSM guidelines (1). The results were used for assigning physical activity intensity during the study days.

Study Protocol

Subjects were fasted and free from caffeine consumption for 12 hours, and free from alcohol consumption and structured exercise for at least 24 hours. Subjects were instructed to record their dietary intake for three days prior to each testing day, and this record was used as a guide to consume similar foods and amounts in the days leading up to the two remaining visits. Subjects were weighed at the beginning of each testing day.

Upon arrival to the lab on testing days, subjects had a Teflon catheter inserted in their antecubital vein by a registered nurse. Baseline blood samples (10 ml) were drawn prior to the ingestion of the first meal, and at 10 min intervals thereafter. Liquid meals were used for each trial and were matched for energy content. Liquid meals were chosen, as we employed a frequent meal consumption pattern and wanted to ensure the meal was digested and absorbed prior to the

subsequent meal. The energy-matched meal conditions consisted of 6276 kJ of a high carbohydrate liquid (15% protein (PRO), 65% carbohydrate (CHO), 20% fat (FAT)), consumed in evenly-spaced intervals as six small meals (~1046 kJ/meal). Small, frequent meal consumption was chosen as a consistent model for the ingestion of liquid meals in this study, to reflect current trends in meal consumption, and as this project was an extension of an earlier project from our lab (119). The macronutrient composition presented was very similar to that used in previous work (119, 226). The liquid meals chosen for the present study were Wegmans Nutritional Beverage (Wegmans, Rochester, NY, USA), with the majority of carbohydrate coming from sucrose and corn syrup, and the majority of protein from soy and whey. All of the subjects were required to remain in the lab, and participated in quiet, sedentary activities including reading, studying, playing board games, and watching movies, with extremely limited physical activity (e.g. walking to restroom) outside of designated experimental protocols.

On the EX study day, the exercise bout (1 h) was performed immediately following the baseline blood sample and the first liquid meal; while on the PA study day the first bout of exercise (5 min) was also following the baseline blood sample and first liquid meal of the study day, and then hourly for the remainder of the PA day. The work intensity of these exercise bouts was 60-65% of the VO_2 peak value, and was confirmed during a visit prior to the initial study day. The treadmill speed and grade was maintained for all additional physical activity bouts during the PA study day to ensure that the subjects completed the same duration and intensity of activity during both physical activity conditions.

Blood samples (2.5 ml) were obtained every 10 minutes throughout the 12 h study visit, transferred to serum separator tubes (BD Vacutainer, Franklin Lakes, NJ), separated by centrifuge, divided into two sets of polypropylene tubes, and stored at -80ºC for subsequent

analysis. Samples were assayed in duplicate for serum glucose using a commercially available glucose oxidase assay (Sigma-Aldrich Corp., St. Louis, MO). A second set of samples were briefly centrifuged (3000g, 5 min, 4°C) and assayed for serum insulin and c-peptide concentrations using Luminex xMap Technology (Linco Research, St. Charles, MO) on a Luminex 100/200 platform (Luminex Corporation, Austin, TX). All procedures followed manufacturer's instructions (Millipore, Billerica, MA), with quality controls within expected ranges for each assay. Inter-assay and intra-assay coefficients for insulin and c-peptide were 4.9 and 8.5%, and 6.0 and 8.9%, respectively. The lowest limits of detection in this assay were 137 and 69 pg/ml for insulin and c-peptide, respectively. All samples for a given subject were run in the same assay series.

The insulin pulse profile was analyzed using a multi-parameter deconvolution technique (AutoDecon, Pulse_XP software; University of Virginia) designed to derive quantitative estimates of attributes of c-peptide secretory pulses and half-life from measured c-peptide concentrations (138). The prevailing serum c-peptide concentration was assumed to approximate a Gaussian distribution of secretory rate, and match insulin at a 1:1 ratio. Basal secretion was estimated for each condition, and assumed not to change during the exercise days (138). C-peptide pulses were considered significant if they were able to be distinguished from zero with a 95% statistical significance as previously reported (83, 202). Each analysis determined six secretory parameters, including 1) the number and temporal location of each discrete secretory burst, 2) half-width of each secretory pulse 3) half –life of clearance (minutes) 4) pulse amplitude (peak per secretory burst), 5) pulse mass (total c-peptide release), and 6) production rate (pulse mass/secretory pulses) (83). Basal secretion was estimated for the control day and

was assumed not to change during the exercise days (202). Similar protocols have been utilized to characterize the deconvolution of hormonal patterns during exercise (83, 202).

Statistical Analysis

Area under the curve (AUC; 12 h and 2 h) for serum glucose, insulin and c-peptide were calculated as both the total area (total AUC; tAUC) and the area above baseline (incremental AUC; iAUC) using the trapezoidal method for all three study conditions (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, California, USA). In addition, 2 h baseline and peak values were calculated for serum glucose, insulin and c-peptide. A one-way ANOVA with repeated measures assessed the differences in the baseline, peak, 12 h tAUC and iAUC for glucose, insulin and c-peptide concentrations, and deconvolution analysis parameters between meal conditions. A two-way ANOVA (time*intervention) was used to assess differences between 2 h tAUC and iAUC values for glucose, insulin and c-peptide. When significant main effects across measures were found, a least-squares analysis of the differences between conditions was utilized in order to determine the magnitude and direction of differences. Significance levels in all statistical tests were accepted at $\alpha=0.05$. Statistical analyses were performed with SPSS for Windows, version 16.0 (SPSS, Inc., Chicago, USA), and all data are reported as mean ± standard error of the mean.

Results

Subject Characteristics

Eleven subjects completed all trials. Three subjects were women, and eight were men. All subjects were considered obese by definition (BMI>30 kg/m^2; Table 1), and each individually fit the outlined health criteria for participation. According to the fasting blood glucose values, nine subjects were considered to have prediabetes and two were above the threshold for type 2 diabetes (6.28±0.29 mmol/L). The average HOMA-IR value across all subjects was 0.42±0.12, while the average QUICKI value was 2.97±0.10. There were no differences between genders in the subject characteristics, except that the men had a higher percent body fat than the women. When examined across testing days, there were no significant differences in body weight in any of the subjects (less than ±2% change). According to self-report dietary logs and physical activity questionnaires, subjects consumed meals (defined as energy-intakes at least 15-minutes apart) an average of 4.7±0.38 times per day, and participated in physical activity 3.3±0.57 times per week at a Borg RPE (6-20) level of 13.4±0.48.

VO_2 Max Testing and Exercise Intensity

The female subjects had a VO_2 peak of 25.5±1.8 ml/kg/min during the exercise stress test, while the male subjects achieved a VO_2 peak of 32.6±2.5 ml/kg/min (P>0.05). During the study days, the mean treadmill speed across all subjects was 3.2±0.1 mph, with a grade of 2.9±1.0%.

Glucose Concentrations

Baseline glucose concentrations following the overnight fast were not significantly different at the beginning of each study day (Figure 1). Although the pattern of response was not different across study conditions, there was a significant effect of condition on the 12 h total glucose tAUC concentrations, such that the 12 h tAUC was attenuated in the PA compared to the EX condition ($P<0.05$), but not the SED ($P>0.05$). When the study day was broken up into 2 h intervals around the meals, the EX condition resulted in greater glucose concentrations in these 2 h intervals than in the PA condition (Figure 2; $P<0.05$), but was not different than the SED condition ($P>0.05$). Additionally following each meal the glucose value after 120 minutes in each 2 h interval (2 h tAUC) was significantly higher in the EX condition than in the other two conditions ($P<0.05$).

Insulin Concentrations

Baseline fasting insulin concentrations were not significantly different at the beginning of each study day (Figure 3). Following each meal, there was a significantly greater peak insulin concentration in the SED compared to the EX and PA conditions ($P<0.05$). While the EX condition did not significantly reduce the 12 h insulin iAUC compared to the SED condition, a significant reduction in 12 h insulin iAUC was demonstrated in the PA compared to the SED condition (Figure 3, $P<0.05$). In the EX condition, there was an attenuated insulin iAUC in the 2 h interval following the initial meal (0-2 hr; Figure 4; $P<0.05$) which rose substantially during the remaining five 2 h intervals. In the PA condition, the insulin iAUC across all six 2 h intervals was significantly reduced in comparison to the SED condition ($P<0.05$).

C-Peptide Concentrations

Baseline c-peptide concentrations following the overnight fast, and at the start of each 2 h interval during the day, were not different across the three experimental conditions (Figure 5). Peak serum c-peptide concentrations across time were significantly different within the EX condition ($P<0.05$). Pairwise comparisons revealed that differences between the 0-2 and 4-6 hr, and the 0-2 and 10-12 hr (Figure 6; $P<0.05$), indicating an increase in c-peptide in the 4-6 and 10-12 intervals as compared to the 0-2 hr interval where exercise was performed. In addition, the 2 h total c-peptide tAUC in the EX condition was significantly attenuated when compared to the SED condition across all time blocks (Figure 6; $P<0.05$). During the EX condition 2 h total c-peptide tAUC was higher in the 2-4, 4-6, and 10-12 hr than the 0-2 hr interval (0-2 14.82±2.04 vs. 2-4 19.05±2.30, 4-6 18.28±2.12, 10-12 20.50±2.57 nmol/L*2 h; $P<0.05$). When 2 h iAUC was examined above baseline, both the EX and PA conditions had significantly reduced iAUC concentrations than the SED condition (Figure 6; $P<0.05$). Also when compared above baseline, a reduced c-peptide concentration was found in the 4-6 than the 0-2 hr interval in the SED condition (4.20±1.35 vs. 11.77±1.54 nmol/L*2 h; $P<0.05$).

Deconvolution analysis was employed to estimate insulin secretion from measured c-peptide concentrations. There were no significant differences noted in the number of secretory pulses in 12 h, half-width of c-peptide secretion, half-life of c-peptide secretion, mass of c-peptide secretion per pulse, mean amplitude of c-peptide secretion, or c-peptide production rate. As c-peptide and insulin are secreted in a 1:1 ratio, it was determined that there were no significant differences in c-peptide secretion between experimental conditions.

Discussion

Chronically elevated glucose levels, and a subsequent increase in insulin secretion are characteristic of the progression towards type 2 diabetes. Our recent study demonstrated that an increase in meal frequency, often recommended as a means of improving glucose tolerance, could alter glucose excursions such that a 6 meal daily pattern resulted in a ~30% increase over the course of a 12 h period (119). However, during that study physical activity of the subjects was limited and this may have contributed to the inappropriate glucose excursions. Long periods of physical inactivity have been shown to have a deleterious effect on blood glucose levels, and the ability of the body to clear glucose following an OGTT (111). Therefore, we compared glucose concentrations and insulin secretion during frequent meal consumption in obese individuals with prediabetes over the course of 12 h (SED); and in response to intermittent physical activity (PA) or a continuous, morning exercise bout (EX). The novel findings of this study are that the EX condition augmented the baseline and glucose tAUC concentrations compared to the SED and PA conditions. Additionally the iAUC for insulin was reduced in the PA condition, compared to the SED and EX conditions. There were no significant differences in c-peptide secretory parameters between conditions, which may point to an improvement in insulin clearance with short, purposeful exercise bouts during the day.

Increased glucose concentrations and tAUC during the sedentary period following exercise in the EX condition were surprising, as 1 h of exercise participation meets or exceeds recommendations designed to reduce hyperglycemia in individuals with or at risk for type 2 diabetes (1). This finding may be partially explained by the procedures designed to control baseline values, namely the 12 h fasting period prior to each study condition. While subjects consumed their first small liquid meal following initial baseline sampling, it only accounted for a

small amount of carbohydrate intake (~40 g, ~1046 kJ). On the exercise day, subjects began their continuous hour of treadmill walking within 5 minutes of ingesting this initial small meal.

The potential for reduced blood glucose levels under these conditions point to an increased demand for endogenous glucose production to maintain homeostasis during the exercise condition; especially when compared to the sedentary condition and relatively short (8.3% duration of the EX condition) activity bout performed during the physical activity condition. We speculate that an increase in the activity of glycogen phosphorylase and gluconeogenesis, designed to liberate glycogen stores, respectively may have been augmented in this condition only. It is well established that hepatic glucose output is controlled by systemic glucose levels (133).

Thus, our finding of morning exercise increasing day-long glucose concentrations is in agreement with previous work examining the effect of a moderate-intensity exercise bout in a glucose depleted state (specifically following a 12 h fast) on glycogen phosphorylase concentrations and gluconeogenic activity (247). Trimmer et al (247) reported that highly trained males (>60 ml/kg/min VO_{2max}) responded to 90 minutes of moderate-intensity exercise with a 128% increase in absolute gluconeogenesis, and 203% increase in glycogenolysis (characterized by a 135% increase in the activity of glycogen phosphorylase). Similarly the 60-minute moderate-intensity exercise condition in the present study may have stimulated a comparable response, despite the fact that the subjects had a liquid meal immediately prior to the exercise. Our obese subjects with prediabetes may have experienced similar increases in gluconeogenesis and glycogenolysis in order to meet the immediate glucose demands of exercise (247). This is particularly likely given the low insulin concentrations during exercise, which indicate a

favorable physiological environment for gluconeogenesis and increased glycogen phosphorylase activity.

Surprisingly, in these obese individuals with prediabetes, glucose levels remained elevated over the course of the day with small, frequent meal consumption. Borer et al (31) highlighted a 20% increase in glucose concentrations when glucose-tolerant, obese females performed 2 h of low-intensity walking (40-45% VO_{2max}) an hour after a high-carbohydrate meal. The repeated small, high carbohydrate ingestions used in the present study may have further prolonged these augmented glucose levels. This emphasizes the need for more research focusing on the interaction between meal consumption and exercise on insulin secretion and glucose excursions in both obese normoglycemic, and obese insulin-resistant populations.

The differential findings of the beneficial effect of physical activity on insulin concentrations when performed as either one continuous morning session followed by sedentary behavior, or twelve short, purposeful bouts designed to break up sedentary duration is remarkable. As expected, there was an initial reduction in the insulin concentration concurrent with exercise in the EX condition. When the EX and PA conditions were compared to SED, there was a 29.3 and 30.7% reduction, respectively, in the insulin concentrations above fasting baseline levels over the 12 h day. While these values are similar, the prevailing insulin levels following each individual meal were lower in the PA condition compared to the SED condition. While the addition of intermittent exercise resulted in attenuated insulin levels throughout the 12 h day and when 2 h meal responses were compared to SED, insulin iAUC rose with each subsequent meal following the initial 2 h interval in the EX condition. These findings imply that the accumulation of short, frequent bouts of activity was more beneficial in controlling insulin levels than the 1 h of morning exercise followed by sedentary behavior. Similarly, Healy et al

(111) demonstrated that indices of impaired glucose tolerance (2 h glucose response to a 75g OGTT) are inversely related to the daily number of breaks in sedentary time. Individuals with metabolic syndrome have been shown to spend longer periods of the day in uninterrupted sedentary behavior (based on accelerometry counts) when compared to their healthy peers (14). These findings highlight the importance of breaking daily sedentary time with light ambulatory activity on reducing circulating insulin levels, either as a stand-alone therapy, or in concert with a structured physical activity program (14, 111).

A major strength of the present study was the inclusion of deconvolution analysis of c-peptide to model insulin secretion over the course of the 12 h period. As insulin and c-peptide are secreted in a 1:1 ratio, the measurement of c-peptide (which has a longer half-life) allows an accurate estimation of the pattern of insulin secretion (83). As no differences were demonstrated in the secretory parameters of insulin between experimental conditions, it appears as though an increase in the clearance of insulin (either hepatic or in the muscle) may have been present in the intermittent physical activity condition. Previously, Kahle and colleagues reported that obese male adolescents experienced an improvement in hepatic insulin clearance following a mixed meal test (calculated as decreased insulin levels, with increasing c-peptide levels) following 15-weeks of mild-intensity exercise training (142). As it is likely that this effect was mediated by an accumulated response to repeated, acute exercise participation, this current finding may represent an acute, transient effect of physical activity.

While there were no statistically significant differences between the secretion parameters of insulin in response to continuous or intermittent physical activity, there were some changes that might be deemed clinically relevant across conditions. For instance, when examining the effect of continuous exercise on insulin secretion, it is apparent that an additional insulin pulse

may be present during the 12 h condition of many subjects, which corresponds with the non-significant trend for reduced insulin across 12 h (Figure 9) and a reduction in the amplitude of each insulin pulse released. Again, while not significantly different, the half-life of insulin clearance in the EX condition was also 17% lower than the SED and 13% lower than the PA conditions, respectively. This may highlight an increase in insulin clearance hypothesized previously, and deserves further study.

Similarly, in the PA condition, an average of 0.6 more pulses per 12 h was present compared to the SED condition. As reported earlier, there was a significant reduction in the insulin concentrations between the PA and SED conditions. This was confirmed by a 59% reduction in the pulse amplitude of insulin in the PA condition, and a 22% increase in the rate of insulin production during the 12 h period.

Conclusion

In conclusion, our findings support the hypothesis that short, purposeful bouts of physical activity over the course of the day will attenuate the glucose excursions and reduce insulin concentrations in obese individuals with prediabetes, likely through an improvement in insulin clearance. In addition, a 1 h continuous exercise bout of morning exercise followed by sedentary behavior may exacerbate glucose responses to meal consumption in this population.

Table 1: Subject Characteristics

	Female (n=3)	Male (n=8)	Total (n=11)
Age (yrs)	25±3.1	25.3±1.5	25.2±1.3
Height (cm)	170±0.5	176.0±2.7	174.4±2.1
Weight (kg)	103.6±10.2	104.5±4.7	104.3±4.1
BMI (kg/m^2)	35.8±3.3	33.8±1.4	34.3±1.3
Body Fat %	42.0±3.3	28.9±2.7*	32.5±2.8
Fasting Blood Glucose (mmol/L)	6.25±0.52	6.30±0.33	6.28±0.29
Resting Systolic BP (mmHg)	125.3±7.7	120.8±3.1	122.0±2.9
Resting diastolic BP (mmHg)	68.3±3.8	65.9±1.8	66.5±1.6
Total Cholesterol (mg/dL)	181±2.4	127.3±2.6*	145.2±8.6
LDL Cholesterol (mg/dL)	91.5±10.2	51.3±5.7*	64.7±7.7
HDL Cholesterol (mg/dL)	57.5±8.6	38.8±4.3	45±4.5
Cholesterol Ratio (TC/HDL)	3.3±0.4	3.5±0.6	3.41±0.4

All values reported as means and standard error of the mean. *P<0.05 vs. female subjects.

Table 2: Deconvolution Variables for C-Peptide

	Sedentary	**Exercise**	**Physical Activity**
C-peptide Pulses per 12 h	6.2±0.25	6.9±0.40	6.8±0.36
Pulse Half-Duration, min	9.75±4.23	14.71±5.52	18.70±6.61
C-peptide Half-Life, min	48.79±5.17	41.59±6.50	47.01±7.86
C-peptide Pulse Mass per pulse, nmol/L	1.92±0.27	1.63±0.17	2.12±0.38
C-peptide Pulse Amplitude, nmol/L/*min^{-1}	0.46±0.11	0.31±0.08	0.29±0.09
12 h C-peptide Production Rate (mass/pulses)	11.34±1.97	11.34±1.50	13.83±2.27

Mean±SEM. No significant differences between conditions.

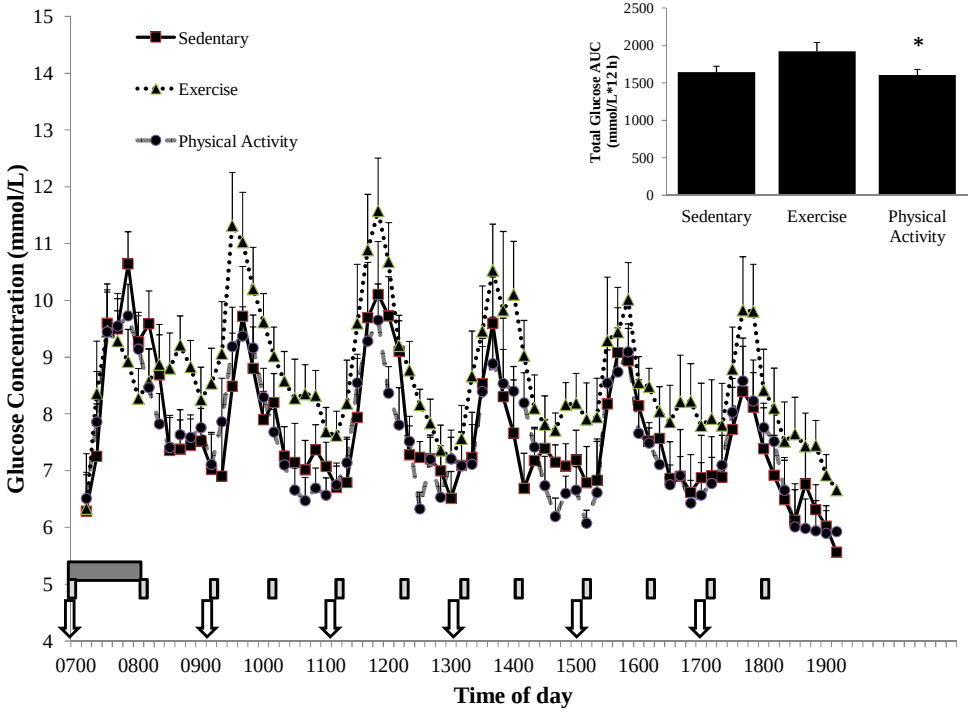

Figure 7: Glucose pattern of response over 12 h by condition. Inset: 12 h glucose tAUC by condition.

Mean±SEM. *P<0.05 vs. EX condition.

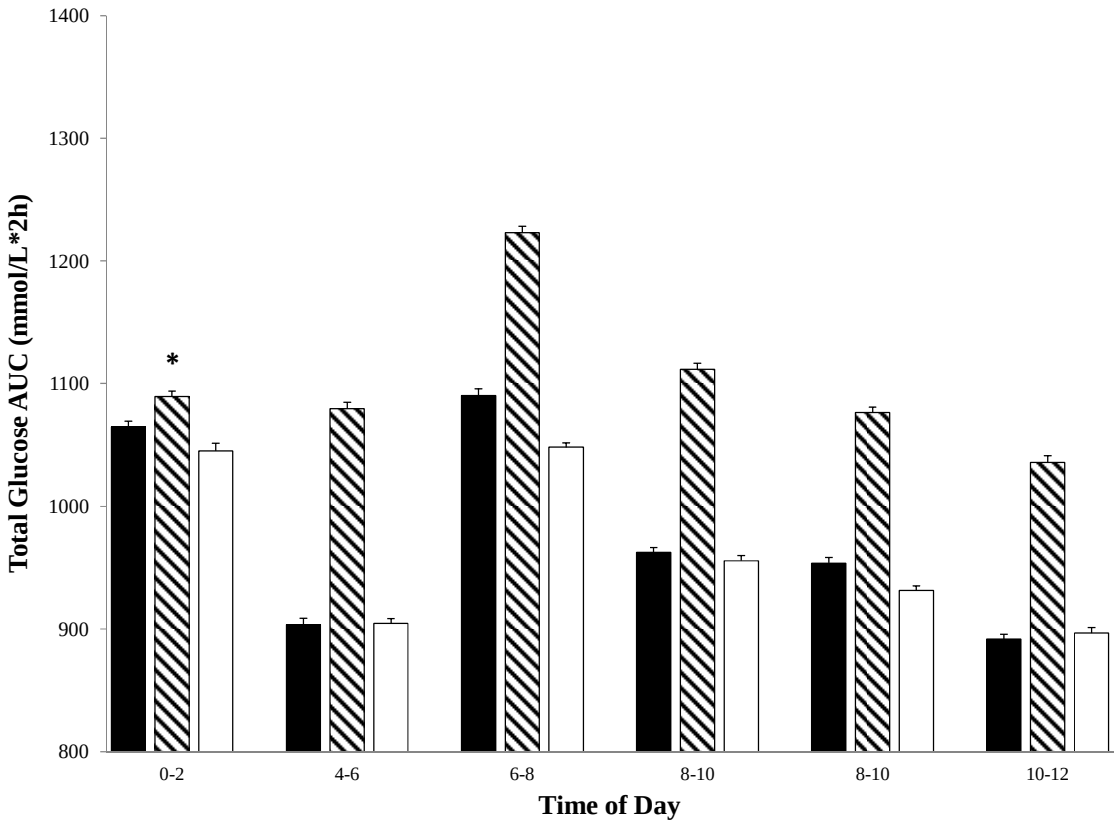

Figure 8: Two-hour total serum glucose AUC across time.

Black=SED, Hatched=EX, White=PA. Mean±SEM. *P<0.05 vs. PA condition across time.

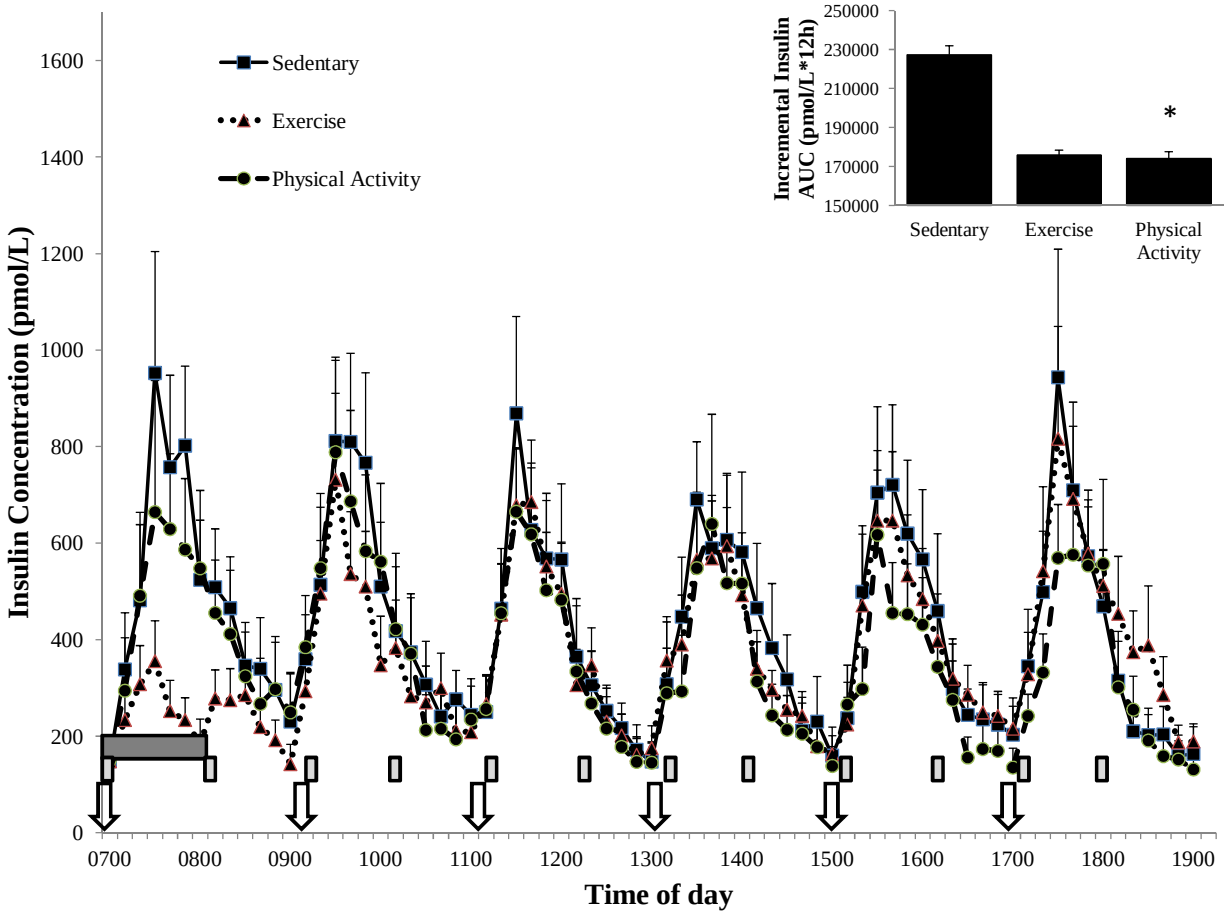

Figure 9: Insulin pattern of response over 12 h by condition. Inset: 12 h insulin iAUC by condition.

Mean±SEM. *P<0.05 vs. SED condition.

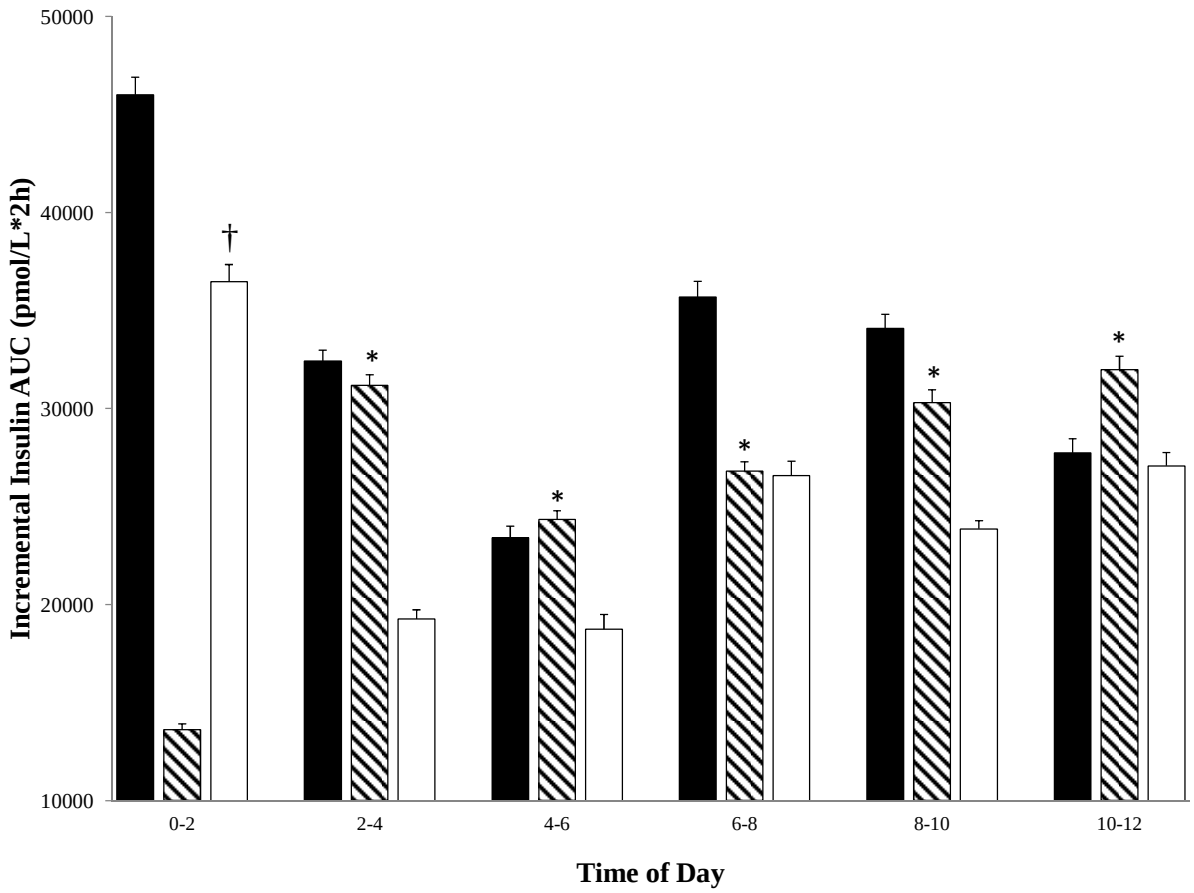

Figure 10: Two-hour incremental serum insulin AUC across time.

Black=SED, Crosshatching=EX, White=PA. Mean±SEM. *P<0.05 vs. 0-2 hr interval in EX condition. †P<0.05 vs. SED condition across time.

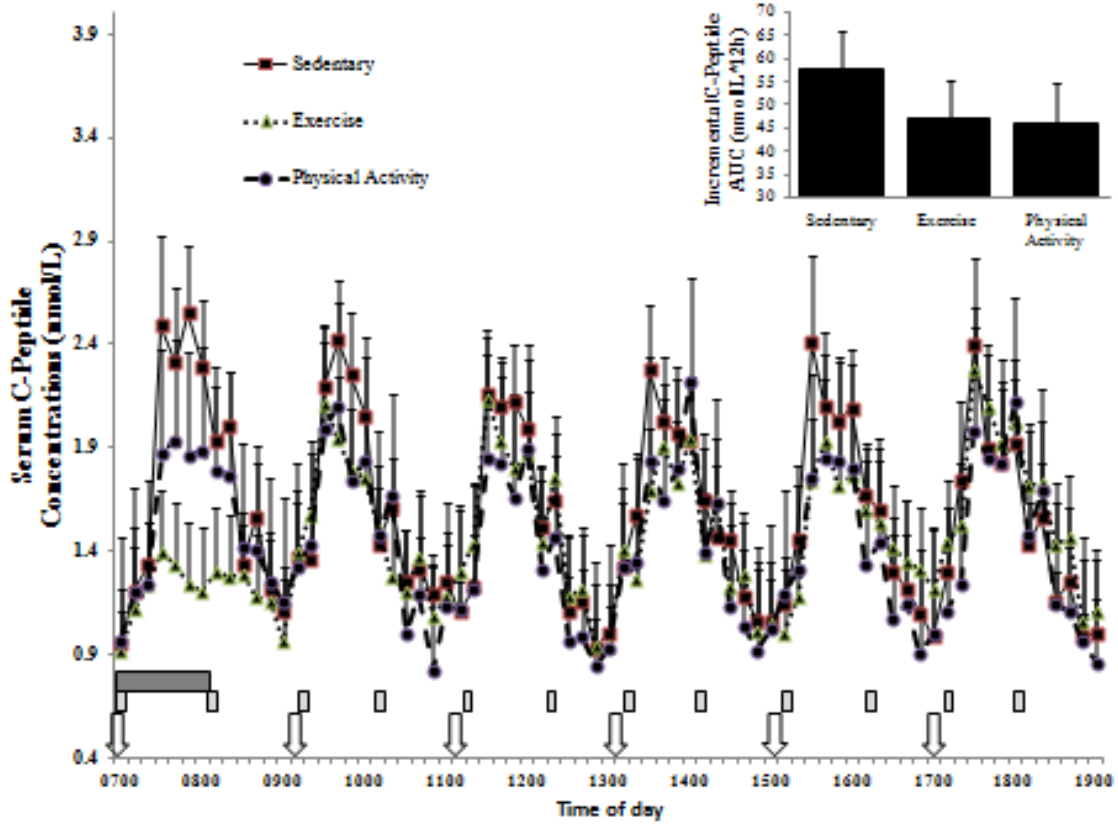

Figure 11: C-peptide pattern of response over 12 h by condition. Inset: 12 h c-peptide iAUC by condition.

Mean±SEM.

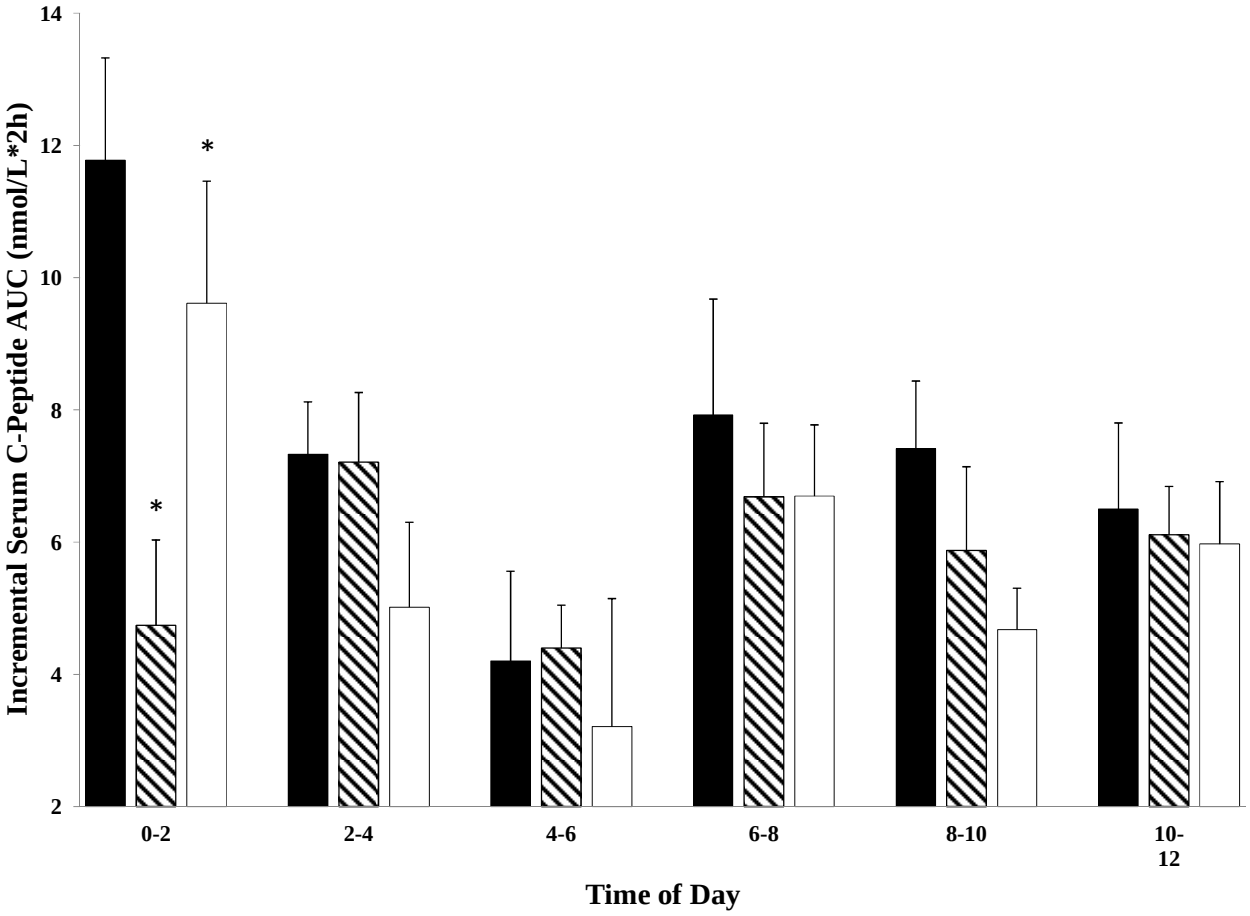

Figure 12: Two-hour incremental serum c-peptide AUC across time.

Black=SED, Crosshatching=EX, White=PA. *P<0.05 vs. SED condition across time.

Chapter IV: Effect of continuous v. intermittent physical activity on the total pancreatic polypeptide-yy and subjective appetite response to frequent meal consumption in obese individuals with prediabetes

Long, uninterrupted bouts of sedentary behavior may negatively impact appetite, although alterations in insulin release with exercise may increase satiety hormones and improve appetite regulation. The influence of exercise duration and timing on this response, particularly during frequent meal consumption, remains equivocal. Thus, the purpose of this study was to determine physiological and subjective appetite responses to physical activity (1 h continuous exercise v. intermittent exercise throughout the day) and meal consumption in obese individuals with prediabetes. **Methods:** Eleven obese subjects (>30 kg/m^2) underwent 3, 12-hour study days including sedentary behavior (SED), exercise ((EX) 1h morning exercise, 60-65% VO_2 max), and physical activity ((PA) 12 hourly, intensity-matched 5-minute bouts). Meals were provided every 2 h. Blood samples were taken every 10 min for 12 h and measured for insulin and peptide YY (PYY), while subjective ratings of appetite (VAS scales) collected every 20 min. Baseline, total area under the curve (tAUC), area under the curve above baseline (iAUC), and magnitude of change (absolute and %) for serum insulin and total PYY concentrations, and subjective appetite ratings were calculated. **Results:** There were no significant differences in indices of total PYY between experimental conditions, though subjective measures of hunger and satiety were reduced with the addition of continuous and intermittent exercise. The relationship between PYY and insulin, as well as PYY and appetite ratings was altered with a continuous morning exercise bout. **Conclusion:** Both continuous and intermittent exercise mechanisms that reduce subjective

satiety in obese individuals with prediabetes are not related to changes in concentrations of the anorexigenic hormone PYY.

Introduction

Obesity has been shown to result in attenuated peptide YY (PYY) concentrations in both the fasting and in the postprandial state (177, 214), yet similar to lean individuals, obese individuals respond to PYY infusion with increased indices of satiety and a reduction in subsequent energy intake (19). In obese individuals, positive energy balance may blunt the expected increase in PYY subsequent to meal consumption (132), and contribute to these low PYY concentrations and increased appetite. Exercise participation may provide obese individuals the opportunity to alter these hormone levels and promote increased post-meal satiety.

Exercise has been promoted as a means of increasing satiety hormone concentrations and maintaining appetite control (84, 105, 253). Two recent, independent studies have demonstrated that sixty minutes of moderate-intensity exercise in healthy, normal-weight individuals augmented plasma PYY concentrations, and transiently reduced subjective scores for hunger (35, 84). Though obese individuals are known to have low baseline PYY levels, researchers (254) have reported increased PYY levels with moderately-intense exercise (60 minutes of 50 v. 65% VO_{2max}) in both lean and obese individuals. In light of this ability to influence the satiety hormone response with exercise, participation in a long, continuous exercise bout is often impractical for an obese individual, and the accumulation of physical activity over the course of the day may be a more appropriate alternative. A paucity of research exists concerning the potential beneficial effects of intermittent exercise on satiety hormones and perception, and whether these small, accumulated exercise bouts over the course of a day provide the same improvements in satiety found with long, continuous exercise bouts.

In addition, a relationship between insulin and PYY has been reported. Studies examining the mechanism of PYY action on insulin levels report no effect on baseline insulin, glucose, or glucagon secretion, but an inhibitory effect on glucose-stimulated insulin production (100, 190). More specifically, increases in PYY concentration may inhibit pancreatic insulin production through a neuropeptide-Y mechanism acting at the level of the pancreatic islets (36). A physical activity-induced increase in endogenous PYY may contribute to a decrease in postprandial insulin concentrations, and would be a beneficial response, particularly in an obese, or insulin-resistant population who may chronically be in a positive energy balance.

Thus, the purpose of this study was twofold: 1) to establish the effect of physical activity (continuous v. intermittent) on the PYY and subjective hunger/satiety response to meal consumption in obese individuals with prediabetes and 2) to establish the relationship between PYY and insulin, and glucose; and subjective (VAS scores for hunger/satiety) measures of appetite in response to continuous and intermittent physical activity. It was hypothesized that continuous and intermittent physical activity would increase concentrations of PYY over the course of 12 h as compared to a control day, and that this would translate to increased indices of satiety and a reduction in subjective hunger. In addition, it was expected that an inverse correlation would exist between the change in PYY and change in insulin concentrations in the obese. Further, that these changes in PYY (+) and insulin (-) would correlate with changes in satiety, and that both continuous and intermittent exercise would improve these relationships.

Methodology

Study subjects

All subjects were required to complete an informed consent document, approved by the Syracuse University Institutional Review Board prior to taking part in this study. Young (18-35 years old), weight-stable, obese (BMI >30 kg/m^2) individuals who were free from gastrointestinal problems and orthopedic limitations to normal walking activity were recruited into this study. All subjects were regularly active, meeting general guidelines for moderate physical activity (3-5 days per week, approximately 150 minutes total).

Each subject was required to have a 12 h fasting blood glucose concentration above 5.55 mmol/L. Subjects were screened for a healthy resting blood pressure (Omron HEM automatic blood pressure monitor -Omron, Kyoto, Japan; values <140/90), the absence of cardiovascular disease (medical questionnaire), and lipid profile (Cholestech LDX -Cholestech Corporation, Hayward, CA**)**; total cholesterol value>200mg/dL, low-density lipoprotein cholesterol value>160 mg/dL). Glucose tolerance, blood pressure, etc. were measured without the use of medication. In addition, female subjects did not use oral contraceptive agents, and were tested within the first eight days of their menstrual cycle to minimize the effect of estrogen on glucose tolerance.

Experimental Design

Three 12 h testing days were completed by each subject. Participants reported to the Human Performance lab following a 12 h fast and caffeine withdrawal, and 24 h of abstinence from exercise, at 0700 h. Subjects randomly completed either a sedentary (SED), exercise (EX;

60-65% VO_2 peak; 1 h continuous bout from 0705-0805 h), or physical activity protocol (PA; 12 5 min intermittent bouts, hourly). The exercise duration and intensity were matched between the EX and PA study days. Subjects were required to track their dietary intake for the three days prior to each experimental condition, and match intake prior to subsequent testing as closely as possible. Each subject was weighed at the start of each experimental condition.

Study Design

During the subject screening phase, general health, physical activity, and inactivity levels were recorded through the use of questionnaires. Individuals were measured for height and weight, and body mass index was used to determine obesity (BMI> 30 kg/m^2). Subjects were measured using air-displacement plethysmography (BODPOD system, Life Measurement, Inc. Concorde, CA) according to manufacturer's specifications (89). Exclusion criteria for health measures (blood pressure, glucose tolerance, cholesterol), were assessed as previously described.

Expired gases and heart rate were measured during the conduction of a continuous protocol to assess VO_2 peak for the determination of exercise intensity during the experimental conditions. A previously described incremental protocol was used to elicit a maximal exercise response (20). Subjects exercised until volitional fatigue, and testing was deemed successful according to ACSM guidelines (1).

Study Protocol

At 0700 h, a registered nurse placed a Teflon catheter into the antecubital vein of each subject. Baseline blood samples (10 ml) were drawn prior to the ingestion of the first meal. Meals were energy-matched across conditions, and each subject consumed 1500 kcals of a mixed, high-carbohydrate liquid (15% protein (PRO), 65% carbohydrate (CHO), 20% fat (FAT)) (Wegmans Nutritional Beverage, Wegmans, Rochester, NY, USA) during each 12 h session. The meals were spaced evenly, with 250 kcals (~1046 kJ/meal) consumed every 2 h. Outside of prescribed activity during the EX and PA conditions, subjects participated in quiet, sedentary activities over the course of the 12 h study days. Each subject performed one hour of moderate-intensity (60-65% VO_2 peak) exercise during the EX and PA conditions, while no activity was performed on the SED day. All exercise sessions started immediately following baseline blood sampling and the first daily meal, with 1 continuous hour from 0700-0800 in the EX condition, and an hourly 5 min bout completed in the PA condition. Exercise was intensity-matched by treadmill speed and grade, and duration identical across the two exercising conditions.

Blood samples were drawn from the indwelling catheter at 10 min intervals over the course of each study day. Samples were assayed in duplicate for serum glucose using a commercially available glucose oxidase assay (Sigma-Aldrich Corp., St. Louis, MO). Additionally, samples were centrifuged (3000g, 5-minutes, 4°C) and assayed for serum total PYY and insulin concentrations (Millipore, Billerica, MA) using Luminex xMap Technology (Linco Research, St. Charles, MO) on a Luminex 100/200 platform (Luminex Corporation, Austin, TX). All procedures followed manufacturer's instructions, with quality controls within expected ranges for each assay. Inter-assay and intra-assay coefficients for insulin and total PYY were 4.0 and 9.5%; and 6.0 and 5.3%, respectively. The lowest limits for detection of this assay

were 137 and 14 pg/ml, for insulin and total PYY, respectively. All samples for a given subject were run in the same assay series.

Subjective Hunger and Satiety (VAS scores)

Subjects were asked to record an index of their hunger and satiety at 20-minute intervals throughout each study day using a visual analogue scale (212). Participants made a mark along a 100 mm line indicating the intensity of their hunger and satiety on a scale on 1-100. The VAS for hunger asked 'How hungry do you feel?' and indicators of 'Not at all hungry' and 'As hungry as I have ever felt' were included at the left and right ends of the scale, respectively. Similarly, the satiety VAS asked 'How full do you feel?' with 'Not at all full' and 'As full as I have ever felt' included as scale anchors. Following each subject condition, each mark in the 100mm line was measured from the left, and the mm length recorded as the VAS value.

Statistical Analysis

Both total area under the curve (tAUC; 12 h and 2 h) and area under the curve above baseline (iAUC; 12 h and 2 h) for serum total PYY and VAS scores for hunger and satiety were calculated using the trapezoidal method across meal conditions (GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, California, USA). The magnitude of change (Δ) in PYY, insulin, and glucose concentrations; as well as VAS scores for hunger and satiety were calculated for each 2 h meal interval by subtracting 2 h baseline from 2 h peak (or nadir) values. Subsequently, percentage change values were calculated from these Δ values by dividing the

(peak-baseline)/baseline values. A one-way ANOVA with repeated measures assessed the differences in the baseline, peak, 2 h and 12 h AUC for total serum PYY concentrations and VAS scores between activity conditions. Two-tailed Pearson's correlations between the average magnitude of change, and average percentage change between 2 h PYY concentrations and insulin, glucose, and VAS scores for satiety across study days were calculated. Significance levels in all statistical tests was accepted at α=0.05. Statistical analyses were performed with SPSS for Windows, version 16.0 (SPSS, Inc., Chicago, USA), and all data are reported as mean ± standard error of the mean.

Results

Subject Characteristics

Eleven subjects completed all trials (3 women, 8 men). All subjects were considered obese by definition (BMI>30 kg/m^2; Table 1), and each individually fit the outlined health criteria for participation. Female subjects had a higher percentage of body fat, and concentration of total and LDL cholesterol than their male peers (Table 1; P<0.05). Nine of eleven subjects were deemed prediabetic according to fasting glucose concentrations, while two demonstrated levels indicative of type 2 diabetes. None of the study subjects had more than a ±2% change in weight between testing days. Subjects in the present study report an average of 4.7±0.38 meals per day, and participation in 3.3±0.57 bouts of moderate-intensity physical activity per week.

VO_2 Max Testing and Exercise Intensity

The female subjects had a VO_2 peak of 25.5±1.8 ml/kg/min during the exercise stress test, while the male subjects achieved a VO_2 peak of 32.6±2.5 ml/kg/min. During the study days, the mean treadmill speed across all subjects was 3.2±0.1 mph, with a grade of 2.9±1.0%.

PYY Concentrations

Baseline total PYY values did not differ across conditions following the overnight 12 h fast. The pattern of hormone release was similar across conditions, with no significant differences noted in baseline PYY values in response to each individual meal (Figure 1; $P>0.05$). Similarly, there were no significant differences in the magnitude of change in PYY between conditions (Table 2; $P<0.05$).

PYY tAUC and iAUC over the course of the day did not differ significantly across the three experimental conditions. 12 h total PYY tAUC and iAUC were comparable among the SED, EX, and PA conditions, and examination of the 2 h PYY responses to each meal similarly revealed no significant differences between conditions during any of these meal blocks (Figure 2; $P>0.05$).

VAS Scores

There were no differences in baseline hunger VAS following the 12 h fast between conditions (Figure 3; SED 49.2±22.9, EX 42.8±24.9, PA 51.7±17.3 mm; $P>0.05$). When tAUC for the VAS scores for hunger were compared, no significant differences were noted across

physical activity conditions (SED 1705.4±315.8, EX 1744.9±584.2, PA1490.617.8 mm*12h; P>0.05). In addition, the iAUC for hunger scores above baseline did not differ among experimental conditions (SED 1622.5±298.8, EX 1669.2±573.2, PA 1402.4±623.2 mm*12h; P>0.05).

As the day progressed, a significant main effect of condition was noted in the hunger response between 1300-1500 h (SED 318.6±82.3, EX 360.2±126.6, PA 272.3±132.1 mm*2h; P<0.05), where intermittent PA resulted in a reduced hunger tAUC than in the EX condition. Between 1500-1700 h, the tAUC for hunger was lower in response to the PA compared to the SED or EX conditions (SED- 354.5±84.4 EX-360.8±114.3 PA- 284.4±145.6; P<0.05). A similar trend of a lower 2 h tAUC in the PA condition approached significance (P=0.068) in the hunger response between 1500-1700 h.

No significant differences were noted between conditions for the baseline satiety VAS at the beginning of each testing day (Figure 4; SED 39.1±24.1, EX 28.8±17.7, PA 29.0±19.0 mm; P>0.05). In a similar manner to the hunger VAS, there were no significant differences between conditions when tAUC or iAUC for satiety over the course of the study days were compared. When satiety was examined in response to each 2 h meal interval, several improvements in satiety were demonstrated late in the day. Between 1300-1500 h, the 2 h tAUC for satiety was higher in response to the PA than the EX condition (177.9±114.4 v. 141.5±102.4 mm*2h, respectively; P<0.05). In addition, there was a main effect for condition found during the 1500-1700 h interval, wherein both the SED and PA conditions (P<0.05) resulted in a higher 2 h tAUC for satiety than the EX condition (SED 198.6±117.2, EX 139.3±99.5, PA 173.9±117.4 mm*2h; P<0.05).

Relationship between PYY and Insulin, Glucose, and Satiety

A significant inverse relationship was reported in the magnitude of change for total PYY and insulin within each 2 h meal interval in the EX ($r=-0.808$; $P=0.05$), but not the SED ($r=0.697$; $P>0.05$) and PA conditions ($r=0.724$; $P>0.05$). This finding was strengthened by the fact that the percentage change values for PYY and insulin during each 2 h interval were also significantly correlated in only the EX condition ($P=-0.875$; $P<0.05$).

There were no significant correlations reported between the magnitude of change, or percentage change in PYY and glucose across the 2 h meal intervals in any of the experimental conditions, though the correlations found in the PA condition ($r=0.587$ and 0.629) were stronger than those found in the SED ($r=0.209$ and 0.189) and EX conditions ($r=0.107$ and 0.189), respectively.

The magnitude of change in PYY was strongly correlated with the change in VAS scores for satiety across 2 h meal intervals ($r=0.800$, $P<0.05$) in the EX condition. Likewise, the correlation between the magnitude of change in insulin and subjective satiety approached significance in the EX condition ($P=0.06$). There were no significant correlations noted between PYY and satiety; or insulin and satiety in the SED and PA conditions ($P>0.05$).

Discussion

Whether short, frequent bouts of exercise designed to break up long sedentary periods during the day influences PYY and subjective measures of appetite in the same manner as a continuous exercise bout is unknown. In the present study, the addition of an hour of morning

exercise (EX) or intermittent hourly 5-minute exercise bouts throughout the day (PA) did not alter PYY concentrations over the course of the day; although exercise affected both subjective measures of appetite and the relationship between appetite perception and PYY. Intermittent exercise resulted in a reduced perception of hunger as the day progressed, with a robust attenuation of hunger perception (32.3%) demonstrated between the PA and EX conditions between 1500-1700 h, and a 24.6 and 26.9% reduction in hunger between 1700-1900 h when compared to the SED and EX conditions, respectively. Intermittent activity also demonstrated improved satiety perception, with increased indices of meal-induced satiety reported in the PA compared to the EX and SED conditions. Between 1500-1700 h, intermittent exercise resulted in a 25.7% increase in satiety VAS when compared to EX. Similarly, in the 2 h period between 1700-1900 h, the perceived score for satiety was increased by 42.6 and 24.8% over the EX condition in the SED and PA conditions, respectively.

Previous studies have demonstrated that prolonged, moderate-intensity exercise increases PYY concentrations (52, 253, 254), though this has not been demonstrated exclusively (152) and these concentrations are often elevated transiently (254). While PYY was elevated immediately following the morning exercise bout (EX) when compared to the SED (11.9%) and PA conditions (19.3%), this effect was negated by the 2 h mark. When the 12 and 2 h AUC values were compared, there were no differences in PYY concentrations found between conditions in line with previous findings (254). Our study demonstrated that intensity- and duration-matched physical activity participation, when spread over the course of an entire 12 h day, did not have any noticeable effect on measured PYY values.

While Martins et al (84) did not report differences between VAS measures of hunger and satiety in response to a 1 h bout of moderate exercise (65% of max heart rate), their

measurements were restricted to a 3 h period surrounding the exercise bout. The present study evaluated these measures over the course of 12 h. In concert with the findings of the aforementioned study (84), one-hour of moderate-intensity exercise did not result in changes in the hunger and satiety perception of our obese subjects with prediabetes. Intermittent exercise participation, however, was shown to decrease hunger perception and increase satiety later in the course of the day. The potential effects of short, frequent bouts over the course of the day to moderate appetite are promising.

Interestingly, the present study did not confirm the previously reported inhibition of glucose-mediated insulin secretion by PYY in obese individuals in the SED condition or PA conditions (100, 190).Though low levels of PYY have been reported in the obese, the effect of the physiological release of PYY on insulin concentrations in the obese remains unexplored. In this examination, the addition of one-hour of morning exercise in our obese cohort with prediabetes resulted in a strong, inverse correlation between the change in PYY and change in insulin over the course of the day; whereas a larger increase in PYY was associated with an attenuated increase in insulin during each meal condition. Despite the fact that there were no differences in the indices of PYY (12 h or 2h AUC, magnitude or percentage of change per meal, etc.), or subjective satiety between any of the study conditions, the continuous physiological stimulus of the 1 h morning exercise bout may have been sufficient in increasing the concentration or action of PYY towards the inhibition of insulin production. Regardless, future investigations of the relationship between PYY and insulin in obese individuals, and with exercise remain promising.

Limitations

The present study analyzed total PYY concentrations, which differs from previous published work which may report PYY 3-36. In addition, the choice and the wording of the subjective VAS questions, though standardized, may have been interpreted differently by the subjects. Additional questions have been used in previous studies in order to round out the information gathered by VAS, including 'how much do you feel you can eat' (84). The use of an additional method of appetite measurement, for example a subsequent buffet meal (84) may have added important information to the relationship between physiological and subjective measurement of hunger, and should be utilized in further assessments of the effect of physical activity on appetite perception.

This study was limited to the analysis of total PYY and insulin. Many unique, and sometimes redundant physiological mediators and pathways exist in the control of appetite regulation (274), it is likely that the reduction in hunger and increase in satiety found during the PA condition may have been under the influence of any number of orexigenic and anorexigenic hormones which were outside of the scope of this research.

Conclusion

In conclusion, short, purposeful bouts of physical activity over the course of the day can improve subjective measures of appetite when compared to a 1 h morning bout of moderate-intensity exercise, though this may be achieved through means unrelated to the concentrations of PYY. The hypothesis that short, frequent bouts of activity designed to break up long sedentary periods in the day would increase concentrations of PYY to a greater extent than morning exercise was

not substantiated in these findings. The relationship between PYY and insulin in obese individuals with prediabetes following a continuous 1 h exercise bout; as well as the causality and application of this relationship remains to be elucidated.

	Female (n=3)	Male (n=8)	Total (n=11)
Age (yrs)	25±3.1	25.3±1.5	25.2±1.3
Height (cm)	170±0.5	176.0±2.7	174.4±2.1
Weight (kg)	103.6±10.2	104.5±4.7	104.3±4.1
BMI (kg/m^2)	35.8±3.3	33.8±1.4	34.3±1.3
Body Fat %	42.0±3.3	28.9±2.7*	32.5±2.8
Physical Inactivity (self-report hours)	19.7±1.6	16.9±0.9	17.6±0.8
Fasting Blood Glucose (mmol/L)	6.25±0.52	6.30±0.33	6.28±0.29
Resting Systolic BP (mmHg)	125.3±7.7	120.8±3.1	122.0±2.9
Resting diastolic BP (mmHg)	68.3±3.8	65.9±1.8	66.5±1.6
Total Cholesterol (mg/dL)	181±2.4	127.3±2.6*	145.2±8.6
LDL Cholesterol (mg/dL)	91.5±10.2	51.3±5.7*	64.7±7.7
HDL Cholesterol (mg/dL)	57.5±8.6	38.8±4.3	45±4.5
Cholesterol Ratio (TC/HDL)	3.3±0.4	3.5±0.6	3.41±0.4

Table 3: Subject Characteristics

Mean±SEM. *P<0.05 vs. female subjects.

Time of Day	0700-0900	0900-1100	1100-1300	1300-1500	1500-1700	1700-1900
PYY (pg/ml)- SED	17.7	11.4	0.3	6.2	5.4	11.7
EX	22.4	-2.7	5.5	7.3	5.0	10.2
PA	16.4	13.0	5.3	10.4	14.8	-6.7
Insulin (pg/ml)- SED	804.5	581.3	624.8	593.6	561.1	740.9
EX*	207.3	584.3	477.3	418.6	484.4	600.7
PA*	513.8	539.0	430.8	494.8	479.0	441.1
VAS Satiety (0-100)- SED	9.2	3.2	10.0	10.2	11.3	15.3
EX	1.0	-8.7	-10.6	-8.3	-5.1	-7.7
PA	9.0	5.8	5.4	3.1	12.1	8.4

Table 4: Magnitude of Change in PYY, Insulin, and Satiety per Meal

Mean±SEM. *P<0.05 vs. SED condition.

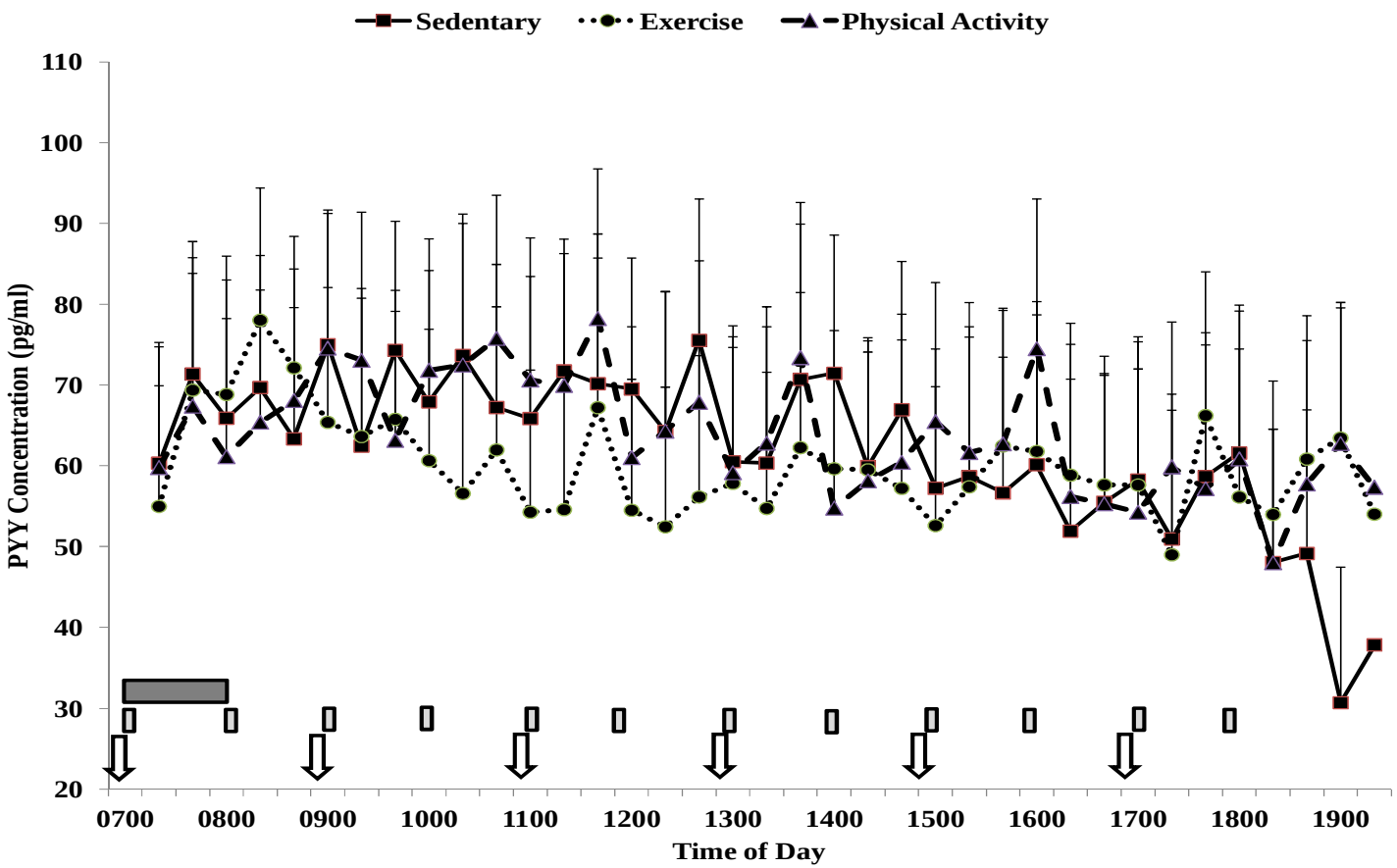

Figure 13: Total PYY pattern of response over 12 h by condition.

No significant differences across condition (P>0.05).

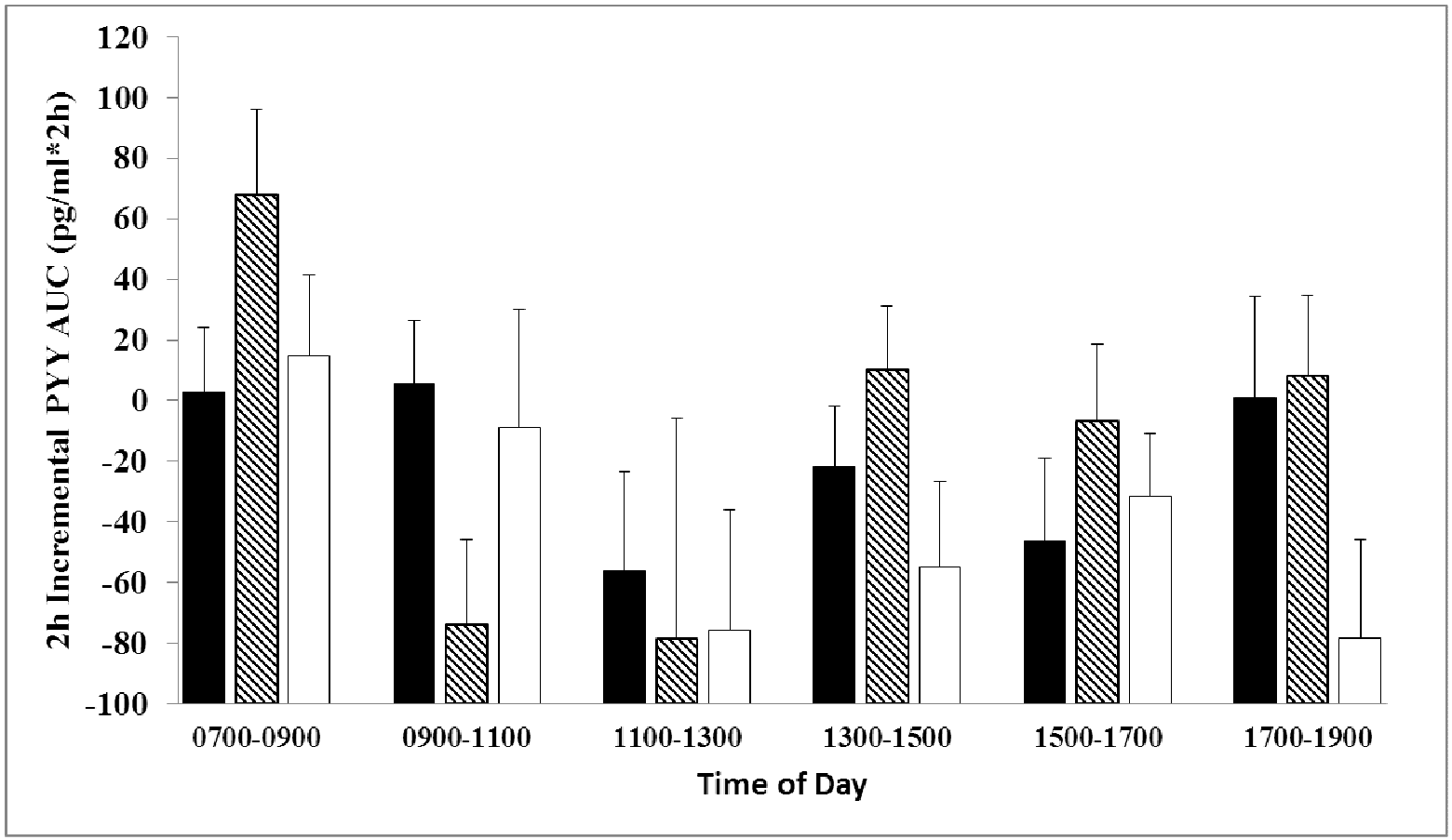

Figure 14: Two-hour incremental total PYY AUC by condition.

Black=SED, Crosshatching=EX, White=PA.

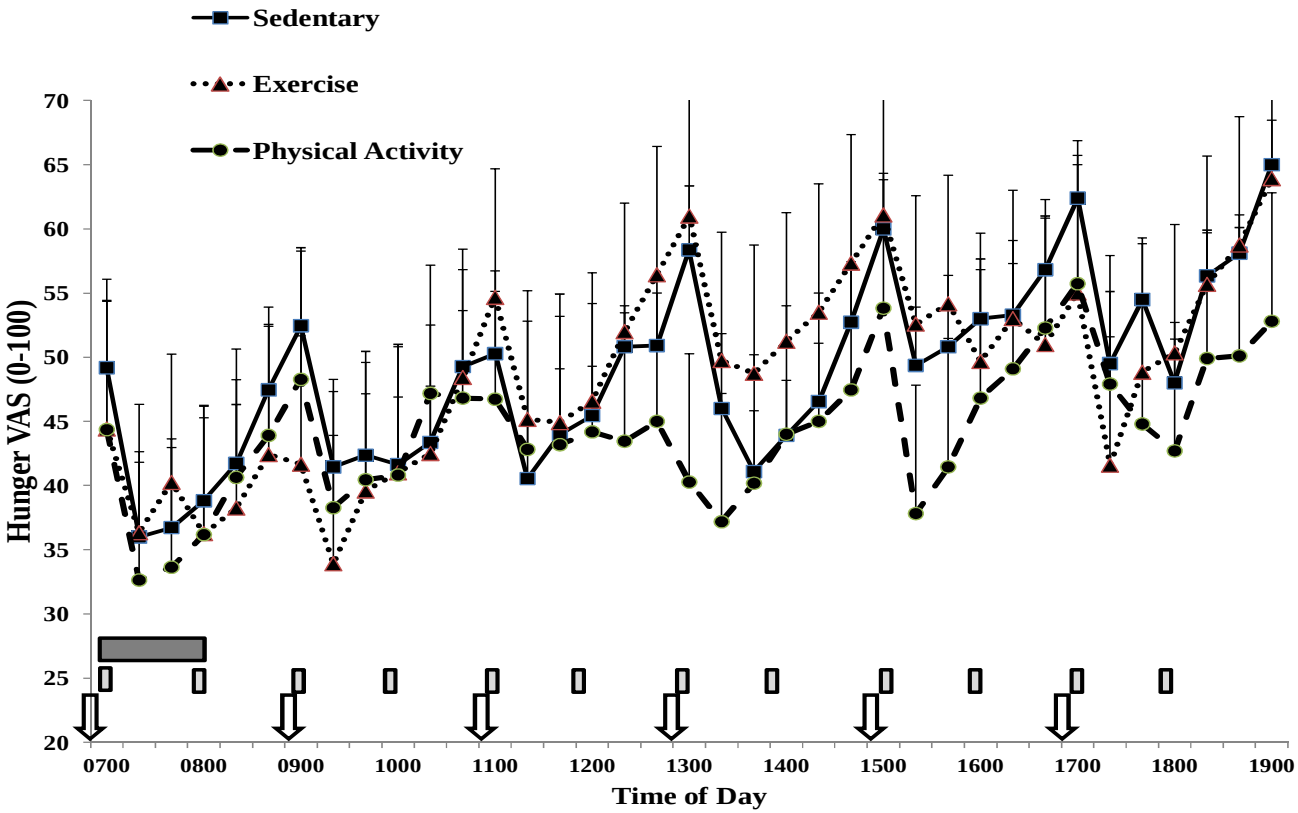

Figure 15: VAS hunger pattern of response over 12 h by condition.

No significant differences by condition (P>0.05).

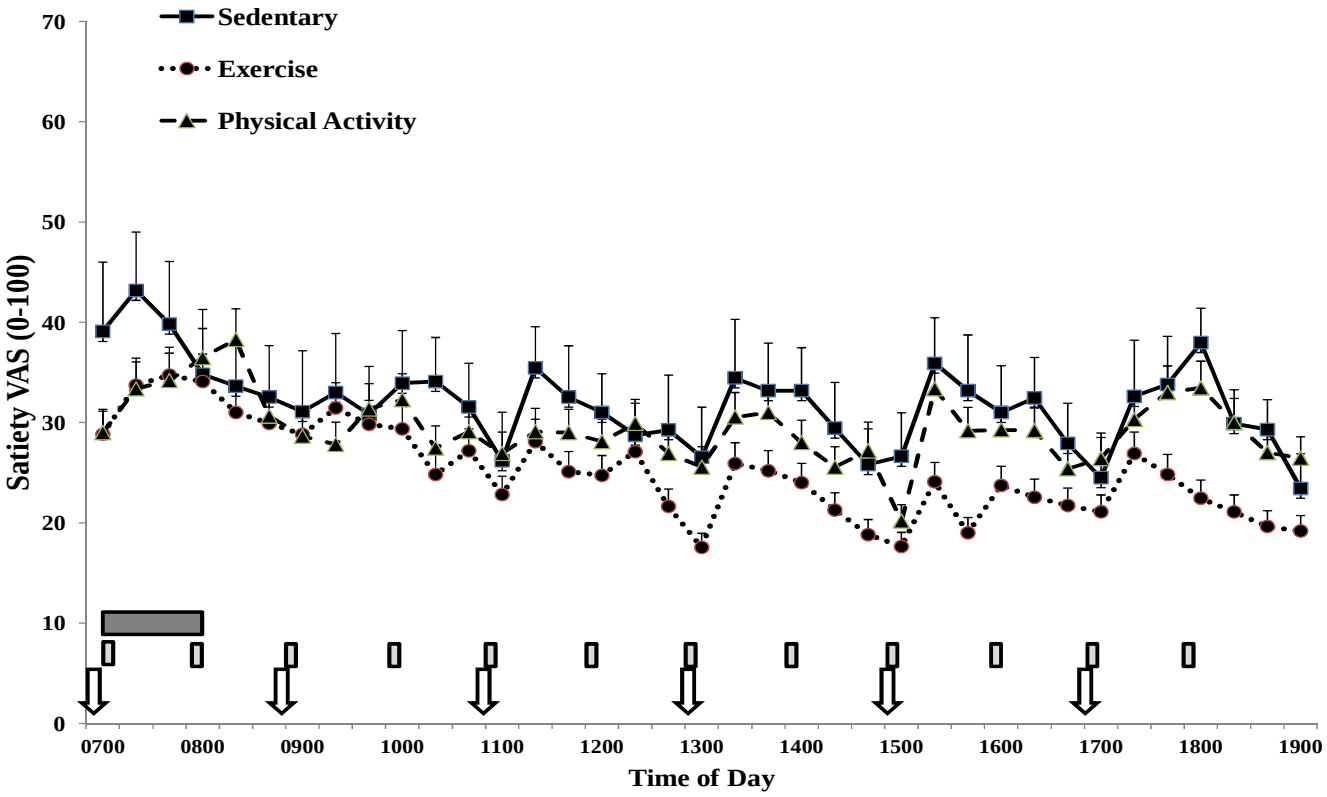

Figure 16: VAS satiety pattern of response over 12h by condition.

No significant differences by condition (P>0.05).

Chapter V: Conclusions, strengths/limitations, and future directions

This dissertation project clearly demonstrated that hormonal and appetite outcomes differ in response to a continuous bout of exercise versus the same amount and intensity of activity partitioned over the course of the day. Interestingly, moderate-intensity morning exercise that meets current physical activity guidelines, followed by a long period of sedentary behavior, may not be as beneficial in obese individuals with prediabetes as short, purposeful bouts of activity. Anecdotally, while all of the subjects in the study were able to complete the 60-minute morning bout of exercise, they all reported a preference for the short, frequent bouts of activity throughout the day.

The primary aim of this work was to examine the effect of intermittent physical activity on the responses of the glucose/insulin mechanism during frequent meal consumption. Breaking up sedentary behavior with short, purposeful exercise bouts, as hypothesized, resulted in reduced glucose and insulin concentrations over the course of the day. Insulin secretion, however, was not different between experimental conditions, and therefore the reduction in insulin found with intermittent activity may have been associated with an increase in insulin clearance. Contrary to our hypothesis, one hour of morning exercise followed by sedentary behavior resulted in augmented blood glucose levels over the course of the day. Despite these increases in glucose, measured insulin concentrations were reduced, albeit not as consistently across the day as the intermittent exercise.

Secondly, PYY, which operates through the inhibition of NPY neurons, was not affected by 1 h of continuous or intermittent moderate activity in the obese individuals in the present study. However, intermittent exercise resulted in an augmented satiety response and reduction in hunger when compared to the continuous, morning exercise bout. While this reduction in satiety

was not related to changes in PYY, additional pathways that influence orexigenic and anorexigenic mechanisms, or additional environmental or psychological mediators, may have been responsible for these changes in subjective satiety measures.

Thirdly, no clear relationship was established between the change in insulin and PYY, except when one hour of morning exercise was performed. A reduction in satiety occurred in the intermittent activity condition compared to the exercise condition over the course of the day; however, the one hour of morning exercise resulted in a strong relationship between changes in the subjective measures of satiety and changes in both the insulin and PYY concentrations. While subjective satiety and hunger were lower in the PA condition, the potential for a continuous morning exercise bout to improve appetite regulation outside of a controlled experimental procedure remains. Further examination of the differential appetite responses to continuous and intermittent exercise, and how these responses translate to actual food consumption following physical activity is needed.

Strengths/Limitations

Human studies have a wide range of strengths and limitations. The present study used experimentally manipulated physical activity conditions and a consistent controlled meal intake in order to measure the glucose, insulin, and PYY responses to meals. This is a definite strength of the study, as this work did not have to rely on self-reported dietary or physical activity records (10, 16) which are known to be unreliable.

In addition, the use of frequent sampling (every 10-minutes) allowed the calculation of insulin pulsatility, and a provided a detailed picture of the glucose, insulin, and PYY responses to physical activity over the course of 12 h. The use of deconvolution analysis using c-peptide

values allowed determination of insulin secretion, and not a complete reliance on circulating levels of insulin, which could reflect secretion or clearance.

Obese individuals with prediabetes occupy an important place along the spectrum between the lean, healthy individual and the obese individual with type 2 diabetes. Thus, this is an important subject cohort for the evaluation of meal and activity intervention. Despite the importance of this cohort, the potential for variability in glucose tolerance and insulin sensitivity, and the response of appetite hormones in this heterogeneous group may have contributed to large subject variability with our relatively small sample size.

Despite the seemingly small sample used in this study, this was an appropriate number. A within subjects ANOVA design is a powerful statistical tool when extraneous variables are controlled across experimental conditions, as it removes between-subjects variance, and allows a subject's testing days to be compared *within* the context of his/her own values, for all intents and purposes acting as his/her own control group. The frequent sampling employed in this project also considerable power to the sample size calculation. A power calculation was conducted to provide an estimate of the minimum sample size for a within subjects (1x3) ANOVA design. Based on our previous work (119), plasma insulin levels were used as the primary outcome variable to determine the power of this study. Insulin values between three different meal conditions were utilized, using the most conservative standard deviation of their within subjects differences. With an effect size of .80, it was determined that a total sample size of 9 would have 95% power to detect significant differences in glucose levels between our 3 physical activity conditions. A conservative number of 11-12 individuals was chosen to account for the addition of the unknown variance that physical activity would add to the protocol.

This study was designed to include more or less equal number of male and female subjects, however, difficulty was encountered with testing in females. Therefore, a proportionally large number of female subjects (7 of 10) who screened into the study and began data collection had to drop out as we had difficulty keeping an IV line patent for twelve-hour testing days. There were no difficulties encountered with the blood draws in any of the male subjects. One male subject who began the protocol and came to the lab for one testing day was not successfully contacted following his first visit, and therefore was considered a dropout. As such, a total of 22 individuals were consented into the present protocol, and those who completed (n=11) represented the group that

An additional limitation of this study may be the use of liquid meals as the nutrient stimulus during frequent meal consumption. While there is evidence that the consumption of liquid meals does not result in differences in the gastrointestinal emptying rate (46) or the glucose response to meal ingestion (33, 78), controversy remains as to the insulin (33, 78) and appetite (244) outcomes between solid and liquid consumption. While in the context of this controlled study, the use of liquid meals was justified; it is understood that this response may not fully reflect the outcomes of consuming traditional whole or processed foods. In the same manner, the decision to give subjects a standard 250-kcal meal, and not to titrate caloric intake to body mass, was determined in order to relate this work to previous findings. In the real world, food items are not sold to an individual according to their body mass. It is likely that increasing the caloric intake to reflect the potential difference in subject's body mass may have altered the findings presented herein, though we expect that a blanket increase in caloric intake in all subjects across testing days (e.g. 3000 v. 1500 kcal consumed daily) would not alter the differences we found between conditions.

The use of a frequent feeding model in this study reflected a growing change in the pattern of meal consumption seen in the United States and other developed nations (56, 275). This was a strength in that the glucose and insulin responses to frequent feeding have been questionable {{}}, and point to a negative potential for chronically elevated glucose levels. In contrast, additional work examining the effect of exercise on different meal patterns including the traditional, three-square meal pattern may be advisable.

The scope of the substances and hormones measured in this doctoral dissertation was notable, and presented many opportunities for analysis, comparison, and correlation. In particular, the use of PYY was justified due to its powerful anorexigenic nature. PYY is known to change in response to short-term interventions, including meal consumption (115), overfeeding (39), and particularly exercise (34, 52, 152). In addition, PYY is known to correlate well with subjective rating of appetite (VAS scales), as demonstrated by Leidy and colleagues (161) who reported that daily fullness ($P<0.05$) and PYY ($P<0.05$) increased in response to increased protein content in a meal, and a reduction in isocaloric meal frequency in overweight and obese men. Despite the advantages of using PYY, we were somewhat limited in our ability to make definitive statements about the effect of activity on various appetite hormones and appetite mechanisms during frequent consumption. Further examination of additional hormones (e.g. ghrelin, neuropeptide-Y, incretins (GIP, GLP-1)), and neurohumoral hunger (e.g. agouti-related peptide) and satiety (e.g. arcuate and paraventricular nuclei) pathways may elucidate the mechanisms behind this change in appetite.

Future Directions

These studies built upon previous work that pointed to the detrimental effects of long periods of sedentary behavior, and found some interesting outcomes pointing to the efficacy of intermittent exercise, particularly in relation to glucose control, insulin levels, and subjective appetite in the obese. Curiously, 1 h of exercise followed by sedentary behavior resulted in augmented glucose excursions and concentrations over the course of the day. This response in and of itself deserves further attention as adherence to traditional exercise guidelines often includes an acute continuous bout of exercise, and may be detrimental to glucose levels, particularly during the commonly prescribed dietary recommendation of frequent meals. Within the scope of possibility for this direction is obviously an examination of the effect of acute exercise followed by sedentary behavior in obese individuals with impaired glucose tolerance and T2D, and lean individuals across the spectrum of glucose tolerance. Also, the effect of exercise at difference times of day, and in response to different frequencies of meal consumption may yield important findings. Clearly more work related to the interaction between frequent meal consumption and physical activity participation, in a variety of cohorts, demands attention. Specific exercise recommendations for certain groups (e.g. obese healthy, T2D), which account for their dietary intake and frequency, might be more beneficial that current broad guidelines.

Furthermore, a better understanding of the relationship between insulin and appetite hormones, their potential effects on subjective means of appetite, and changes initiated with acute continuous and intermittent exercise and exercise training may be fruitful in further refining recommendations to better align diet and physical activity. The mechanisms which influence hunger and satiety in an intermittent exercise program versus a continuous protocol also deserve further merit as an area of great potential.

Chapter VI: Appendix

This section contains the list of definitions and all of the IRB approval and informed consent documents of the study. In addition, the complete nutritional information for the Wegman's beverage and a sample of the VAS scale for hunger and satiety are included.

List of Definitions/Abbreviations

Bolus- dose, often used in the context of meal intake or exercise prescription as a large, single administration as opposed to small, frequent increments

Cachexia- a wasting syndrome, loss of weight and/or muscle associated with a loss of appetite, often in response to a disease condition e.g. cancer, acquired immune deficiency syndrome, or chronic obstructive pulmonary disorders

C-peptide- 'connecting peptide', peptide component of proinsulin, which splits from insulin upon exocytosis from the pancreas, released in a 1:1 ratio, extended half-life makes c-peptide an excellent marker of insulin secretion

Euglycemic-hyperinsulinemic clamp- a method for quantifying insulin sensitivity of tissue which involves a continual infusion of insulin (to maintain 100μU/ml) and subsequent measureme of the rate of glucose infusion necessary to reach homeostasis

Exercise- goal-specific physical activity participation

Ghrelin- potent appetite-stimulating peptide hormone produced in the gastrointestinal tract

Glucose excursions- variation in the glucose concentration from baseline in response to an acute event, such as a meal or exercise bout

HOMA-IR- 'homeostatic model assessment', used to approximate insulin resistance using fasting glucose and insulin levels

Incretin- subset of gastrointestinal hormones that increase the production of insulin in the endocrine pancreas following meal consumption e.g. glucagon-like peptide-1, and gastric inhibitory peptide

Insulin- primary hormone responsible for the regulation of carbohydrate and fat metabolism

Insulinotropic- responsible for initiating an increase in insulin production

Insulin resistance- a metabolic disorder where the body does not utilize insulin effectively, thought to be related to signaling cascades subsequent to ligand-receptor binding

Isocaloric- containing the same number of calories as another experimental meal

Meal frequency- the habitual number of meals and snacks consumed by an individual on a daily basis, aka: meal periodicity, meal timing

Obese- body mass index above 30 kg/m^2

Orexigenic- hunger-stimulating

Patency/patent- open, free-flowing

Physical activity- coordinated contractions of the skeletal muscles to produce movements; often ambulatory in nature

Postprandial- after the consumption of a meal

PYY- anorexigenic (satiety) peptide hormone that is released by gastrointestinal cells in response to nutrient ingestion

QUICKI- 'quantitative insulin sensitivity check index', used to measure insulin sensitivity using log-transformed indices of fasting glucose and fasting insulin

Sedentary behavior- a condition of either no physical activity, or limited physical activity participation

Thermic effect of feeding- energy expenditure (and heat production) resulting from the metabolism of energy-containing foods and beverages

Thermogenesis- the production of heat

Type 2 diabetes- medical condition characterized by fasting blood glucose concentrations above 126 mg/dl, and insulin resistance

Volitional fatigue- voluntary termination of an exercise test due to subject's perception of exercise difficulty and ability to continue

SYRACUSE UNIVERSITY
Institutional Review Board
MEMORANDUM

TO: Stefan Keslacy
DATE: July 30, 2009
SUBJECT: **Expedited Protocol Review-Approval of Human Participants**
IRB #: 09-176
TITLE: *The Effects of Physical Activity on the Insulin Response to Frequent Meals*

The above referenced protocol, submitted for expedited review, has been evaluated by the Institutional Review Board (IRB) for the following:

1. the rights and welfare of the individual(s) under investigation;
2. appropriate methods to secure informed consent; and
3. risks and potential benefits of the investigation.

Through the University's expedited review process, your protocol was determined to be of no more than minimal risk and has been given **expedited approval.** It is my judgment that your proposal conforms to the University's human participants research policy and its assurance to the Department of Health and Human Services, available at: http://www.orip.syr.edu/humanresearch.html.

Your protocol is approved for implementation and operation from **July 30, 2009** until **July 29, 2010**. If appropriate, attached is the protocol's approved informed consent document, date-stamped with the expiration date. This document is to be used in your informed consent process. If you are using written consent, Federal regulations require that each participant indicate their willingness to participate by signing the informed consent document and be provided with a copy of the signed consent form. Regulations also require that you keep a copy of this document for a minimum of three years.

CHANGES TO APPROVED PROTOCOL: Proposed changes to this protocol during the period for which IRB approval has already been given, cannot be initiated without IRB review and approval, except when such changes are essential to eliminate apparent immediate harm to the participants. Changes in approved research initiated without IRB review and approval to eliminate apparent immediate hazards to the participant must be reported to the IRB within five days. Protocol changes are requested on an amendment application available on the IRB web site; please reference your IRB number and attach any documents that are being amended.

CONTINUATION BEYOND APPROVAL PERIOD: To continue this research project beyond **July 29, 2010**, you must submit a renewal application for review and approval. A renewal reminder will be sent to you approximately 60 days prior to the expiration date. *(If the researcher will be traveling out of the country when the protocol is due to be renewed, please renew the protocol before leaving the country.)*

UNANTICIPATED PROBLEMS INVOLVING RISKS: You must report any unanticipated problems involving risks to subjects or others within 10 working days of occurrence to the IRB at 315.443.3013 or orip@syr.edu.

Office of Research Integrity and Protections
121 Bowne Hall, Syracuse, New York 13244-1200
(Phone) 315.443.3013 ♦ (Fax) 315.443.9889
orip@syr.edu ♦ www.orip.syr.edu

STUDY COMPLETION: The completion of a study must be reported to the IRB within 14 days.

Thank you for your cooperation in our shared efforts to assure that the rights and welfare of people participating in research are protected.

Diane S. Young

Diane S. Young, Ph.D.
Chair

Note to Faculty Advisor: This notice is only mailed to faculty. If a student is conducting this study, please forward this information to the student researcher.

DEPT: Exercise Science, 201 Women's Bldg. **STUDENT: Michael Holmstrup**

315-443-3013 Fax 315-443-2382
regcomp@syr.edu

SYRACUSE UNIVERSITY
Institutional Review Board
MEMORANDUM

TO:	Stefan Keslacy
DATE:	October 12, 2009
SUBJECT:	**Amendment Approval-Use of Human Participants**
AMENDMENT #	2:Other-Modify Inclusion Criteria; Age
IRB #:	09-176
TITLE:	*The Effects of Physical Activity on the Insulin Response to Frequent Meals*

The amendment(s) submitted to the above referenced human participants protocol for review by the Institutional Review Board (IRB) is approved.

This protocol must still be renewed yearly, based on the original expiration date of **July 29, 2010**. If applicable, attached is the protocol's approved, amended informed consent document, date-stamped with the expiration date. This amended document replaces the original approved document and is to be used in your informed consent process. If you are using written consent, Federal regulations require that each participant indicate their willingness to participate by signing the informed consent document and be provided with a copy of the signed consent form. Regulations also require that you keep a copy of this document for a minimum of three years.

CHANGES TO APPROVED PROTOCOL: Any additional proposed changes to this protocol during the period for which IRB approval has already been given, cannot be initiated without IRB review and approval, except when such changes are essential to eliminate apparent immediate harm to the participants. Changes in approved research initiated without IRB review and approval to eliminate apparent immediate hazards to the participant must be reported to the IRB within five days. Protocol changes are requested on an amendment application available on the IRB web site; please reference your IRB number and attach any documents that are being amended.

CONTINUATION BEYOND APPROVAL PERIOD: To continue this research project beyond **July 29, 2010**, you must submit a renewal application for review and approval. A renewal reminder will be sent to you approximately 60 days prior to the expiration date. *(If the researcher will be traveling out of the country when the protocol is due to be renewed, please renew the protocol before leaving the country.)*

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Diane S. Young

Diane S. Young, Ph.D.
Chair

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DEPT: Exercise Science, 201 Women's Bldg. **STUDENT:** Michael Holmstrup

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regcomp@syr.edu

SYRACUSE UNIVERSITY
Institutional Review Board
MEMORANDUM

TO:	Stefan Keslacy
DATE:	April 9, 2010
SUBJECT:	**Amendment Approval-Use of Human Participants**
AMENDMENT #	3:Other-Data collection change (Ernie Davis Lab replaces 306 Women's Bldg.)
IRB #:	09-176
TITLE:	*The Effects of Physical Activity on the Insulin Response to Frequent Meals*

The amendment(s) submitted to the above referenced human participants protocol for review by the Institutional Review Board (IRB) is approved.

This protocol must still be renewed yearly, based on the original expiration date of **July 29, 2010**. If applicable, attached is the protocol's approved, amended informed consent document, date-stamped with the expiration date. This amended document replaces the original approved document and is to be used in your informed consent process. If you are using written consent, Federal regulations require that each participant indicate their willingness to participate by signing the informed consent document and be provided with a copy of the signed consent form. Regulations also require that you keep a copy of this document for a minimum of three years.

CHANGES TO APPROVED PROTOCOL: Any additional proposed changes to this protocol during the period for which IRB approval has already been given, cannot be initiated without IRB review and approval, except when such changes are essential to eliminate apparent immediate harm to the participants. Changes in approved research initiated without IRB review and approval to eliminate apparent immediate hazards to the participant must be reported to the IRB within five days. Protocol changes are requested on an amendment application available on the IRB web site; please reference your IRB number and attach any documents that are being amended.

CONTINUATION BEYOND APPROVAL PERIOD: To continue this research project beyond **July 29, 2010**, you must submit a renewal application for review and approval. A renewal reminder will be sent to you approximately 60 days prior to the expiration date. *(If the researcher will be traveling out of the country when the protocol is due to be renewed, please renew the protocol before leaving the country.)*

UNANTICIPATED PROBLEMS INVOLVING RISKS: You must report any unanticipated problems involving risks to subjects or others within 10 working days of occurrence to the IRB at 315.443.3013 or orip@syr.edu.

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Kathleen King, Ph.D.
IRB Chair

Note to Faculty Advisor: This notice is only mailed to faculty. If a student is conducting this study, please forward this information to the student researcher.

DEPT: Exercise Science, 201 Women's Bldg.

STUDENT: Michael Holmstrup

315-443-3013 Fax 315-443-2382
regcomp@syr.edu

SYRACUSE UNIVERSITY
Institutional Review Board
MEMORANDUM

TO:	Stefan Keslacy
DATE:	August 2, 2010
SUBJECT:	**Renewal Approval - Expedited Review**
IRB #:	09-176
TITLE:	*The Effects of Physical Activity on the Insulin Response to Frequent Meals*

The request for renewal of your human subjects protocol has been reviewed by the Institutional Review Board (IRB) and has been evaluated for the following:

1. the rights and welfare of the individual(s) under investigation;
2. appropriate methods to secure informed consent; and
3. risks and potential benefits of the investigation.

Your protocol is approved for implementation and operation for a period of one year, from **August 2, 2010** to **August 1, 2011**. If appropriate, attached is the protocol's approved informed consent document, date-stamped with the expiration date. This document is to be used in your informed consent process. If you are using written consent, Federal regulations require that each participant indicate their willingness to participate by signing the informed consent document and be provided with a copy of the signed consent form. Regulations also require that you keep a copy of this document for a minimum of three years.

CHANGES TO APPROVED PROTOCOL: By its very nature, research involving human participants often requires change in plans and procedures. You are reminded of your responsibility to obtain IRB approval of any changes in your protocol prior to implementing them, except when such change is essential to minimize harm to the participants. Changes in approved research initiated without IRB review and approval to eliminate apparent immediate hazards to the participant must be reported to the IRB within five days. Protocol changes are requested on an amendment application available on the IRB web site; please reference your IRB number and attach any documents that are being amended.

CONTINUATION BEYOND APPROVAL PERIOD: To continue this research project beyond **August 1, 2011**, you must submit a renewal application for review and approval. A renewal reminder will be sent to you approximately 60 days prior to the expiration date. *(If the researcher will be traveling out of the country when the protocol is due to be renewed, please renew the protocol before leaving the country.)*

UNANTICIPATED PROBLEMS INVOLVING RISKS: You must report any unanticipated problems involving risks to subjects or others within 10 working days of occurrence to the IRB at 315.443.3013 or orip@syr.edu.

STUDY COMPLETION: The completion of a study must be reported to the IRB within 14 days.

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Kathleen King, Ph.D.
IRB Chair

Note to Faculty Advisor: This notice is only mailed to faculty. If a student is conducting this study, please forward this information to the student researcher.

DEPT: Exercise Science, 201 Women's Bldg. **STUDENT:** Michael Holmstrup

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SYRACUSE UNIVERSITY
Institutional Review Board
MEMORANDUM

TO: Stefan Keslacy
DATE: July 22, 2011
SUBJECT: **Protocol Closed**
IRB #: 09-176
TITLE: *The Effects of Physical Activity on the Insulin Response to Frequent Meals*

Your request to close your human subjects research protocol was received. This letter informs you that the above referenced protocol will be closed on **August 1, 2011.**

If you decide to pursue this research at any time, you will need to submit a new, updated application to the Institutional Review Board. Application materials are available at: http://orip.syr.edu/human-research/human-research-irb.html.

Thank you for your cooperation in our shared efforts to assure that the rights and welfare of people participating in research are protected.

Kathleen King, Ph.D.
IRB Chair

Note to Faculty Advisor: This notice is only mailed to faculty. If a student is conducting this study, please forward this information to the student researcher.

DEPT: Exercise Science, 201 Women's Bldg. **STUDENT: Michael Holmstrup**

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SYRACUSE UNIVERSITY

SCHOOL OF EDUCATION
Exercise Science

Syracuse University Department of Exercise Science

CONSENT/AUTHORIZATION FORM

STUDY TITLE: The effects of physical activity on the insulin response to frequent meals

PRINCIPAL INVESTIGATOR: Stefan Keslacy Ph.D.

BACKGROUND/PURPOSE

You are being asked to participate in a study examining the impact of both structured and unstructured physical activity on your insulin response to frequent meals. This study is recruiting obese individuals (21-35 yr old) and will take place over a 2-3 month period of time. Involvement in the study is voluntary, so you may choose to participate or not. This form will explain the study to you. Please feel free to ask questions about the research if you have any. I will be happy to explain anything in greater detail if you wish.

The purpose of this study is to investigate how physical activity can alter your glucose and insulin response to frequent meals. We have previously found in non-obese individuals that when fed frequent meals, meals consumed later in the day required a greater insulin response to handle blood glucose levels. In this study, subjects remained sedentary all day. Thus we are interested in examining in overweight/obese individuals if physical activity throughout the day will alter the insulin response.

PROCEDURES

On your first visit to the lab (4 hours), you will complete a medical history questionnaire, physical activity questionnaire, sedentary behavior questionnaire, and height/weight measurement. On this visit we will ask you to come to the Human Performance Lab to have your fasting blood glucose checked in the morning. This will be done by getting a blood sample by finger stick. Using these assessment tools, if you are too physically active (regularly active > 3 times a week), have medical issues affecting the desired measurements (e.g. a high fasting glucose level, some medications, inability to exercise, etc), or a height:weight ratio outside of our acceptable limits, then we will have to exclude you from further participation in the study. Following the fasting blood glucose measurement, we will give you a 75g glucose drink (oral glucose tolerance test). After 2 hours we will reassess your blood sugar level to see how well your body handles a glucose load.

If we can include you in the study, you will then complete an exercise stress test on the treadmill in the Human Performance Lab. During this test, you will start walking very slowly on the treadmill and the speed will increase every 2 minutes until a speed of 3.5 mph (a brisk walking pace), then the grade

(or incline) of the treadmill will begin to increase every 2 minutes until you reach fatigue. This test takes about 10-12 minutes. You will feel fatigued at the end of this test but this will be a passing feeling and you will completely recover in about 3-5 minutes. You should not feel sore the next day. You will also have a face mask on during this test so that we can measure all of the air you breathe out. This will allow us to estimate your fitness level. In addition we will ask you to participate in a test where we can measure your percent body fat using the BodPod. In this test you will wear a bathing suit and sit in capsule quietly while the machine makes the measurement. You will also be asked to puff gently through a tube a few times. This part of the testing takes about 10 minutes.

After this initial testing, you will be asked to make 3 visits to the Human Performance Lab. Each visit will last 12 hours at the lab where your meals (1500 kcals of liquid drinks) will be provided for you. For each visit, you will arrive in the early morning (7am) following an overnight fast (from 7pm the previous night). You will have your height and weight measured. Using sterile procedures, a nurse will put a heparin lock (a small catheter in a vein) into your forearm so that we can draw blood samples. By using a heparin lock, this allows us to take multiple blood samples but not have to stick you again. Initially we will draw one large sample (about 10 ml or 2 teaspoons) so that we can measure your triglyceride levels, cholesterol levels, and glucose levels. You will be asked to drink the first meal after this sample, blood samples will be taken every 10 minutes (about 2.5 ml of blood (about a ¼ of a tsp)) for the following 12 hours. On each of the 3 study days, you will be provided with a meal drink every 2 hours, along with as much water as you like. During the visit, you will sit quietly during these 12 hours and can study, read, watch movies, and perform quiet, sedentary activities. During another visit, you will perform a 1-hour, moderate-intensity exercise bout at 0745 hours, after which you will spend the rest of the day in seated, quiet activity. During another visit, you will sit quietly during the 12 hours, however, each hour, will walk for 5-minutes at moderate-intensity, in order to "break up" your sedentary time. These three visits will be presented to you in a random order.

RISKS

The risks to you for participating in this study are:

1. The risks associated with the exercise test and exercise training include increased blood pressure, rapid heart beat and rarely heart arrhythmias (abnormal heart beats).
2. The BodPod is safe, although you may feel some discomfort if you do not feel comfortable in small spaces. A well- trained technician will conduct this test and can minimize this anxiety.
3. Blood sampling may cause fainting, and some discomfort and/or bruising at the site on your arm where the blood was taken. Rarely an infection occurs at the blood drawing site. A trained Registered Nurse will use sterile technique to place the catheter. On each of these visits about 180 ml of blood will be taken; this is about 1/2 of what would be taken if you were to donate blood. No more than 2 testing days will occur within one month, then we will wait 6-8 weeks for the next session in order to ensure adequate recovery of blood.

BENEFITS:

Your participation in this study will be of no direct benefit to you; however, participation in the max test, and exercise bouts may teach you more about your exercise capacity and body fat percentage. This study may make a significant contribution to our knowledge regarding the effects of physical activity on

the glucose and insulin responses to frequent meals throughout the day. Subjects will be paid $200 upon completion of the study (or at agreed pro-rated levels for partial participation).

VOLUNTARY PARTICIPATION:

Your participation in this study is entirely voluntary and you may refuse to participate or discontinue participation at any time without penalty or loss of benefits to which you would normally be entitled. Your decision about whether or not to participate in the study will not affect your relationship with Syracuse University.

COSTS/PAYMENTS:

There are no costs to you or your insurance carrier for participating in this study.

You will be paid for the initial visit and each 12 hour visit.
Visit 1-$15
Visit 2-$55
Visit 3-$55
Visit 4-$75

If one of the study days needs to be terminated before the completion of 12 hour, we will pay you $4/hour for the time completed.

In addition, by accepting payment for participating in this study, certain identifying information about you may be made available to professional auditors to satisfy audit and Federal reporting requirements, but confidentiality will be preserved. Please note that if you earn $600 or over in a calendar year as a research subject, you may have to pay taxes on these earnings.

QUESTIONS:

If you have any questions about the research, or in the event of a research-related injury, please contact Michael Holmstrup (315) 443-4540 (meholmst@syr.edu) or Stefan Keslacy Ph.D. (skeslacy@syr.edu)

If you have any questions about your rights as a participant, or if you have any questions, comments, or complaints and wish to address someone other than the investigator, or if you cannot reach the investigator, or when appropriate, if there are research related injuries, please contact the Syracuse University Office of Research Integrity and Protections (315) 443-3013.

PRIVACY AND CONFIDENTIALITY:

All data will be coded with an identifier and the subject's name will be taken off of their data sheet. Only Dr. Keslacy, and Michael Holmstrup will have access to this code. The subject key will be retained for a maximum time of five years after the study is published at which time all subject data will be destroyed. In reports or publications, only group data will be used, and no subject identifiers will be

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included. During the research testing, data collection is performed in individual rooms where other individuals present can not see or access your data. Since there is a common room, it is possible for subjects to see each other and talk while they are waiting or potentially when we are doing the 12 h blood draws. However, no personal data will be available at this time.

IN CASE OF INJURY:

You will be responsible for any costs not paid by your insurance company. No other compensation is offered by Syracuse University. Syracuse University has no plans to give you money if you are injured. You have not waived any of your legal rights by signing this form.

Consent To Participate In Research & Authorization To Use And Share Personal Health Information:

I am over 18 years of age.

I hereby give my consent to participate in this research study and agree that my personal health information can be collected, used and shared by the researchers and staff for the research study described in this form. I will receive a signed copy of this consent form.

_____
Printed name of the subject

_____ _____
Signature of subject Date

_____ _____
Signature of Person Obtaining Consent/Authorization Date

_____ _____
Signature of Witness Date

Nutrition Facts for Wegmans Nutritional Beverage

Serving size: 1 can
Servings per container: 6

Nutrient	Qty	%DV
Calories	250	
Calories from Fat	50	
Total Fat	6 g	9%
Saturated Fat	0.5 g	3%
Polyunsaturated Fat	1.5 g	
Monounsaturated Fat	3.5 g	
Cholesterol	-5 mg	2%
Sodium	200 mg	8%
Potassium	410 mg	12%
Total Carbohydrate	40 g	13%
Dietary Fiber	-1 g	4%
Sugars	18 g	
Protein	9 g	18%
Vitamin A		25%
Vitamin C		50%
Calcium		30%
Iron		25%

Vitamin D	25%
Vitamin E	25%
Biotin	25%
Chloride	6%
Chromium	25%
Copper	25%
Folate	25%
Iodine	25%
Magnesium	25%
Manganese	60%
Molybdenum	50%
Niacin	25%
Pantothenic Acid	25%
Phosphorus	25%
Selenium	25%
Vitamin B12	25%
Vitamin B6	25%
Zinc	25%
Riboflavin	25%
Thiamin	25%

Vitamin K	25%

Ingredients

Water, Corn Syrup Solids, Sugar (Sucrose), Milk Protein Isolate, Soy Protein Isolate, Maltodextrin (Corn), Canola Oil, High Oleic Sunflower Oil, Natural & Artificial Flavor, Potassium Citrate, Corn Oil, Magnesium Phosphate Dibasic, Calcium Phosphate Tribasic, Sodium Citrate, Soy Lecithin, Salt (Sodium Chloride), Soy Mono- and Diglycerides, Carrageenan, Ascorbic Acid, Magnesium Chloride, Potassium Chloride, Choline Chloride, Ferric Pyrophosphate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Vitamin A Palmitate, Niacinamide, Manganese Sulfate, Cupric Sulfate, Calcium Pantothenate, FD&C Yellow No. 6, Vitamin D3, Thiamin Hydrochloride, Lutein, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Biotin, Potassium Iodide, Chromium-Amino Acid Chelate, Molybdenum-Amino Acid Chelate, Phylloquinone, Selenium-Amino Acid Chelate and Cyanocobalamin.

Subject_____ **Date**_____ **Condition**_____ **Time**_____

1. How hungry do you feel?

|_____|

Not at all hungry — As hungry as I have ever felt

2. How full do you feel?

|_____|

Not at all full — As full as I have ever felt

1. How hungry do you feel?

|_____|

Not at all hungry — As hungry as I have ever felt

2. How full do you feel?

|_____|

Not at all full — As full as I have ever felt

1. How hungry do you feel?

|_____|

Not at all hungry — As hungry as I have ever felt

2. How full do you feel?

|_____|

Not at all full — As full as I have ever felt

1. How hungry do you feel?

|_____|

Not at all hungry — As hungry as I have ever felt

2. How full do you feel?

|_____|

Not at all full — As full as I have ever felt

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Chapter VIII: Curriculm Vitae

CURRICULUM VITAE

Michael E. Holmstrup M.S.

Delaware State University

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Dover, DE 19901 — Email: mholmstrup@desu.edu

Education

Institution	Degree	Years
Syracuse University, Syracuse, NY Exercise Science Department	Ph.D. Science Education/ Exercise Science	8/2005-Present A.B.D. Status
East Stroudsburg University of Pennsylvania	M.S. Exercise Physiology	1/2004-5/2005
East Stroudsburg University of Pennsylvania	B.S. Exercise Physiology Summa Cum Laude	1/2001-12/2003

Employment History

Full-Time Instructor

Delaware State University
1/2011-Present

Classroom Instructor

MVSC 202: Anatomy and Physiology II
MVSC 218: Sport and Fitness Nutrition
MVSC 355: Physiology of Exercise
MVSC 466: Health and Fitness Specialist
MVSC 463: Neuromuscular Adaptations to StrengthTraining

Teaching Associate

Syracuse University
9/2008-12/2010

Lab Coordinator and Lab Instructor-

Lab Coordinator: Ernie Davis Teaching Lab (2010)
PPE 497: Physiology of Exercise (Fall 05'-07'; Fall 09')

Teaching Assistant

Syracuse University
9/2005-9/2008

Lab Instructor/Teaching Assistant-

PPE 500: Obesity and Body Composition (Fall 05')
PPE 685: Systemic Physiology (Fall 06', 08', 10')
PPE 693: Research/Quantitative Methods (Spr 08')
PPE 753: Cardiovascular Physiology (Spr 08')

Classroom Instructor-

PED 291: Individualized Fitness (Spr 06', Fall 10')
HEA 332: Personal Health & Safety (F07'-Sp08'; F10')
PPE 295: Intro to Exercise Science (Spr 09'-Sum 09')

Syracuse University Teaching Fellow

8/2008-12/2010

Responsible for the instruction and acclimatization of new foreign and domestic Teaching Assistants (all academic departments) during the ten-day, **All-University TA Orientation Program** at Syracuse University. This nationally-recognized program involves instruction including, but not limited to, focused small-group interactions, 'microteaching' exercises, language proficiency examinations, and diversity training. The goal of this program being the preparation of incoming graduate students for the rewarding role of Teaching Assistant at Syracuse University.

Research Assistant

9/2005-12/2010

Research responsibilities include: working in the SU Human Performance Laboratory assisting with a variety of research projects and current grants in the areas of **obesity**, heart rate variability, baroreceptor sensitivity, **insulin responses to meal frequency**, self-selection of walking pace in overweight and obese individuals**,** and the metabolic and cardiovascular responses to fructose ingestion. Currently working on manuscripts branching from several recent research projects in the department, and collecting data for my dissertation project.

Fitness Director
6/2007-6/2009

Fit Families Program- Multidisciplinary, joint effort between Syracuse University and SUNY Upstate Medical University incorporating behavioral, nutrition and exercise education

Responsibilities included: Developing and implementing a curriculum designed to teach healthy exercise habits and lifestyle changes to an ever-growing group of overweight and obese children and teenagers, and their families.

Graduate Assistant	**East Stroudsburg University Human Performance Lab-**
1/2004-5/2005	Assisted with the teaching of Undergraduate Exercise Physiology sections and assisting with student research
E.S.U. Recreation Center 8/2003-5/2005	**Recreation Attendant-** Ensured the maintenance and safe use of exercise equipment on the fitness floor, answered client's fitness questions, acclimatized new employees and clients to the fitness center **Personal Trainer-** Performed 6 hours per week designing and implementing resistance, aerobic and flexibility programs

Professional Service

Appointed as **Student Representative** of Mid-Atlantic Regional Chapter of the American College of Sports Medicine- 2007-2009

Position responsibilities included:

-Attending and participating in chapter and national planning meetings as a voting member of the Executive Committee

-Coordinating and running 'book raffle' at annual chapter meeting
-Attending and reporting on national Student Affairs Committee meeting
-Generating newsletter articles geared towards increasing student involvement in the chapter
-Coordinating and presenting during a special 'student seminar' at regional meetings.

New Initiatives:

-Organizing a concurrent session 'Effects of Exercise on Cellular Function' (11/08)
-Developing a successful regional 'College Bowl' tournament for chapter undergraduates
-Developing a successful 'Meet the Experts' informative session for chapter undergraduates
-Developing a chapter 'Facebook' page to improve communication to students and professionals

Elected as **Member-at-Large** of Mid-Atlantic Regional Chapter of the American College of Sports Medicine for 2009-2011, Appointed as Co-Chair (09') and Chair (10') of the **Exposition Committee**

Position responsibilities include:

-Attending and participating in chapter and national planning meetings as a voting member of the Executive Committee
-Developing business relationships with universities, and product vendors to help support the regional chapter's annual conference; contributed to largest ever corporate and university EXPO at the annual MARC meeting (11/06/09-11/07/09, and 11/05/10-11/06/10)
-Organizing and moderating a concurrent session 'Periodization and Exercise Modalities for the Competitive Mixed Martial Artist' (11/06/09)
- Organizing and moderating a concurrent session 'Inactivity Physiology' (11/06/10)

Elected as **Secretary/Treasurer** of Mid-Atlantic Regional Chapter of the American College of Sports Medicine for 2011-2014

Position responsibilities include:

-Voting member of MARC Executive Committee responsible for meeting minutes
- Planning and implementing the 2010 and 2011 MARC Student Fitness Challenge

Academic Honors

Syracuse University

Reviewer: Journal of Human Nutrition and Dietetics

2011 **MARC ACSM** Doctoral Research Award Finalist

2009 **MARC-ACSM** President's Award for Outstanding Doctoral Student- $500

2009 **MARC-ACSM** Research Award for Outstanding Research Proposal- $500

2009 School of Education Research & Creative Grant- $1000
School of Education- Syracuse University- 4/28/09

2008 School of Education Research & Creative Grant- $800
School of Education- Syracuse University- 4/28/08

Accepted into **Future Professoriate Program,**
School of Education/Graduate School- Syracuse University- Completed Spring 2010

Appointed as a **Senior All-University Teaching Mentor** for 2010-2011- $1300
Appointed as a **Senior All-University Teaching Fellow** for 2009-2010- $1300
Appointed as an **All-University Teaching Fellow** for 2008-2009- $1200
Office of Professional Development Programs, Syracuse University

Graduate Student Travel Grant- $400
2009-2010- American College of Sports Medicine Regional Conference, Harrisburg, PA
2008-2009- American College of Sports Medicine National Conference, Seattle, WA
2007-2008- American College of Sports Medicine National Conference, Indianapolis, IN
2006-2007- American College of Sports Medicine National Conference, New Orleans, LA

Summer Grant Writing Award, 2007- $1500
School of Education- Syracuse University- May 2007

East Stroudsburg University

First Place Honors- Graduate Research Award- $100
Frederick Douglass Institution- East Stroudsburg University of PA- 4/14/05

President's Certificate of Recognition- East Stroudsburg University of PA
Employee Service to University- 4/27/05

E.S.U. Certificate of Exceptional Academic Achievement- 4/25/03

Publication Record

Peer-Reviewed Articles

"Relationship of Habitual Meal Frequency and Body Composition in Middle-Aged Males" **Michael E. Holmstrup M.S.**, Timothy J. Fairchild Ph.D., Gregory B. Dwyer Ph.D., Joanne Smith Ph.D., Shala E. Davis Ph.D. *Journal of Applied Nutrition.* 55(3): 2008. #081607

"Caloric Estimation Bias of Realistic Meal and Beverage Preparations"
Michael E. Holmstrup, Kay Stearns-Breuning, Timothy J. Fairchild Ph.D. *Topics in Clinical Nutrition.* 23(3): July/September 2008.

"Plasticity of heart rate signaling and complexity with exercise training in obese individuals with and without type 2 diabetes"JA Kanaley, S Goulopoulou, RM Franklin, T Baynard, **ME Holmstrup**, R Carhardt, RS Weinstock, B Fernhall. *International Journal of Obesity.* 33: 1198-1206, 2009.

"Assessment of endothelial function and blood metabolite status following acute ingestion of a fructose-containing beverage"
Bidwell AJ, **Holmstrup ME**, Doyle RP, Fairchild TJ. Acta Physiol (Oxf) 200(1): 35-43, 2010.

"Effect of meal frequency on glucose and insulin excursions over the course of a day" **Michael E. Holmstrup**, Christopher M. Owens, Timothy J. Fairchild, Jill A Kanaley. Clinical Nutrition. 5(6): e277-e280, 2010.

Submitted Articles

Do overweight individuals select a moderate intensity workload when exercising? Hall CM, **ME Holmstrup**, J Koloseus, D Anderson, and JA Kanaley. Submitted to International Journal of Obesity. In Review.

Size perception does not predict caloric estimation accuracy. **ME Holmstrup,** AJ Bidwell, TJ Fairchild. Submitted to Journal of Nutrition Education and Behavior. In Review.

Characteristics of accurate calorie estimators. **ME Holmstrup,** KS Bruening, J Rozelle. Submitted to Journal of Nutrition Education and Behavior. In Review.

Abstracts

Michael E. Holmstrup, Cameron Hall, Dan Anderson, Jay Koloseus, Jill A. Kanaley, FACSM. Self Selected Versus Moderate Intensity Walking In Overweight Individuals. *Medicine & Science in Sports and Exercise.* 41(5) Supplement: S5, May 2009.

Kanaley JA, Goulopoulou S, Franklin RM, Baynard T, **Holmstrup M**, Fernhall B. Heart rate variability and heart rate complexity following aerobic exercise training in obese individuals with and without type 2 diabetes. The Obesity Society – Annual Scientific Meeting, Phoenix, AZ- October, 2008.

Amy J. Bidwell, **Michael E. Holmstrup,** Timothy J. Fairchild. Metabolic and Cardiovascular Responses to the Ingestion of Fructose. American Physiological Society Meeting- The Integrative Biology of Exercise-V, Hilton Head, SC- September, 2008.

Michael E. Holmstrup, Timothy J. Fairchild, Jill A. Kanaley FACSM. The Acute Insulin Response to Meal Frequency and Composition in Healthy Adults. *Medicine & Science in Sports and Exercise.* 40(5) Supplement: S5, May 2008.

Michael E. Holmstrup, Styliani Goulopoulou, Jill A. Kanaley FACSM. The Relationship between Body Size and the Exercise Pressor Response in Children and Young Adults. *Medicine & Science in Sports and Exercise.* 39(5) Supplement: S5, May 2007.

Professional Presentations

Teaching/ Leadership Presentations

Facilitator/Panelist- Various Sessions- 2007-2010 All-University TA Orientation Program, Syracuse University

Facilitator- "Meet the Experts"
-Student Session; Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November 6, 2008

"Leader of the Pack: Developing Student Leadership Qualities"
-Student Session; Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November 2, 2007

"Teaching in the Sciences"- Invited Speaker/Panelist
-2007 All-University TA Orientation Program, Syracuse University- August 15, 2007

"The Role of Inquiry in the Undergraduate Exercise Science Laboratory"
-Science Education Symposium, Syracuse University, Syracuse, NY- May 2, 2007

Invited Talk: 8 Misconceptions about America's Growing Obesity Problem. Delta Sigma Theta Dover Alumnae Chapter Regional Day of Service. Delaware State University. April 2, 2011.

Scientific Presentations

The Effect of Physical Activity on Insulin Economy in Obese Individuals
- Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November 4, 2011, Doctoral Award Finalist

Body Image Does Not Predict Caloric Estimation Accuracy
- Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November 7, 2009

Is Exercise Enough? Interactions between Physical (In)Activity and Health
-Invited Lecture, Hamilton College, Clinton, NY- October 6, 2009

Self-Selected Versus Moderate Intensity Walking in Overweight Individuals
-American College of Sports Medicine National Conference, Seattle, WA- May 30, 2009
-Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November 7, 2008

The Acute Insulin Response to Meal Frequency and Composition in Healthy Adults
- American College of Sports Medicine National Conference, Indianapolis, IN- May 31, 2008

The Acute Hormonal Response to Meal Frequency and Composition in Non-Diabetic Young Adults
-Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November 1, 2007

The Relationship between Body Size and the Exercise Pressor Response in Children and Young Adults
-American College of Sports Medicine National Conference, New Orleans, LA- June 1, 2007
-Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November, 10, 2006

The Impact of Meal Frequency on Body Composition in Adult Males
-Mid-Atlantic Regional Chapter of the American College of Sports Medicine Conference, Harrisburg, PA- November 12, 2005.