Skeletal muscle metabolism following a high fat diet in sedentary and endurance trained males

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ABSTRACT

Insulin resistance (IR), T2DM, and obesity together form a cluster of interrelated metabolic challenges that may be linked by metabolic inflexibility. Metabolic inflexibility is characterized by the resistance to switching substrate oxidation preference based on substrate availability and can be measured in either fasted or insulin-stimulated conditions. As the largest site for glucose disposal and a primary tissue influencing regulation of blood glucose concentrations, skeletal muscle likely plays a central role in regulating substrate oxidation preference based on substrate availability. Skeletal muscle lipotoxicity caused by an impaired regulation of fat uptake and oxidation is postulated to disrupt insulin signaling and lead to skeletal muscle IR. High dietary saturated fat intake results in reduced basal fat oxidation and a resistance to switching to carbohydrate oxidation during insulin-stimulated conditions in susceptible individuals. This metabolic inflexibility may lead to an accumulation of intramyocellular species that impair insulin signaling. Endurance exercise training improves the capacity for fat oxidation in metabolically inflexible individuals. However, relatively little is known about how endurance exercise training influences substrate oxidation preference when paired with a high fat diet (HFD). Therefore, the purpose of this study was to determine the effects of a HFD on substrate metabolism in skeletal muscle of sedentary and endurance trained (ET)
males. Healthy, sedentary (n=17) and ET (n=7) males first consumed a 10-day moderate carbohydrate diet (55% carbohydrate, 30% total fat, <10% saturated fat) isocaloric to their individual energy requirements and then underwent a 4-hour high fat challenge testing session. During the session, they consumed a high fat meal (820 kcals; 25% carbohydrate, 63% total fat [26% saturated fat]), and skeletal muscle biopsies were taken in the fasted and 4-hour postprandial conditions. Participants then consumed a 5-day HFD (30% carbohydrate, 55% total fat, 25% saturated fat) and repeated the high fat challenge testing session. Substrate oxidation measures were performed on the collected skeletal muscle tissue, and the meal effect, defined as the percent change from the fasting to 4-hour postprandial condition, for each measure was calculated. There was a HFD by physical activity group interaction on meal effect for metabolic flexibility (P<0.05) and a HFD effect on meal effect for glucose oxidation (P<0.05). Meal effects for metabolic flexibility and glucose oxidation were maintained in the ET (20 ± 4% to 41 ± 21% and 128 ± 92% and 41 ± 15%, respectively; both P>0.05) but decreased in the sedentary (34 ± 7% to 4 ± 5% and 78 ± 26% to -21 ± 6%, respectively; both P<0.01) group. There were trends toward HFD effects on reductions in meal effects for total (P=0.062) and incomplete (P=0.075) fat oxidation, which were driven primarily by an increase in fasting total (12.1 ± 2.6 nmol/mg protein/h to 18.5 ± 2.3 nmol/mg protein/h; P<0.01) and incomplete (11.5 ± 2.5 nmol/mg protein/h to 17.6 ± 2.3 nmol/mg protein/h; P<0.01) fat oxidation in the ET group as a result of the HFD. Fasting total and incomplete fat oxidation did not change in the sedentary group (7.3 ± 0.8 nmol/mg protein/h to 7.8 ± 0.8
nmol/mg protein/h and 6.8 ± 0.7 nmol/mg protein/h to 7.2 ± 0.8 nmol/mg protein/h, respectively; both P>0.05). Overall, these findings suggest the ET state attenuates deleterious effects of a short-term HFD on reduced metabolic flexibility and insulin-stimulated glucose oxidation. In addition, a HFD-induced reduction in fat oxidation during the fasted-to-fed transition may be caused by differing mechanisms in sedentary and ET individuals. These findings provide a basis for future work targeting the elucidation of potential mechanistic differences in substrate oxidation preference between sedentary and ET individuals.
Skeletal muscle metabolism following a high fat diet in sedentary and endurance trained males

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GENERAL AUDIENCE ABSTRACT

Type 2 diabetes (T2DM) is a commonly occurring disease worldwide, and treatment of the disease is considerably burdensome for individuals and societies. T2DM is closely related to insulin resistance (IR) and obesity, and in each of these conditions, the characteristic of metabolic inflexibility has been observed. Metabolic inflexibility is a reduced ability to adjust fat or carbohydrate utilization for energy based on the availability of each of these macronutrients. Skeletal muscle may be an important tissue in the regulation of macronutrient utilization since it plays a key role in blood glucose regulation. High dietary saturated fat intake may lead to metabolic inflexibility in skeletal muscle in susceptible individuals. This metabolic inflexibility may result in increased storage of fat within skeletal muscle, which is hypothesized to disrupt insulin signaling. This disruption can lead to IR. Endurance exercise training improves metabolic flexibility. However, little is known about how endurance exercise training influences macronutrient utilization when paired with a high fat diet (HFD). Therefore, the purpose of this study was to determine the effects of a HFD on macronutrient utilization in skeletal muscle of sedentary and endurance trained (ET) males. Seventeen healthy, sedentary males and seven ET males first consumed a 10-day moderate-carbohydrate diet that was provided by the study
investigators and designed to keep each participant weight stable. Participants then underwent a high fat challenge testing session in which they consumed a high fat meal and had skeletal muscle biopsies taken both before and after the meal. Participants then consumed a 5-day HFD, also designed to keep them weight stable, and repeated the high fat challenge testing session. Macronutrient utilization measures were performed on the collected skeletal muscle samples. Overall, metabolic flexibility was reduced in the sedentary group but was maintained in the ET group, which suggests that ET individuals may be protected against developing a HFD-induced metabolic inflexibility in skeletal muscle and its associated downstream negative effects on insulin signaling. In addition, fat utilization during the high fat challenge meal decreased in both sedentary and ET individuals as a result of the HFD. However, fat utilization in the fasted state was higher in ET individuals after the HFD compared with baseline, but fat utilization was the same in sedentary individuals before and after the HFD. This suggests there may be differences between sedentary and ET individuals in the mechanisms involved in the adjustment of fat utilization to dietary fat intake. Further research is needed to understand these differences, as they may play important roles in understanding how IR and T2DM develop.
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Several co-investigators contributed to the experimental design and data collection for Chapter 3. Matthew Hulver, Ph.D., and Kevin Davy, Ph.D., were responsible for the concept and study design. Brenda Davy, Ph.D., R.D., oversaw the development of the diets and the metabolic kitchen in which the controlled diets were prepared. Ryan McMillan, Ph.D., conducted substrate metabolism experiments for data collection.
CHAPTER 1: INTRODUCTION

Background

The prevalence of type 2 diabetes (T2DM) is increasing nationwide and worldwide, and healthcare for this condition is costly. Coinciding with the increasing growth of population and the number of older adults, the worldwide prevalence of diabetes increased nearly four-fold between 2004 and 2014, from 108 million people to an estimated 422 million. In 2012, diabetes caused 1.5 million deaths worldwide, and poor blood glucose control contributed to another 2.2 million deaths. Poorly managed T2DM is characterized by chronic hyperglycemia and results in increased risk for cardiovascular disease, elevated blood pressure, dyslipidemia, nephropathy, neuropathy, and retinopathy. In addition to the individual burden of managing disease treatment and care, diabetes presents a major economic burden in the form of direct medical costs, indirect costs from loss of work productivity, premature mortality, and overall reduced gross domestic product for individuals nations. The direct costs from diabetes in 2014 were estimated to be $825 billion worldwide, with the US having the second highest direct costs of any nation at $105 billion. Given the individual burden of disease, economic costs to societies, and increasing prevalence, effective and economical diabetes prevention, diagnosis, and treatment strategies are vital.

Though some elements of the disease process have been identified, the etiology of T2DM is not fully understood. Insulin resistance (IR), metabolic
syndrome, T2DM, and obesity form a cluster of interrelated metabolic challenges; therefore, the pathogenesis of T2DM complex and interdependent on these other conditions. T2DM is characterized by impaired suppression of hepatic glucose output, peripheral glucose disposal, and pancreatic \( \beta \)-cell insulin secretion. The pathogenesis of the disease generally includes a period of prediabetes, which is characterized by the development of IR and impaired glucose tolerance. Primary T2DM disease prevention focuses on modifying lifestyle behaviors, and treatment centers around pharmacological and behavioral management blood glucose levels. Improving diagnosis, prevention, and treatment of T2DM requires detailed understanding of the pathogenesis of the disease and its relationship with other health conditions.

One physiological phenomenon linking IR, T2DM, and obesity is the resistance to switching substrate utilization preference based on substrate availability, termed metabolic inflexibility. Metabolic inflexibility has been demonstrated in conditions of IR, T2DM, and obesity and is regarded as one of the early signs of metabolic syndrome development. However, whether metabolic inflexibility precedes these conditions or is a result of them is unclear. Susceptible individuals develop indications of reduced metabolic flexibility within a very short time frame of exposure to obesogenic environments (e.g., high fat dietary intake, high calorie intake, or sedentary behavior). Thus, improving our understanding how obesogenic factors may influence the ability to adapt substrate metabolism to substrate availability may shed light on the
relationships among obesity, IR, and T2DM. Understanding these relationships can lead to more targeted prevention and treatment strategies.

**Overview of Metabolic Flexibility**

The term “metabolic flexibility” was initially described by Kelley and Mandarino as the ability to switch substrate oxidation based on substrate availability. In metabolically flexible tissue, fat oxidation predominates under fasting conditions, and fat oxidation is suppressed while glucose oxidation and storage are increased under insulin-stimulated conditions. Similarly, the transition from rest to exercise is associated with increases in rates of glucose and fat oxidation and reductions glycogen and fatty acid storage in skeletal muscle. The concept of metabolic flexibility can involve systems of organs and interactions between environmental stimuli and organ or whole body system response. The general idea is that fuel oxidation responds to fuel availability, which can be measured in a multitude of controlled conditions, including exercise and dietary manipulation. “Metabolic flexibility” is often used to broadly describe a cell’s, tissue’s, or whole body’s ability to adapt substrate oxidation to substrate supply using either glucose or fat as the stimulus.

**Origins in the Randle Cycle**

The beginning concepts of metabolic flexibility stem from the Randle cycle or glucose fatty acid cycle, a model to describe the mechanistic switch between carbohydrate and fat oxidation, which suggests that selection of fuel utilization in
normally-functioning tissues is dependent on competition of substrate supply and can operate independently of hormonal control. Randle et al. described nonesterified fatty acids as playing a causal role in the development of metabolic abnormalities to carbohydrate, including impaired insulin sensitivity, impaired glucose tolerance, and diabetes, and being a driver of the glucose fatty-acid cycle model. Within the model, insulin serves to bolster glucose uptake in both skeletal muscle and adipose tissue, inhibits lipolysis of adipose tissue, and promotes lipogenesis in adipose and skeletal muscle tissue. Growth hormone, corticosteroids, and epinephrine stimulate lipolysis from adipose and skeletal muscle tissue and may provide further regulation of the system by inhibiting glucose uptake in skeletal muscle. The overall purpose of this model is to maintain constant plasma glucose levels. Historically, this homeostatic model has been used to explain the presence of IR associated with obesity and T2DM; however, research in the decades following Randle’s observation suggested that increased fat oxidation via the Randle cycle may not be the instigator in the development of IR.

While Randle’s initial cycle model focused on availability of free fatty acids as the driver to increasing fatty acid oxidation and decreasing glucose oxidation, the concept was later expanded to reflect the challenge of glucose to fatty acid oxidation. Based on the Randle cycle, it was assumed that elevated levels of free fatty acids found in T2DM would suppress glucose metabolism. However, using a leg balance technique to estimate muscle glucose uptake, oxidation, and storage, Kelley and Mandarino found that in individuals with T2DM characterized
by fasting hyperglycemia, glucose oxidation persisted during fasting conditions when fat oxidation would have predominated according to the Randle cycle.\textsuperscript{25} Kelley and Simoneau\textsuperscript{11} also reported in individuals with T2DM suppressed fat oxidation during post-absorptive conditions and further demonstrated increased fat oxidation during insulin-stimulated conditions when glucose oxidation should have predominated.

**Importance of Skeletal Muscle**

Skeletal muscle comprises approximately 40% of total body mass and plays an important metabolic role in fat and carbohydrate storage and utilization. Thus, the switch between oxidation of different substrates under different conditions in skeletal muscle is important to understanding whole-body metabolism. Skeletal muscle is the largest tissue for glucose disposal,\textsuperscript{26} and when glycogen stores are fully replete, can comprise approximately 75% of the total body glycogen stored.\textsuperscript{27} Skeletal muscle accounts for an average of \( \sim 65\% \) of total systemic insulin-mediated glucose utilization in non-obese individuals (\( \sim 57\% \) in females and \( \sim 77\% \) in males).\textsuperscript{26} The oxidative capacity of skeletal muscle, compared to other tissues, plays an important role in adjusting fat oxidation in response to fat supply and can potentially influence insulin signaling,\textsuperscript{28} and accumulation of lipid species impairs insulin signaling (as described below).\textsuperscript{27,29-31} Highly oxidative skeletal muscle, categorized as type I fibers, are associated with greater insulin sensitivity and greater metabolic flexibility;\textsuperscript{32} conversely, glycolytic (type II) fibers are associated with presence of
metabolic syndrome risk factors. These major contributions to glucose and fat metabolism are indicative of skeletal muscle’s importance in regulating insulin sensitivity.

**Lipotoxicity Alters Insulin Signaling**

The oxidative capacity of skeletal muscle, compared to other tissues, plays an important role in adjusting fat oxidation in response to fat supply and can influence insulin signaling. The rate at which fat oxidation matches fat availability is a key determinant of skeletal muscle lipotoxicity and subsequent IR because accumulation of lipid in skeletal muscle tissue occurs when oxidative tissue does not appropriately adjust fat oxidation to fat supply. Intramyocellular lipids are elevated in states of obesity and T2DM, and increased lipotoxicity from ceramides and diacylglycerol (DAG) leads to impaired insulin signaling and subsequent IR. While intramuscular triglycerides (TAG) are typically measured as indicators of intramyocellular lipids presence, other lipid intermediates, including ceramides, DAG, and fatty acyl-CoA, are generally regarded as the culprits of disrupted insulin signaling, though there is some debate as to which particular species are the most problematic. Insulin receptors in skeletal muscle from obese and T2DM individuals have higher levels of phosphorylation on the serine and threonine residues and have lower tyrosine-kinase activity, which may be due to protein kinase C (PKC) beta action. This PKC activity is directly linked to increased intramyocellular fatty acyl-CoA concentrations.
Kelley et al. observed that rates of fatty acid uptake are similar between non-obese and obese individuals, yet measures of leg respiratory quotient (RQ) indicate reduced fatty acid oxidation in obese compared to lean subjects, indicating free fatty acids (FFA) are possibly still being released through lipolysis. Additionally, oxidative enzyme capacity and carnitine palmitoyl transferase I (CPT I) activity are lower in obese individuals compared to non-obese controls.

Other work, however, suggests increased fatty acid uptake may also contribute to intramyocellular lipid accumulation. Elevated levels of lipid intermediates were found in moderately obese individuals (body mass index (BMI) 30.2 ± 0.81 kg/m²) despite no declines in fat oxidation compared to non-obese controls, yet extremely obese individuals (BMI 53.8 ± 3.5 kg/m²) had reduced elevated lipid intermediates as well as reduced capacity for fatty acid oxidation. Additionally, Koves et al. suggested lipid oversupply promotes lipid oxidation, which disconnects the coupling of β-oxidation and the tricarboxylic acid (TCA) cycle and causes incomplete oxidation of acylcarnitine intermediates. Lipid oversupply within skeletal muscle may partially result from increased fatty acid transporter CD36 (FAT/CD36)-mediated fatty acid uptake. An overabundance of fatty acids within the cytosol are not fully oxidized. Bonen et al. observed that skeletal muscle strips from obese donors exhibited increased rates of fatty acid esterification without a similar increase in rates of fat oxidation. Impairments in fat oxidation that were initially attributed to reduced capacity for fat oxidation in
the mitochondria\textsuperscript{46,47} are perhaps related to reduced mitochondrial content rather than mitochondrial dysfunction.\textsuperscript{48,49}

Lipid and acylcarnitine intermediates may alter insulin signaling and glucose transport mechanisms. Overall, it appears that skeletal muscle lipotoxicity may occur due to a reduced rate of fat oxidation, incomplete fat oxidation, and increased fat uptake.

**Summary**

T2DM sits within a cluster of interrelated metabolic conditions, but the relationship among these conditions is not well defined. A resistance to switching substrate oxidation to match substrate availability at the whole-body and skeletal muscle level, termed metabolic inflexibility, is demonstrated in individuals in each of these conditions. Thus, impaired substrate oxidation preference may be an important link in understanding the development of these conditions, particularly T2DM.
CHAPTER 2: REVIEW OF LITERATURE

Overview

Understanding the conditions in which metabolic inflexibility develops and the specific mechanistic responses that lead to altered substrate oxidation preference will aid in the understanding the pathophysiology of T2DM and development of better-targeted prevention and treatment strategies. High fat dietary intake and sedentary behavior may contribute to impaired substrate oxidation preference in skeletal muscle.

Metabolic Flexibility Assessment Methods

The definition of metabolic flexibility is broad, and as such, a variety of approaches fit within the constraints of the definition and have been implemented to assess metabolic flexibility. The basic structure of providing a stimulus as a “challenge” and then measuring the adaptive metabolic response is consistent throughout all methods, but differences in measurement paradigms must be considered when synthesizing findings among studies. Key differences in methods include the stimulus type and method of delivery and the level of measurement (i.e., whole-body vs. tissue-specific measurement).

Administration of carbohydrate and fat can each be used as a stimulus to assess metabolic flexibility. Metabolic flexibility to carbohydrate is classically assessed under insulin-stimulated conditions with the hyperinsulinemic-euglycemic clamp. With this method, metabolic flexibility is assessed as the ability to suppress fat oxidation in favor of glucose utilization. This method can
most directly relate metabolic flexibility to insulin sensitivity, but one limitation is the need to control insulin-stimulated glucose disposal rate to appropriately account for glucose availability. The hyperinsulinemic-euglycemic clamp allows for measurement of metabolic flexibility to glucose in a highly controlled environment but does not account for meal pattern variations in daily dietary intake; therefore, while being beneficial in an experimental environment, the hyperinsulinemic-euglycemic clamp may not be as physiologically applicable as other methodologies that incorporate dietary intake. Evidence that fat metabolism may play a more pertinent role in the relationship between substrate oxidation and IR supports the use of lipid infusion during a hyperinsulinemic-euglycemic clamp. However, the challenges of interpreting results outside of a highly controlled clinical setting remain. Given that an aerobic exercise bout drastically increases energy demand, the transition from rest to a state of aerobic exercise to assess changes to reliance on fat oxidation has also been used as a measure of metabolic flexibility.

Respiratory quotient (RQ) measured via indirect calorimetry, either at the whole-body or tissue level, is a classic measure of substrate oxidation in the resting state. Due to the higher availability of fatty acids during overnight fasting, basal RQ has been argued as a measure of metabolic flexibility to fat, and a lower basal RQ, indicative of greater fat oxidation, has been utilized in many studies as a descriptor of a metabolically flexible state. However, RQ is highly affected by energy balance and dietary composition and, as such, may be a poor measure of chronic basal substrate oxidation unless energy balance is
tightly controlled\textsuperscript{28,60}. Therefore, change in RQ measured in response to a meal challenge, several days of controlled dietary intake, or controlled insulin-stimulated conditions may be considered more appropriate indicators of metabolic flexibility than basal substrate oxidation rates alone.

Due to these constraints on interpreting RQ, Bergouignan et al.\textsuperscript{61} proposed as another assessment of metabolic flexibility an index that reflects the relationship between RQ and blood insulin concentrations in order to account for the dynamic physiological and metabolic response to dietary intake. In this method, improved metabolic flexibility is defined as a higher variance in RQ coupled with a lower variance in insulin concentrations in response to controlled, standardized meals. Thus, improvements in metabolic flexibility are measured in interventions where individuals serve as their own controls.

The relationship between IR and whole-body metabolic flexibility likely begins with alterations in cellular mechanisms at the level of tissues and organs.\textsuperscript{17,62-64} Specifically, liver, adipose tissue, and skeletal muscle have all been implicated in the development of whole-body metabolic inflexibility and IR, thought there is still considerable question as to which tissue is the primary contributor to whole-body IR.\textsuperscript{17,65} Skeletal muscle has been given particular attention given its relationship with glucose metabolism (as discussed in Chapter 1) and its implications in T2DM development.\textsuperscript{65} Importantly, interrogation of substrate oxidation at the skeletal muscle level may provide insight into changes to cellular mechanisms that shed light on links between metabolic flexibility and IR.
Macronutrient Oxidation and Balance

The basis for the development of metabolic inflexibility and IR lies in the regulation of macronutrient oxidation preference. Under conditions of energy balance, macronutrient oxidation eventually matches macronutrient intake (i.e., RQ matches food quotient (FQ)). This concept is key to measuring metabolic flexibility because if fuel oxidation eventually matches fuel intake, metabolic flexibility can be measured within a short time span (i.e., within 5-7 days). As long as dietary protein intake is adequate, nitrogen balance is achieved spontaneously, and protein oxidation contributes minimally, but consistently, to the macronutrient mix being oxidized. Therefore, the relationship between metabolic inflexibility and IR depends on the coordination of fat and carbohydrate storage and utilization.

The framework for substrate balance centers around maintaining appropriate blood glucose concentrations and, by extension, glycogen stores. Therefore, the preferred fuel source for oxidation under varying conditions can be considered within the context of maintaining adequate storage of glycogen, as suggested by the model developed by Flatt. Glycogen provides a critical fuel reserve for sudden energy demands produced by a bout of exercise; however, glycogen stores are capped at ~500-1000 grams throughout the body. Dietary carbohydrate consumed in excess of glycogen repletion requirements has one of 2 fates: either increased carbohydrate oxidation or storage as fat.
While dietary carbohydrate intake can promote carbohydrate oxidation, dietary fat intake does not promote fat oxidation but storage instead. Dietary fat stored in abdominal adipose tissue is lower in obese compared with lean individuals, suggesting a potential increase in ectopic storage in obese individuals. In addition, lipid infusion results in increased intramuscular triglyceride (IMTG) accumulation and acute peripheral IR.

When glycogen stores are reduced, fat oxidation is promoted to preserve glycogen. In addition, fat balance is closely tied with energy balance. Flatt et al. reported that over a 9-hour postprandial period, total fat oxidation in humans was the same regardless of whether a low fat or high fat meal was initially provided, but fat balance differed significantly (-287 kcal in low-fat vs. +60 kcal in high fat) and was closely matched to energy balance (-310 kcal in low fat vs. ~+40 kcal in high fat). Bennett et al. reported that throughout a 6-hour RQ measurement after a high fat meal, subjects were in positive fat balance and did not demonstrate increased fat oxidation. In the 24-hour period following the high fat meal, total energy expenditure remained the same, but daily energy and fat balance was positive.

Importantly, these preceding observations of the relationship between dietary macronutrient intakes and their oxidation have all been made at the whole-body level. Given that glycogen storage in skeletal muscle accounts for ~80% of total body carbohydrate storage, the relationship between glycogen storage capacity and fat oxidation specifically within skeletal muscle may be especially important in considering the development of IR.
Influence of Lifestyle Factors on Metabolic Flexibility

Two key features of an obesogenic environment that contribute to an altered macronutrient oxidation preference are high dietary fat intake, specifically saturated fat, and physical inactivity or sedentary behavior. Overall, tight regulation of carbohydrate oxidation compared to carbohydrate intake and fat storage compared to overall energy intake (i.e., maintaining metabolic flexibility) is crucial to combating environments of high-fat, high-sugar diets and physical inactivity, even if these environments are only encountered for a short period of time.\textsuperscript{28}

In individuals who are metabolically flexible, fat oxidation decreases in the postprandial or insulin-stimulated state and increases in the postabsorptive state or in response to initiation of a bout of exercise.\textsuperscript{60,80} Basal fat oxidation, however, is suppressed in skeletal muscle and at the whole-body level of individuals who are insulin resistant,\textsuperscript{8} are obese,\textsuperscript{14,31,34} have lost weight,\textsuperscript{81} or have a family history of T2DM.\textsuperscript{82,83} Exercise training, on the other hand, improves metabolic flexibility in metabolically inflexible individuals.\textsuperscript{61,84-86}

High Fat Diet

Given the link between intramuscular lipid species accumulation and development of IR in skeletal muscle, a HFD intervention has been utilized to assess alterations in substrate oxidation preference based on nutrient availability. Blundell et al.\textsuperscript{87} reported that habitual HFD consumers had higher rates of fat
oxidation after a high fat load compared with habitual low fat diet consumers, but the ability to oxidize carbohydrate after a high carbohydrate load was not different between habitual diet groups. These results suggest there are specific mechanistic adaptations to high dietary fat consumption that promote metabolic flexibility to fat, and equally, poor adaptation to dietary fat intake results in a reduced capacity for fat oxidation. Overall, these adaptations are not well-understood, but fat availability, uptake, storage, and oxidation within skeletal muscle all play important and integrated roles.

According to the Randle cycle, an increase in fat availability should lead to increased fat oxidation. However, IR may arise when adipose tissue lipolysis and subsequent free fatty acid by skeletal muscle result in increased ectopic storage as insulin signaling-interfering lipid intermediates rather than an increased fat oxidation rate. In insulin-stimulated conditions, a similar disruption of insulin signaling can occur when adipose tissue does not appropriately take up dietary fat postprandially—or during lipid infusion—and adipose tissue lipolysis is also not suppressed, resulting in increased circulating free fatty acids. Observations of these events are primarily from studies utilizing lipid infusion during a hyperinsulinemic-euglycemic clamp to increase fatty acid availability well above potential oxidative capacity. Other evidence, however, suggests elevated plasma triglyceride concentrations and uptake by skeletal muscle, rather than free fatty acids, are associated with disrupted insulin signaling.

In line with the notion that elevated triglyceride activity may play a prominent role in altered fat oxidation capacity and intramyocellular lipid
accumulation, the type of dietary fat consumed may result in different fatty acid partitioning for storage and oxidation in skeletal muscle.\textsuperscript{72} Specifically, Bakke et al.\textsuperscript{92} reported that palmitic acid (the most prominent saturated fat in a Western dietary pattern) is oxidized at a much lower rate than oleic acid (a prominent dietary omega-9 monounsaturated fat) in cultured myotubes. Montell et al.\textsuperscript{38} and Gaster et al.\textsuperscript{93} reported that palmitic acid more readily accumulated as diacylglycerol and TAG in cultured myotubes compared with oleic acid. Gaster et al.\textsuperscript{93} proposed that because oleic acid accumulated as free fatty acid, it may be more readily available for fat oxidation. In general, however, monounsaturated fat tends to be more readily stored as triglycerides than saturated fats.\textsuperscript{27} Hulver et al.\textsuperscript{29} reported a higher expression of stearoyl-CoA desaturase-1 in skeletal muscle of females with severe obesity compared with normal-weight females. Stearoyl-CoA desaturase-1 converts long-chain saturated fatty acids into monounsaturated fatty acids. Elevated stearoyl-CoA desaturase-1 expression was associated with decreased fat oxidation and increased TAG synthesis.\textsuperscript{29} The results of the preceding studies appear to be in contrast with each other; however, it may be that dietary intake incorporating multiple fat types, including polyunsaturated fats specifically, may divert more saturated fats toward TAG storage rather than lipid species that appear to affect insulin signaling.\textsuperscript{38,94} Therefore, the ratio of fat types within a dietary pattern may be important in determining the influence of fat on insulin signaling.

The downregulation of genes associated with mitochondrial oxidation and biogenesis by a high fat diet is mostly attributed to the effect of saturated fat in
Interestingly, a meal high in medium chain triglycerides, a saturated fat readily hydrolyzed into free fatty acids early during gastrointestinal transit, results in increased whole-body fat oxidation during the immediate postprandial period; however, a meal high in long chain triglycerides, a saturated fat more readily incorporated into the liver triglyceride pool, did not affect postprandial rates of fat oxidation. Rather, long chain triglycerides were diverted toward storage. Therefore, even within the classification of saturated fats, different chain lengths may result in varied partitioning. Clearly, more studies aimed at characterizing the effects of varying dietary fat composition on skeletal muscle fat availability and storage are needed.

The rate of adjustment of fat oxidation in response to high dietary fat intake is individually variable. In response to a 5-day isocaloric high fat diet, genes linked to fat oxidation (including pyruvate dehydrogenase kinase 4, uncoupling protein 3, peroxisome proliferator-activated receptor α, and peroxisome proliferator-activated receptor gamma coactivator-1-α (PGC-1α)) increased in skeletal muscle of non-obese men and women but responded in a highly variable manner in obese men and women, indicating that as a whole group, non-obese subjects were able to shift toward upregulation of fat oxidation in response to the high fat diet. However, upregulation of expression of these genes is conflicting among research and may be due to varying methods of assessment. Sparks et al. found that PGC-1α mRNA was decreased by 20-25% and six genes involved in oxidative phosphorylation were downregulated by 20-30% in skeletal muscle after a 3-day high fat diet in healthy, normal weight
males. These findings are interestingly consistent with those of individuals with T2DM, who appear to have a downregulation of oxidative phosphorylation and PGC-1α gene expression. Lipid infusion also resulted in elevated FFAs and downregulation of PGC-1α and other oxidative genes in healthy, non-obese males.

Fat trafficking into the cell and into the mitochondria may be a point of regulation of adjusting fat oxidation to fat availability. Fatty acid translocase CD36 (FAT/CD36) and carnitine palmitoyltransferase 1 (CPT1) are two key transport proteins that move long-chain fatty acids into the muscle cell and into the mitochondria, respectively, and are some of the most well-studied of the fatty acid transport proteins. While other fatty acid transport proteins are certainly involved in the regulation of fat trafficking, these two have specifically defined functions under exercise and insulin-stimulated conditions. Under both skeletal muscle contraction and insulin stimulation, FAT/CD36 is translocated to the skeletal muscle cell surface to facilitate transport of long-chain fatty acids into the cytoplasm, and these conditions may have an additive effect with each other on FAT/CD36. Thirty minutes of electrical-induced contractions mimicking exercise conditions resulted in an increase in the rate of fatty acid transport into the sarcolemma, up to a 1.8-fold increase from baseline, which was returned to resting rate after 20 minutes of cessation of electrical stimulation. This occurrence was associated with a translocation of FAT/CD36 to the plasma membrane. In skeletal muscle of individuals with obesity and T2DM, an increase in FAT/CD36 on the plasma membrane was correlated with an increase
in fatty acid uptake and IMTG accumulation.\textsuperscript{44} In addition, these effects were associated with permanent relocation of FAT/CD36 to the plasma membrane.\textsuperscript{44} CPT1 moves long chain fatty acyl-CoAs across the outer mitochondrial membrane. CPT1 catalyzes the transesterification of fatty acyl-CoA to acylcarnitine; acylcarnitine then moves to the inner mitochondrial membrane for transport toward oxidative machinery of the mitochondria by carnitine palmitoyltransferase 2.\textsuperscript{102} Increased concentrations of malonyl-CoA inhibits the activity of CPT1. Increased flux through the TCA cycle generates increased citrate, which can be transported to the cytosol; in the cytosol, citrate stimulates the activity acetyl-CoA carboxylase 2 to convert cytosolic acetyl-CoA into malonyl-CoA.\textsuperscript{88} When CPT1 activity, and thus \(\beta\)-oxidation, are reduced, a mismatch between fat uptake into the myocyte and fat oxidation may occur and lead to accumulation of fat storage as TAG, DAG, and ceramides.\textsuperscript{88} Therefore, the activity of CPT1 plays a central role in regulation of fat oxidation and accumulation of lipid species that may interfere with insulin signaling.

Overall, individuals with rapid adjustment to high dietary substrate intake (i.e., are metabolically flexible) are likely less prone to intramuscular lipid accumulation, lipotoxicity, and the resulting derangements in insulin signaling when confronted with high dietary fat intake.\textsuperscript{28}

*Endurance exercise training*

Exercise improves insulin sensitivity in a majority of the population.\textsuperscript{20,105-107} Endurance exercise is well-recognized as a mechanism for increasing insulin sensitivity in the short term\textsuperscript{108,109} and is associated with increased fat
Given the relationships among exercise, insulin sensitivity, and fat oxidation, many have acknowledged that improving metabolic flexibility may be one of the benefits of regular exercise; however, the mechanisms by which exercise influences metabolic flexibility are not well understood. Interestingly, in cases where IR was present, exercise improved metabolic flexibility for those who were characterized by either the impaired fasting glucose or impaired glucose tolerance phenotype, but not in individuals for whom both were present.

Metabolic flexibility may play a role in the improvements in insulin sensitivity in part due to the impact of endurance exercise on oxidative capacity of skeletal muscle. There are several adaptations to endurance exercise that alter substrate handling and may play a role in metabolic flexibility in terms of the ability of skeletal muscle to adapt quickly to altered dietary intake patterns (e.g., a short-term HFD).

The general hypothesized paradigm is that adaptations induced by chronic endurance exercise training increase the capacity for fat oxidation during exercise and at rest such that fat utilization and fat availability within the myocyte are better matched. This results in a reduction in intramyocellular levels of lipid species that perturb the normal insulin signaling cascade. Thus, endurance exercise improves metabolic flexibility and protects against the development of IR. Paradoxically, IMTG are strongly linked to IR, but IMTG is also found in highly trained endurance athletes, who exhibit high insulin sensitivity. This suggests some difference between pathways and circumstances leading to IMTG.
accumulation in insulin resistant individuals versus endurance-trained individuals and that IMTG likely is simply a marker of insulin sensitivity in deranged muscle as opposed to a player within the pathway. In fact, ET individuals utilize IMTG as a substrate source during exercise, which correlates with increased storage capacity for IMTG.\textsuperscript{35} Transcriptional changes leading to structural changes of skeletal muscle allow this increased storage capacity.\textsuperscript{35} In IR, elevated plasma FFA may reduce the oxidative rate of IMTGs due to FFA coming from adipose tissue lipolysis.\textsuperscript{35} Fatty acid metabolites, specifically ceramides and diacylglycerides, increase in storage in insulin resistant individuals, and these metabolites may be what negatively affect insulin signaling, as discussed previously.\textsuperscript{35} While the storage of IMTG appear similar in states of IR and endurance trained, the root cause and development of IMTG storage and utilization differs.\textsuperscript{35} IMTG presence in IR is indicative of diacylglycerol and ceramide formation, which result in lipotoxicity and influence insulin signaling.\textsuperscript{35} Additionally, IMTG accumulation in endurance trained individuals is caused by transcriptional changes to induce skeletal muscle structural changes to store more triglycerides for substrate utilization during exercise.\textsuperscript{35} With the onset of exercise training in the presence of IR, IMTG stores do not consistently decline; however, insulin sensitivity and oxidative capacity improve.\textsuperscript{35} Improving fat oxidation to reduce accumulation of intramyocellular lipids may be an important strategy in mitigating the progression toward IR.

First observed by Holloszy et al.\textsuperscript{115} in 1967, a key metabolic adaptive response to endurance exercise training is mitochondrial biogenesis. Endurance
exercise training results in a 50-100% increase in mitochondrial content over a 6-week training regimen.\textsuperscript{116} Central to mitochondrial biogenesis is the activity of AMPK and PGC-1\textalpha.\textsuperscript{117,118} Acute endurance exercise results in increases in concentrations of ADP and AMP, which then activates AMPK. AMPK then regulates certain transcription factors that increase PGC-1\textalpha gene expression, which initiates the cascade of remodeling events that leads to the production of new mitochondria.\textsuperscript{118} Thus, sequential acute bouts of endurance exercise result in increased mitochondria content. Indeed, PGC-1\textalpha gene expression is higher in ET compared with sedentary individuals. In addition to promoting mitochondrial biogenesis, PGC-1\textalpha activates nuclear respiratory factors that increase expression of OXPHOS genes within mitochondria as well as several specific PPARs that regulate gene transcription fatty acid transport proteins.

In addition, the plasticity of skeletal muscle allows it to adapt to endurance exercise training. Of the 3 general myosin heavy chain-based isoforms of muscle fibers (Type I, Type IIa, and Type IIx), Type I muscle fibers possess the highest oxidative capacity.\textsuperscript{119} Though chronic endurance training can reduce the proportion of Type IIx muscle fibers and increase Type IIa, it is generally accepted that Type II fibers do not become Type I fibers, at least in any measurable and physiologically meaningful way.\textsuperscript{118} Nevertheless, endurance exercise training results in increased proportions of fiber types that exhibit higher mitochondrial activity, capillary density within the fiber, fatigue resistance and oxidative capacity.\textsuperscript{118,120} The calcineurin-NFAT pathway is the primary contributor to the regulation of muscle fiber type; however, PGC-1\textalpha functions as a co-
activator in inducing fiber type changes toward higher oxidative capacity fibers.121,122

Oxidative muscle fibers have greater capillary density compared with glycolytic fibers, and not surprisingly, endurance trained individuals have higher capillary density compared with untrained individuals.118 Exercise-stimulated angiogenesis is primarily regulated by PGC-1α and its co-activator ERR α, which both bind to DNA binding regions to upregulate the expression of VEGF and other angiogenic factors.123 In addition, endurance exercise promotes angiogenesis though concentrations of HIF-1, which also regulate VEGF and other angiogenic factor expressions. During exercise, the partial pressure of oxygen decreases, which triggers a release of VHL from HIF-1. HIF-1 can then bind to DNA binding sites to increase angiogenic factor expression.118 Nitric oxide produced from endothelial cells due to shear stress caused by an exercise bout can also stimulate angiogenesis.118

Fatty acid transport proteins located on plasma and mitochondrial membranes are also increased as a result of endurance exercise. As previously discussed in this literature review, FAT/CD36 translocation can be induced by skeletal muscle contraction.104 In addition, Jong-Yeon et al. reported that CPT1 activity as well as long-chain (palmitate) and medium-chain (octanoate) fatty acid oxidation was higher in ET compared with sedentary individuals.110

The overall effect of these adaptations to endurance exercise training in concert is increased fat oxidation both during a bout of exercise124,125 and at rest.59,126,127 Modulation of fat oxidation during exercise is a function of intensity
and duration.\textsuperscript{114} Generally about a 15\% improvement in maximal oxygen uptake occurs as a result of training,\textsuperscript{128} which is indicative of both the oxidative capacity of skeletal muscle as well as improved delivery of substrate to working muscle. Endurance exercise also improves capacity for glucose oxidation during insulin-stimulated conditions.\textsuperscript{129} Smith et al.\textsuperscript{97} found that 76\% of the variance in fat balance in response to a 4-d eucaloric HFD in healthy males was attributed to VO2max. As would be expected, fat balance was negatively correlated with VO2max ($r=-0.873$); in other words, a higher VO2max was predictive of high fat oxidation.

Relatively little is known about how exercise impacts substrate oxidation preference when paired with a HFD. Battaglia et al.\textsuperscript{86} reported that skeletal muscle fat oxidation increased in response to a 3-d high fat diet in non-obese but not obese participants; however, exercise training improved skeletal muscle fat oxidation similarly in both groups. This increase did not correspond to enhanced metabolic flexibility in either the non-obese or obese group when a high fat diet was included in addition to the exercise training. Goedecke et al.\textsuperscript{130} reported within 5-10 days of initiating a HFD in trained cyclists, there was a shift from reliance on carbohydrate oxidation during exercise to a reliance on fat oxidation.

\textit{Sedentary behavior}

Observations of metabolic inflexibility among obese, diabetic, and insulin resistant individuals often do not account for physical activity status; therefore, it is possible that sedentary behavior contributes to the metabolic inflexibility
reported in these studies. Metabolic flexibility responds in a linear manner to level of physical activity. Bedrest studies suggest that with decreased chronic physical activity or detraining, there is a decrease in fat oxidation and increased reliance on carbohydrate oxidation. In addition, physical inactivity may reduce gene expression fatty acid transport proteins, including FAT/CD36 and CPT1, and increase intramuscular lipid storage, which may include lipid species that negatively affect insulin signaling. In a study by Bergouignan et al., various levels of physical activity, from detraining of trained non-obese individuals to training sedentary overweight individuals for 1-2 months, resulted in a linear response of metabolic flexibility to physical activity level. Training regimens that followed current physical activity guidelines did not produce significant improvements in metabolic flexibility, as measured by variance in insulin and RER in response to moderate- and low-fat meals (i.e., 55% carbohydrate and 32% fat and 72% carbohydrate and 11% fat), though there were trends toward improvements. The authors suggested that a combination of change in body composition and exercise level is needed to produce the intended changes in metabolic flexibility. Of note, it appears that metabolic flexibility may respond to detraining and increased sedentary behavior more than exercise training or increasing physical activity level.

**Conclusion**

Macronutrient oxidation eventually matches macronutrient intake in order to establish energy balance. In metabolically flexible individuals, a coordination of
adapations within skeletal muscle allow substrate oxidation to match substrate availability within a relatively short time frame. However, the time in which substrate oxidation and substrate availability remain mismatched may be a critical period in which positive fat balance results in ectopic accumulation of lipid species within skeletal muscle. High dietary saturated fat intake may result in reduced metabolic flexibility and increased accumulation of specific intramyocellular lipids associated with impaired insulin signaling in susceptible individuals. Sedentary behavior is also associated with reduced metabolic flexibility; however, endurance exercise training results in physical and metabolic adaptations to skeletal muscle that may support the maintenance of metabolic flexibility.
CHAPTER 3: Skeletal muscle substrate oxidation response to a high fat diet in sedentary and endurance trained males

Abstract

Metabolic inflexibility, a reduced ability to adjust substrate oxidation preference to dietary macronutrient intake, is associated insulin resistance (IR), type 2 diabetes (T2DM), and obesity. Sedentary behavior is associated with reduced capacity to oxidize fats; however, endurance exercise training improves the capacity for fat oxidation. Therefore, the purpose of this investigation was to determine the effects of a HFD on substrate metabolism in skeletal muscle of sedentary and endurance trained (ET) males. Healthy, sedentary (n=17) and ET (n=7) males first consumed a controlled 10-day moderate-carbohydrate diet (55% carbohydrate, 30% total fat, <10% saturated fat) isocaloric to their individual energy requirements and provided by a metabolic kitchen. Participants then underwent a 4-hour high fat challenge testing session in which they consumed a high fat meal (820 kcals; 25% carbohydrate, 63% total fat [26% saturated fat]), and skeletal muscle biopsies were taken in the fasting and 4-hour postprandial conditions. Participants then consumed a controlled high fat diet (30% carbohydrate, 55% total fat, 25% saturated fat) isocaloric to individual energy requirements for 5 days and repeated the high fat challenge testing session. Substrate oxidation measures were performed on the collected skeletal muscle tissue, and meal effects, defined as the percent change from the fasting to 4-hour postprandial condition, were calculated for each measure. There was a
HFD by physical activity group interaction on meal effect for metabolic flexibility (P<0.05) and a HFD effect on meal effect for glucose oxidation (P<0.05). Meal effects for metabolic flexibility and glucose oxidation were maintained in the ET (20 ± 4% to 41 ± 21% and 128 ± 92% and 41 ± 15%, respectively; both P>0.05) but decreased in the sedentary (34 ± 7% to 4 ± 5% and 78 ± 26% to -21 ± 6%, respectively; both P<0.01) group. There were trends toward reductions in meal effects for total (P=0.062) and incomplete (P=0.075) fat oxidation as a result of the HFD in the overall sample; however, fasting total and incomplete fat oxidation increased after the HFD in the ET (12.1 ± 2.6 to 18.5 ± 2.3 and 11.5 ± 2.5 to 17.6 ± 2.3 nmol/mg protein/h, respectively; both P<0.01) but not sedentary (7.3 ± 0.8 to 7.8 ± 0.8 and 6.8 ± 0.7 to 7.2 ± 0.8 nmol/mg protein/h, respectively; both P>0.05) group. These findings suggest that the ET state attenuates the deleterious effects of a short-term HFD on reduced metabolic flexibility and blunted glucose oxidation in the insulin-stimulated condition. It is possible that a HFD-induced reduction in fat oxidation during the fasted-to-fed transition may be caused by differing mechanisms induced by habitual sedentary and endurance exercise behavior.

**Keywords**
Substrate oxidation; physical activity; metabolic flexibility; skeletal muscle; high fat diet

**Introduction**
A high fat diet (HFD) has been linked to an accumulation of intramyocellular lipid species, impaired insulin signaling, and subsequent insulin
resistance (IR). Individuals who are obese, insulin resistant, and have a family history of type 2 diabetes (T2DM) demonstrate a reduced ability to increase fat oxidation in response to high fat availability. This inability to adjust substrate oxidation to substrate availability, termed metabolic inflexibility, has been suggested as a key metabolic feature of T2DM, obesity, and IR. However, the directional nature of this relationship is unclear.

A central factor linking metabolic inflexibility to IR is skeletal muscle lipotoxicity, an accumulation of intramyocellular lipid species that disrupt normal function of the insulin signaling cascade. Lipotoxicity results when rates of fat oxidation are not matched to fat availability, which can be caused by a reduced rate of fat oxidation, incomplete fat oxidation, or increased fat uptake. In addition, skeletal muscle is the largest contributor to glucose disposal and, thus, plays an important role in determining peripheral insulin sensitivity.

Mounting evidence suggests that sedentary behavior is linked to adverse cardiometabolic outcomes, including reduced insulin sensitivity and T2DM. In addition, bedrest studies have reported reduced insulin sensitivity, reduced whole body lipolysis, decreased levels of fatty acid transport proteins (e.g., carnitine-palmitoyl transferase 1 (CPT1) and fatty acid transporter CD 36 (FAT/CD36)), reduced fat oxidation in favor of increased carbohydrate oxidation, and increased accumulation of intramuscular lipid species. Therefore, sedentary behavior has been suggested as a primary factor leading to metabolic inflexibility.
Conversely, aerobic exercise improves insulin sensitivity in a majority of the population, and the mechanisms by which this occurs may be in part related to improvements in metabolic flexibility. In fact, improved metabolic flexibility and fat oxidation as a result of endurance exercise training have been shown in obese individuals, individuals engaging in weight loss, individuals with T2DM, and lean, sedentary individuals. Endurance exercise training improves the capacity for fat oxidation in skeletal muscle during exercise and in the postabsorptive state. In addition, endurance exercise training results in mitochondrial biogenesis, angiogenesis, increased tricarboxylic acid cycle (TCA) enzyme activity, increased substrate transport into cells and mitochondria, and increased glycolysis and fat oxidation. Whether or not skeletal muscle fiber type switching occurs as a result of endurance training is still debated, but predominantly glycolytic fibers become more oxidative with endurance exercise training. Thus, skeletal muscle of endurance trained (ET) individuals possesses many characteristics that may mitigate the effect on altered substrate utilization induced by a HFD. Therefore, the purpose of this study was to determine the effects of a short-term HFD intervention on ex vivo substrate metabolism in skeletal muscle of sedentary and ET males. We utilized a high fat meal challenge to determine changes in substrate oxidation capacity in the fasted and insulin-stimulated state.
Methods and Materials

Participants

Seventeen non-obese, healthy, sedentary (≤2 hours of physical activity per week for at least the last 3 months and no planned, intentional exercise) and seven endurance-trained (running or cycling for ≥5 hours per week and participating in ≥2 races of ≥10 kilometers in the last year) males aged 18-40 years completed the study. Participants were weight stable for the last 6 months (±2.5 kg) and had a BMI between 18 and 40 kg/m². In addition, participants had blood pressure <140/90 mm Hg, fasting blood glucose <126 mg/dL, LDL cholesterol <130 mg/dL, total cholesterol <240 mg/dL, and triglycerides <300 mg/dL. Participants were not taking any medications or supplements known to influence these blood markers or study outcome variables. If antibiotics had been taken within the last 3 months, a 3-month washout period was required before initiating the study protocol. Participants were nonsmokers and had no medical history of cardiovascular disease or hyper- or hypothyroidism. In addition, participants had a habitual dietary intake of ≤40% kcals from total fat and no dietary restrictions that would preclude them from consuming the study diets. The protocol was approved by the Virginia Polytechnic Institute and State University Institutional Review Board (#06-367), and written and verbal informed consent were obtained each study participant.
**Procedures**

**Figure 1** details the study timeline and overall study design. Interested individuals first completed online questionnaires to assess initial eligibility for study participation. Then, interested individuals completed 2 screening visits to further determine if they met study inclusion criteria. During the first screening visit, participants completed a fasting blood draw and anthropometric measures and were given instructions to record habitual dietary intake with 4-day food intake records (3 weekdays and 1 weekend day). In the second screening visit, participants returned their 4-day food intake records for review with a registered dietitian. In addition, body composition was assessed via dual x-ray absorptiometry (DXA; GE Lunar Prodigy), and a treadmill exercise test to volitional exhaustion was utilized to assess maximal oxygen consumption ($VO_2_{max}$) with a metabolic cart (ParvoMedics TrueOne 2400, Sandy, UT). Eligible participants then participated in the intervention protocol in which they consumed a standardized diet for 10 days, followed by a high fat challenge testing session. Participants then completed a 5-day high fat diet feeding period and second high fat challenge testing session.
**Figure 1. Schematic of study design and procedures.** Abbreviations: ICD, informed consent document; FIR, food intake record; CBC, complete blood count; HHQ, health history questionnaire.

**Controlled feeding**

Energy needs for each participant were estimated using the Mifflin-St. Jeor equation.\(^{149}\) Participants first consumed a 10-day standardized diet (55% carbohydrate, 30% total fat, <10% saturated fat) that was isocaloric to their calculated individual energy requirements. A 5-day HFD (30% carbohydrate, 55% total fat, 25% saturated fat) was used as the intervention diet. All foods were assembled in a metabolic kitchen; foods that required preparation were weighed using a digital benchtop scale (Practum 5101-1S, Sartorius; Goettingen, Germany) to the +0.0 to 0.9 grams of planned menu amounts.

Participants reported to the metabolic kitchen daily to eat breakfast and pick up a cooler of food for the remainder of the day. Participants were provided with all foods and drinks, except water, for the duration of the study. Participants
were instructed to consume all required foods provided in the cooler; optional snack modules (~250 kcals each with nutritional composition comparable to that of the total diet) were also provided. Measuring daily body weights, weighing back returned food containers, and verbally checking in with study staff ensured compliance to controlled feeding requirements. For the duration of the study, participants remained within 1 kg of the average of their first 5 days on the standardized diet. Body weights drifting outside this parameter required adjustment of dietary intake by either increasing or decreasing kilocalorie diet level, or by removing or requiring consumption of snack modules.

**High fat meal challenges**

Immediately following the 10-day standardized diet and 5-day HFD, participants underwent high fat meal challenge testing sessions. **Figure 2** outlines the events of each testing session. Participants were asked to refrain from exercise for 36 hours prior to each high fat meal challenge. The high fat meal consisted of two prepackaged sausage, egg, and cheese biscuits (820 kcals; 25% carbohydrate, 63% fat [26% saturated fat]). Blood was drawn in the fasting condition and every hour for four hours after consumption of the high fat meal. Serum insulin **concentration** was measured **via** an Immulite 1000 benchtop immunoassay analyzer (Siemens Healthcare, Erlangen, Germany).

Fasting and postprandial skeletal muscle biopsies were taken from the vastus lateralis with a 5-mm modified Bergström needle (Cadence, Staunton, VA). A biopsy was performed in the right leg in the fasting condition and in the left leg in the postprandial condition. Skin was first cleaned with a povidone-
iodine solution, and approximately 10 mL lidocaine (1%) was injected to locally anesthetize the skin and deeper tissue. A small incision was made with a #10 scalpel, and then the Bergström needle with suction was used to collect skeletal muscle tissue. The incision site was cleaned with a saline solution and bandaged with steri-strips; ice and compression were applied to the incision site for 20 minutes to reduce discomfort and swelling. Tissue samples were washed with a phosphate buffered saline solution to maintain pH and then weighed, partitioned for specific outcome measures, and either kept on ice for immediate analyses or flash frozen in liquid nitrogen for later analyses. Immediately after biopsy extraction, tissue for substrate oxidation assessment was placed in 200 μL SET buffer (0.25 M sucrose, 1 mM EDTA, 0.01 M Tris-HCl, and 2 mM ATP) and kept on ice until homogenization and substrate oxidation assessment. Approximately 75 mg of tissue collected was allocated to substrate oxidation measures and enzyme activity assays utilizing homogenates.

Figure 2. High fat challenge testing session timeline and procedures.
Substrate metabolism measures

Skeletal muscle homogenates were prepared and used for assessment of glucose oxidation, total fat oxidation, complete and incomplete fat oxidation, and pyruvate dehydrogenase activity as previously described.\cite{84,150}

Skeletal muscle samples were homogenized by mincing with scissors and then processing with a homogenizer equipped with a Teflon pestle. Radio-labeled palmitic acid ([1-\textsuperscript{14}C]-palmitic acid), glucose ([U-\textsuperscript{14}C]-glucose), and pyruvate ([1-\textsuperscript{14}C]-pyruvate) was used to measure complete $^{14}$CO\textsubscript{2} production and \textsuperscript{14}C-labeled acid-soluble metabolites. Samples were incubated in the radio-labeled substrate (i.e., palmitic acid, glucose, or pyruvate) along with non-radioactive substrate, 0.5% bovine serum albumin, and a reaction media for one hour, and then perchloric acid was added to the media for one hour to release $^{14}$CO\textsubscript{2}. To measure metabolic flexibility, bovine serum albumin-bound non-labeled palmitic acid was also added to the working buffer for [1-\textsuperscript{14}C]-pyruvate oxidation. $^{14}$CO\textsubscript{2} was trapped using 1M NaOH, and 5 mL of scintillation fluid was added to the tube containing the sample, NaOH, and $^{14}$CO\textsubscript{2}. A scintillation counter was then used to measure concentrations of radiolabeled carbons atoms. Labeled acid-soluble metabolites were measured by determining concentration of radiolabeled carbon atoms contained within the acidified media. All samples were run in triplicate, and data was normalized to protein content using the bicinchoninic acid assay.

Metabolic flexibility was calculated as the percentage reduction in pyruvate oxidation in the presence of free fatty acids and is expressed as a ratio
of CO₂ production of labeled pyruvate over CO₂ production of labeled pyruvate with palmitate added. Therefore, the metabolic flexibility measure in the present study captures the preferential substrate for oxidation in skeletal muscle, or the suppressibility of pyruvate oxidation by free fatty acid. The ratio of complete to incomplete fat oxidation was also determined and is expressed as complete CO₂ production over acid-soluble metabolites.

**Enzyme activity assays**

The activities of citrate synthase, β-hydroxyacyl-CoA dehydrogenase (BHAD), cytochrome c oxidase, and malate dehydrogenase were determined in muscle homogenates spectrophotometrically as previously described, and maximum enzyme activities are reported as μmol/mg protein/min.

Citrate synthase activity was determined by measuring the reduction of 5,5’-dithio-bis (2-nitrobenzoic acid; DTNB) over time. Ten microliters of 1:5 diluted muscle homogenate was placed in 170μL of solution containing 0.1 M Tris buffer (pH 8.3), 1 mM 5,5’-dithio-bis in 0.1 M Tris buffer, and 0.01 M oxaloacetate in 0.1 M Tris buffer. After a 2-minute background reading and calibration of the spectrophotometer (SPECTRAmax ME, Molecular Devices Corporation, Sunnyvale, CA), 3.0 mM acetyl-CoA was added to initiate the reaction. Change in absorbance was measured at 405 nm at 37°C every 12 seconds for 7 minutes. Measurements were performed in duplicate.

BHAD activity was determined as the oxidation of NADH to NAD+. Thirty-five microliters of muscle homogenate were placed in 190 μL of buffer consisting
of 0.1 M triethanolamine-HCL, 5 mM EDTA tetrasodium salt dihydrate, and 0.45 mM NADH (pH 7.0). After an initial background reading and calibration of the spectrophotometer, 15 𝜇L of 2 mM acetoacetyl-CoA was added to initiate the reaction. The change in absorbance was measured every 12 seconds for 6 minutes at 340 nm and 37°C. Measures for BHAD were performed in triplicate.

Cytochrome c oxidase activity was determined by measuring the oxidation of ferrocytochrome c to ferricytochrome c. The change in absorbance was measured every 10 seconds for 7 minutes at 550 nm.

Malate dehydrogenase activity was determined by measuring the rate of oxidation of NADH. Ten microliters of diluted muscle homogenate were placed in 290 𝜇L of reaction media, which consisted of 0.1 M potassium phosphate buffer (pH 7.4) containing 6 mM oxaloacetic acid in potassium phosphate buffer and 3.75 mM NADH in potassium phosphate buffer. Absorbance was measured for 5 minutes at 340 nm and 37°C. Malate dehydrogenase activity measures were performed in triplicate.

Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics software (v. 24 for Mac, 2016, IBM Corporation). Differences in baseline participant characteristics between the two groups were assessed with independent samples t-tests. For substrate oxidation measures and enzyme assays, the meal effect of the high fat challenge meal was determined as percent change from the fasting to 4-hour postprandial state. A mixed-design analysis of
variance was used to compare high fat challenge responses pre- and post-HFD between the two groups. A Bonferroni confidence interval adjustment was used in comparing main effects. Key high fat challenge response variables included measures of substrate oxidation and enzyme activity related to fat oxidation and the TCA cycle. Independent samples t-tests were used as post hoc analyses to further assess differences between groups in each of the high fat challenge response variables, and paired samples t-tests were used as post hoc analyses to further assess differences in high fat challenge response variables within groups before and after the high fat diet. Areas under the curve for serum insulin measures during the high fat challenge were calculated using the trapezoidal rule. For all tests, the significance level was set a priori at $\alpha=0.05$.

Results

Participant characteristics

The study enrollment, group allocation, follow-up, and analysis process are outlined in Figure 3. Baseline participant characteristics are shown in Table 1. Age, body mass index, percent body fat, body weight, HDL-cholesterol, triglycerides, and fasting plasma glucose were similar (all $P>0.05$) between ET and sedentary groups. Total cholesterol ($P=0.005$) and LDL-cholesterol ($P=0.003$) were lower and VO2max was higher in the ET compared with the sedentary group ($P=0.005$). Body weight and percent body fat did not change from baseline throughout the study for either group (all $P>0.05$).
Figure 3. Schematic of study enrollment, group allocation, follow-up, and data analysis.
Table 1. Participant characteristics at baseline.

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<th>Sedentary</th>
<th>Endurance Trained</th>
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<td><strong>Age, years</strong></td>
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<td><strong>BMI, kg/m²</strong></td>
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<td>24 ± 1</td>
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<td>150 ± 6*</td>
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<tr>
<td><strong>LDL-C, mg/dL</strong></td>
<td>111 ± 7</td>
<td>73 ± 7*</td>
</tr>
<tr>
<td><strong>HDL-C, mg/dL</strong></td>
<td>54 ± 3</td>
<td>63 ± 4</td>
</tr>
<tr>
<td><strong>Triglycerides, mg/dL</strong></td>
<td>99 ± 9</td>
<td>68 ± 8</td>
</tr>
<tr>
<td><strong>Fasting Plasma Glucose, mg/dL</strong></td>
<td>88 ± 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82 ± 6</td>
</tr>
<tr>
<td><strong>VO&lt;sub&gt;2&lt;/sub&gt;max, mL/kg/min</strong></td>
<td>39.3 ± 2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>56.7 ± 3.1*</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SEM
<sup>a</sup> n=16 for the group
<sup>b</sup> n=13 for the group
<sup>c</sup> n=4 for the group.

* p<0.05 vs. sedentary group

Abbreviations: BMI, body mass index; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol

Dietary intake

Habitual dietary saturated fat intake was lower in the ET compared with the sedentary group (13 ± 1% of kcal vs. 10 ± 1% of kcal, P=0.018), but habitual dietary carbohydrate intake was higher in the ET compared with the sedentary group (54 ± 4% of kcal vs. 45 ± 1% of kcal, P=0.012). All other habitual macronutrient intakes were similar between groups (All P>0.05). Standardized and high fat dietary intakes are shown in Table 2. The ET group consumed significantly higher dietary amounts of energy and all macronutrients compared with the sedentary group in both the standardized and high fat diets.
Table 2. Standardized and high fat diet energy and macronutrient intakes for sedentary and endurance trained groups.

<table>
<thead>
<tr>
<th></th>
<th>Standardized Diet</th>
<th>High Fat Diet</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Endurance Trained</td>
</tr>
<tr>
<td>Energy, kcals/d</td>
<td>2714 ± 63</td>
<td>3438 ± 184**</td>
</tr>
<tr>
<td>Total Fat, g/d</td>
<td>91 ± 2</td>
<td>119 ± 7**</td>
</tr>
<tr>
<td>Saturated Fat, g/d</td>
<td>26 ± 1</td>
<td>35 ± 2**</td>
</tr>
<tr>
<td>Carbohydrate, g/d</td>
<td>377 ± 9</td>
<td>471 ± 24**</td>
</tr>
<tr>
<td>Protein, g/d</td>
<td>103 ± 3</td>
<td>132 ± 7**</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SEM
*p<0.05 vs. sedentary group
**p<0.01 vs. sedentary group

Metabolic flexibility

There was a significant HFD and physical activity level interaction on meal effect metabolic flexibility (P=0.012); metabolic flexibility within the ET group was not changed after the HFD (P=0.342; Figure 4A), whereas metabolic flexibility in the sedentary group decreased in response to the HFD (P=0.003). The meal effect for metabolic flexibility was not different between groups at baseline or after the HFD (P=0.242 and P=0.129, respectively).

Glucose oxidation

There was a significant main effect of the HFD on the meal effect for glucose oxidation (P=0.015). The meal effect for glucose oxidation was maintained in the ET (P=0.375) compared with the sedentary group, which decreased after the HFD (P=0.002; Figure 4B).
Pyruvate oxidation

There was a significant main effect of physical activity level on pyruvate oxidation (P=0.047). The meal effect for pyruvate oxidation was higher in the ET compared with the sedentary group post-HFD (P=0.012); however, pyruvate oxidation was similar between groups before the HFD (P=0.145; Figure 4C).

Figure 4. Percent changes in substrate oxidation from the fasted to postprandial state before and after the high fat diet. (A) Metabolic flexibility, expressed as percent change in pyruvate oxidation in the presence of free fatty acids; (B) glucose oxidation; (C) pyruvate oxidation. Data are expressed as mean ± SEM. *, p<0.05; **, p<0.01. Abbreviation: HFD, high fat diet.
Fat oxidation

The meal effects for fat oxidation measures are in Table 3. There were no significant effects of the HFD or physical activity level on the meal effects for complete fat oxidation or the ratio of complete to incomplete fat oxidation. However, the meal effects for total fat oxidation (P=0.062) and incomplete fat oxidation (P=0.075) tended to decrease with the HFD.

Because changes in skeletal muscle substrate oxidation are commonly assessed in basal rather than insulin-stimulated conditions, fasting fat oxidation measures were compared between groups at baseline and after the HFD. There was an interaction between the HFD and physical activity level on fasting total fat oxidation (P=0.014). Fasting total fat oxidation was similar between groups at baseline (P=0.115) and significantly increased in the ET, but not the sedentary, group in response to the HFD (Figure 5A). There was an interaction between the HFD and physical activity level on fasting incomplete fat oxidation (P=0.010). Fasting incomplete fat oxidation was not different between groups at baseline (P=0.118) but was significantly higher in the ET group after the HFD (P<0.001; Figure 5C). No changes were observed in fasting complete fat oxidation or the ratio of compete to incomplete fat oxidation (Figures 4B and 4D).
Table 3. Percent changes in fat oxidation from the fasted to postprandial states before and after the high fat diet.

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<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Endurance Trained</th>
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<tbody>
<tr>
<td></td>
<td>Δ Pre-High Fat Diet (%)</td>
<td>Δ Post-High Fat Diet (%)</td>
</tr>
<tr>
<td>Total Fat Oxidation</td>
<td>88.9 ± 29.7</td>
<td>10.6 ± 16.1</td>
</tr>
<tr>
<td>Complete Fat Oxidation</td>
<td>197.4 ± 67.1</td>
<td>17.0 ± 20.8</td>
</tr>
<tr>
<td>Incomplete Fat Oxidation</td>
<td>83.7 ± 28.8</td>
<td>10.5 ± 15.9</td>
</tr>
<tr>
<td>Complete: Incomplete Fat Oxidation</td>
<td>90.3 ± 66.5</td>
<td>6.1 ± 10.1</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SEM
There were no significant interactions or main effects for any measure; however, there were trends toward diet effects for total (p=0.062) and incomplete (p=0.075) fat oxidation.
Figure 5. Fasting fat oxidation before and after the high fat diet. (A) total fat oxidation, (B) complete fat oxidation, (C) incomplete fat oxidation, (D) ratio of complete: incomplete fat oxidation. Data are expressed as mean ± SEM. **, p<0.01; ***, p<0.001.

Enzyme activities

Results for enzyme activities are presented in Table 4. Due to limitations in tissue availability, cytochrome c oxidase and BHAD could not be assessed in the full sample. Thus, results are presented with limited sample sizes for these two measures.

There was a significant interaction between the HFD and physical activity level on citrate synthase activity (P=0.019). The meal effect for citrate synthase
activity significantly was maintained in the ET (P=0.803) compared with the sedentary group, which decreased in response to the HFD (P=0.001; **Figure 6A**). Fasting citrate synthase activity was significantly higher in the ET compared with the sedentary group at baseline (P=0.002; **Figure 6B**).

There was a trend toward a HFD and physical activity level interaction on BHAD activity (P=0.051; **Figure 6C**). In addition, the fasting BHAD activity was similar between groups at baseline (P=0.951; **Figure 6D**).
Figure 6. Oxidative enzyme activities before and after the high fat diet. (A) Percent change in citrate synthase activity from the fasted to postprandial state before and after the high fat diet; (B) fasting citrate synthase activity at baseline in sedentary and endurance trained groups; (C) percent change in BHAD activity from the fasted to postprandial state before and after the high fat diet; (D) fasting BHAD activity at baseline in sedentary and endurance trained groups. Data are expressed as mean ± SEM. *, p<0.05; **, p<0.01; ***, p<0.001.

There were no significant interactions or main effects of the HFD or physical activity level on the meal effect for malate dehydrogenase or cytochrome c oxidase activity in either group (Table 4).
Table 4. Percent changes in enzyme activities from the fasted to postprandial state before and after the high fat diet in sedentary and endurance trained participants.

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<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Endurance Trained</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>△ Pre-High Fat Diet (%)</td>
<td>△ Post-High Fat Diet (%)</td>
<td>△ Pre-High Fat Diet (%)</td>
<td>△ Post-High Fat Diet (%)</td>
</tr>
<tr>
<td>Citrate Synthase</td>
<td>35.80 ± 11.14</td>
<td>-17.61 ± 7.23†‡</td>
<td>2.99 ± 13.65</td>
<td>6.15 ± 11.16</td>
</tr>
<tr>
<td>Cytochrome C Oxidase</td>
<td>2.98 ± 8.16a</td>
<td>15.08 ± 45.16a‡</td>
<td>54.32 ± 22.38</td>
<td>23.03 ± 17.23</td>
</tr>
<tr>
<td>BHAD</td>
<td>8.86 ± 13.54b</td>
<td>-22.52 ± 10.09b†</td>
<td>26.94 ± 19.76</td>
<td>40.10 ± 21.57**</td>
</tr>
<tr>
<td>Malate Dehydrogenase</td>
<td>12.58 ± 5.15</td>
<td>-4.53 ± 5.26</td>
<td>19.71 ± 14.27</td>
<td>15.24 ± 7.32</td>
</tr>
</tbody>
</table>

All values expressed as mean ± SEM
† p<0.05 within group vs. Pre-HFD
‡ p<0.01 within group vs. Pre-HFD
**p<0.01 vs. sedentary
a indicates n=4
b indicates n=12

Serum insulin concentration

Serum insulin concentrations were similar between groups before the HFD. After the HFD, serum insulin concentrations at the 3-hour (p=0.024) and 4-hour (p=0.009) postprandial time points were lower in the ET compared with the sedentary group. In addition, within the ET group, serum insulin concentration at the 4-hour time point was significantly lower post-HFD compared to baseline. However, there were no differences between groups in serum insulin concentration areas under the curve before or after the HFD (Figure 7).
Figure 7. Serum insulin concentration during the high fat challenge meal before and after the high fat diet. Inset: Area under the curve between sedentary and endurance trained groups before and after the high fat diet. Data are expressed as mean ± SEM.

Discussion

The main finding of the present study was that after a 5-day HFD the meal effect for metabolic flexibility in skeletal muscle was maintained in ET individuals compared with sedentary individuals, in which the meal effect for metabolic flexibility decreased. In addition, the meal effect for glucose oxidation was
maintained in ET individuals but decreased in sedentary individuals after the HFD; thus, sedentary individuals demonstrated a blunted response to insulin-stimulated conditions after the HFD. Using similar techniques as the present study to measure skeletal muscle substrate oxidation, Lund et al.\textsuperscript{152} recently reported lower metabolic flexibility and glucose oxidation in cultured myotubes from sedentary individuals (VO2max ≤46 ml/kg/min) compared with ET individuals (VO2max >60 ml/kg/min); however, Lund et al. did not control dietary intake. The pyruvate oxidation measure used in the present study is indicative of pyruvate dehydrogenase activity, which regulates the entry of acetyl-CoA generated from glycolysis-derived pyruvate into the TCA cycle.\textsuperscript{153} The pyruvate dehydrogenase complex serves as a key link among β-oxidation, glycolysis, and the TCA cycle.\textsuperscript{154} In the present study, ET individuals had higher pyruvate oxidation than sedentary individuals, suggesting greater acetyl-CoA derived from glucose during the fasted-to-fed transition of the high fat meal challenge. Taken together, these findings overall suggest that sedentary behavior coupled with dietary choices high in fat, particularly saturated fat, may reduce the ability of skeletal muscle to quickly and adequately adjust substrate oxidation to match substrate availability.

To establish energy balance, macronutrient oxidation will eventually match macronutrient intake;\textsuperscript{66} however, the time in which oxidation rates and fat availability remain mismatched may be a critical period of positive fat balance in which intramyocellular lipid species may accumulate. Lipotoxicity resulting from this ectopic accumulation negatively affects insulin signaling.\textsuperscript{41,155} Increased
rates of fat oxidation during aerobic exercise bouts\textsuperscript{57,120,124,156} and in the postabsorptive state\textsuperscript{59,84,85,126} may reduce lipotoxicity and the downstream deleterious effects on insulin signaling. In the present study, there was an overall trend toward a HFD-induced reduction in meal effect for total fat oxidation. Fasting total fat oxidation increased in response to the HFD in the ET but not sedentary group. These findings suggest there may be divergent effects of a HFD between individuals who are sedentary and ET. In ET individuals, the trend toward a decrease in meal effect for total fat oxidation could be a result of the increase in fasting fat oxidation coupled with no absolute change in postprandial fat oxidation; in other words, if the absolute rate of postprandial fat oxidation remained constant, the increase in fasting fat oxidation was driving the decreased meal effect observed during the fasted-to-fed transition. Conversely, the lack of change in fasting fat oxidation in the sedentary group may indicate that the meal effect trend observed was due to reduced postprandial fat handling and oxidation. Therefore, while ET individuals did not have altered fat handling during the high fat challenge meal, sedentary individuals had a reduced capacity for fat oxidation to handle an acute fat load. Others have also noted an increase in basal fat oxidation as a result of endurance exercise training.\textsuperscript{86,139,157} ET individuals in the present study continued their usual exercise routine throughout the duration of the study and refrained from exercise for 36 hours prior to each high fat challenge session. Therefore, these data suggest that the ability to adjust substrate oxidation preference to match dietary intake, or to remain metabolically flexible, in the face of a short-term HFD may depend on the ability to increase
postabsorptive fat oxidation and fat oxidation during acute aerobic exercise bouts rather than during the postprandial response. Dube et al.\textsuperscript{157} reported an increase in fat oxidation in ET but not sedentary individuals during a hyperinsulinemic-euglycemic clamp with lipid infusion; however, differences in methodologies between a lipid infusion and a high fat meal may explain differing observations between that study and the present study. Blaak et al.\textsuperscript{14} reported a reduction in postprandial whole-body fat oxidation after a high fat challenge meal in obese, insulin resistant individuals. The study also reported a significantly lower physical activity level in obese individuals compared with individuals of normal BMI,\textsuperscript{14} which suggests that sedentary behavior could be a contributing factor to the reduction in postprandial fat oxidation observed.

The trend in incomplete fat oxidation follows a similar pattern as total fat oxidation, in that the meal effect trended toward a decrease due to the HFD in the overall sample and that fasting rates increased in ET but not sedentary individuals in response to the HFD. Since total fat oxidation is the sum of complete and incomplete fat oxidation, these findings suggest that the observations in total fat oxidation are primarily due to incomplete fat oxidation. Complete fat oxidation did not change in either group as a result of the HFD. Increased incomplete fat oxidation occurs when the rate of $\beta$-oxidation of fatty acids is higher than the rate of substrate flux through the TCA cycle, and incomplete fat oxidation results in increased concentrations of acylcarnitines and lipid intermediates.\textsuperscript{43,158} Increased intramuscular acylcarnitine concentrations have been suggested as a link between aberrant mitochondrial metabolism and
IR in states of obesity and T2DM, but it remains unclear whether these metabolites interact with proinflammatory pathways to influence insulin signaling or are simply biomarkers of mitochondrial oxidative stress.\textsuperscript{43,158} Counterintuitively, fasting incomplete fat oxidation was higher in the ET compared with the sedentary group after the HFD and increased in response to the HFD compared with baseline in the ET group. These observations are in agreement with others who have reported similar increases in fasting incomplete fat oxidation, as well as acylcarnitine content, in trained individuals.\textsuperscript{159-161} These results seem to be in conflict with the notion that incomplete fat oxidation is linked with IR.\textsuperscript{43,162} Huffman et al.\textsuperscript{159} reported that acylcarnitine content in the fasted state in skeletal muscle was the strongest biomarker for trained state and that acylcarnitine content changed concomitantly with changes in expression of genes regulating fat uptake and transport into the mitochondria. Short-chain acylcarnitines synthesized by carnitine acetyltransferase have been associated with reduced acetyl-CoA inhibition of pyruvate dehydrogenase and improved metabolic flexibility.\textsuperscript{153} Therefore, increased incomplete fat oxidation in the fasted state may simply indicate an increased capacity for fatty acid handling and a promotion of metabolic flexibility. This remains speculative in the present study, however, as acylcarnitine concentrations were not measured.

Fasting citrate synthase activity was higher in ET individuals compared to sedentary individuals at baseline. There was a significant interaction between the HFD and physical activity level on meal effect citrate synthase activity. Citrate synthase activity was maintained in ET individuals in response to the HFD but
was lower in sedentary individuals, following a similar pattern as that of metabolic flexibility. Though not statistically significant, there was a trend toward an interaction between the HFD and physical activity level in meal effect BHAD activity, suggesting a reduced capacity to increase fat oxidation in sedentary individuals. These findings suggest that ET individuals possessed the ability to adjust enzyme activities to adequately handle high fat availability prior the HFD and were able to maintain it, whereas sedentary individuals were not.

The controlled feeding aspect of the study design was a major strength of this study. First, the highly controlled feeding and daily body weight measurements ensured compliance and weight stability in the present study. Thus, participants were in energy balance at the time substrate oxidation measurements were taken. Second, a 10-day standardized diet was used, which ensured that participants’ baseline substrate oxidation measures were in response to the same substrate availability provided by dietary intake. In addition, the focus on metabolically healthy participants allowed for assessment of effects related specifically to physical activity behaviors (i.e., the sedentary or ET state), and testing sessions occurred at least 36 hours after the last bout of exercise for ET individuals, ensuring the observed effects were a result of habitual training and not acute effects of exercise. A novel aspect of the present study was the use of both a HFD and high fat challenge meal to assess adaptations in substrate oxidation. Substrate oxidation and metabolic flexibility are commonly assessed during hyperglycemic-euglycemic clamps with and without a lipid infusion. However, this technique may not yield an appropriate
view of true physiological responses to substrate metabolism due to bypassing the digestive tract and the potential influence of gut microbiome-derived metabolites or hormones associated with digestive and absorptive processes.

A few limitations should be considered. The sample size of the present study was small and limited to males aged 18-40 years. As such, generalizability of our findings may be limited. In addition, homogenization of tissue prior to substrate oxidation measurement potentially limits the ability to account for substrate trafficking since membranes are destructed during homogenization. Although skeletal muscle comprises a large fraction of total body mass and plays an important role in postprandial nutrient metabolism, the degree to which our ex vivo measurements are reflected in whole body substrate oxidation is unclear.

**Conclusion**

In summary, we observed that metabolic flexibility and glucose oxidation were maintained from the fasted-to-fed transition after a 5-day HFD in skeletal muscle of ET individuals. However, metabolic flexibility and glucose oxidation decreased in skeletal muscle of sedentary individuals in response to 5 days of high dietary fat intake. In addition, total fat oxidation and incomplete fat oxidation tended to decrease in response to the HFD in both ET and sedentary individuals; however, ET individuals had increased fasting total and incomplete fat oxidation after the HFD, whereas sedentary individuals did not. Therefore, the findings of the present study suggest increased fasting fat oxidation may be a primary contributor to the ability of ET individuals to readily adapt macronutrient oxidation to match a short-term HFD. In addition, the increase in fasting fat oxidation in ET
individuals may be a result of increased incomplete, rather than complete, fat oxidation.

Future research should consider the assessment of changes in skeletal muscle substrate oxidation during a bout of exercise after a similar short-term HFD to further elucidate potential differences in fat oxidation incurred by a bout of exercise that may not be detected in the resting condition. Some studies have suggested that dietary fat type influences differing patterns of fat trafficking, storage, and utilization;\textsuperscript{38,92,93,95,163} therefore, future studies should consider the effects of dietary fat composition, and its interaction with habitual physical activity level, substrate oxidation. In addition, future research is needed to understand the impact of sex and age on skeletal muscle substrate oxidation following diets of varying macronutrient composition.
The main purpose of this study was to determine substrate oxidation response to a HFD in sedentary and ET individuals. Previous studies have commonly assessed skeletal muscle substrate oxidation response to high fat availability either in the fasted state after a dietary intervention or utilizing a hyperinsulinemic-euglycemic clamp with lipid infusion. The use of both a HFD and a meal challenge paradigm was a novel approach in the present study. We observed a reduction in metabolic flexibility and blunted glucose oxidation during the fasted-to-fed transition in response to the HFD in sedentary individuals. ET individuals, however, were able to maintain metabolic flexibility and glucose oxidation despite the same HFD. The HFD resulted in a trend toward reduced total and incomplete fat oxidation in both sedentary and ET individuals; however, fasting total and incomplete fat oxidation increased in response to the HFD in ET individuals but were not changed in sedentary individuals. This suggests that the reduction in fat oxidation induced by the HFD may be a result of different mechanisms in sedentary and ET individuals. The findings of this study provide a basis for future work targeting the elucidation of these mechanistic differences in substrate oxidation between sedentary and ET conditions.

Assessing substrate oxidation in sedentary and ET individuals undergoing the same controlled dietary intervention provided a unique perspective of adaptations in substrate oxidation across the physical activity spectrum. Historically, studies with exercise interventions have utilized sedentary behavior
as a control condition, as it resembles physical activity patterns typical of a Western population; however, from an evolutionary perspective, sedentary behavior is a recent attribute of human society. It could be argued that a physically active or ET state is a more evolutionarily appropriate “healthy” control condition for assessing metabolic adaptations like altered substrate oxidation.\textsuperscript{164} To date, a majority of studies assessing the effects of endurance exercise on measures of substrate oxidation and metabolic flexibility have utilized endurance training interventions to improve metabolic flexibility in individuals who demonstrate metabolic inflexibility at baseline.\textsuperscript{59,85,86,112,126,139,143} It is possible that the metabolic inflexibility associated with obese or insulin resistant individuals\textsuperscript{11,14,16,18} is at least partially a result of sedentary behavior. The findings of the present study support this notion.

This work focused exclusively on \textit{ex vivo} substrate metabolism in skeletal muscle; however, metabolic and hormonal interactions among organs and tissues, including the liver, adipose tissue, gut, and pancreas, play an important role in understanding mechanisms that regulate whole-body substrate oxidation. For example, adiponectin released from adipose tissue has been shown to increase fat oxidation in skeletal muscle.\textsuperscript{165} Therefore, studies designed to assess these interactions are necessary. In addition, the degree to which skeletal muscle metabolism reflects whole-body substrate oxidation is not known. Future studies should consider methods to corroborate \textit{ex vivo} substrate oxidation in skeletal muscle with whole-body indirect calorimetry measures.
The present study focused solely on substrate oxidation in young males, but potential sex differences in substrate oxidation preference under various conditions, including exercise, should be investigated. Most studies including ET individuals have either exclusively studied males or include a disproportionately larger sample of males than females. Thus, conclusions made from these studies may be limited. In general, females have higher IMTG concentrations at rest than males regardless of training status\textsuperscript{166} and are more insulin sensitive than males of similar fitness levels.\textsuperscript{111} In addition, it is possible that menstrual cycle phase plays an important role in skeletal muscle lipid content, which could potentially influence insulin signaling, but this remains unknown.\textsuperscript{111} Therefore, these characteristics suggest there may be sex differences in substrate oxidation preference.
REFERENCES


APPENDIX

Approved Institutional Review Board Research Protocol
MEMORANDUM

DATE: September 27, 2012

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Rivero M.D.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires May 31, 2014)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective September 21, 2012, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: September 21, 2012
Protocol Expiration Date: September 20, 2013
Continuing Review Due Date*: August 26, 2013

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.
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<thead>
<tr>
<th>Date*</th>
<th>OSP Number</th>
<th>Sponsor</th>
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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: August 13, 2013

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Rivero M.D.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires April 25, 2018)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective August 12, 2013, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: September 21, 2012
Protocol Expiration Date: September 20, 2013
Continuing Review Due Date*: August 26, 2013

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.
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<td>American Diabetes Association</td>
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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: October 14, 2013

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Rivero M.D.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires April 25, 2018)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective October 14, 2013, the Virginia Tech Institutional Review Board (IRB), at a convened meeting, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: October 14, 2013
Protocol Expiration Date: October 13, 2014
Continuing Review Due Date*: September 29, 2014

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.
### Date* | OSP Number | Sponsor | Grant Comparison Conducted?
--- | --- | --- | ---
03/06/2013 | 13012307 | American Diabetes Association | Not required (Not federally funded)

* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: October 13, 2014

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Rivero M.D.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires April 25, 2018)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective October 13, 2014, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: October 14, 2014
Protocol Expiration Date: October 13, 2015
Continuing Review Due Date*: September 28, 2015

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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<td>03/06/2013</td>
<td>13012307</td>
<td>American Diabetes Association</td>
<td>Not required (Not federally funded)</td>
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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: September 23, 2015

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Rivero M.D.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires July 29, 2020)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective September 23, 2015, the Virginia Tech Institution Review Board (IRB) Chair, David M Moore, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: October 14, 2015
Protocol Expiration Date: October 13, 2016
Continuing Review Due Date*: September 26, 2016

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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<td>American Diabetes Assoc</td>
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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: March 16, 2016

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Rivero M.D., Elaina Lynn Marinik, Mary Elizabeth Baugh, James B Grubb

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective March 14, 2016, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

http://www.irb.vt.edu/pages/responsibilities.htm

(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: October 14, 2015
Protocol Expiration Date: October 13, 2016
Continuing Review Due Date*: September 26, 2016

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: October 14, 2016

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Manuel Rivero, Elaina Lynn Marinik, Mary Elizabeth Baugh, Loren Ashley Weldon, Kristin Osterberg, Nabil E. Boutagy, et. al.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective October 10, 2016, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:
http://www.irb.vt.edu/pages/responsibilities.htm
(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: October 14, 2016
Protocol Expiration Date: October 13, 2017
Continuing Review Due Date*: September 25, 2017

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: December 1, 2016

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Manuel Rivero, Elaina Lynn Marinik, Mary Elizabeth Baugh, Loren Ashley Weldon, Kristin Osterberg, Nabil E. Boutagy, et. al.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective December 1, 2016, the Virginia Tech Institution Review Board (IRB) Chair, David M Moore, approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:
http://www.irb.vt.edu/pages/responsibilities.htm
(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: October 14, 2016
Protocol Expiration Date: October 13, 2017
Continuing Review Due Date*: September 25, 2017

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal/work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.
MEMORANDUM

DATE: November 2, 2017

TO: Kevin Davy, Brenda Davy, Matthew Wade Hulver, Madlyn Irene Frisard, Jose Manuel Rivero, Elaina Lynn Marinik, Mary Elizabeth Baugh, Loren Ashley Weldon, Kristin Osterberg, Nabil E. Boutagy, et. al.

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires January 29, 2021)

PROTOCOL TITLE: Effect of High Fat Diet on Muscle Metabolism

IRB NUMBER: 06-367

Effective October 9, 2017, the Virginia Tech Institution Review Board (IRB), at a convened meeting, approved the Continuing Review request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

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(Please review responsibilities before the commencement of your research.)

PROTOCOL INFORMATION:

Approved As: Full Review
Protocol Approval Date: October 14, 2017
Protocol Expiration Date: October 13, 2018
Continuing Review Due Date*: September 24, 2018

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:

Per federal regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals/work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

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<td>03/06/2013</td>
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<td>American Diabetes Association (Title: Pro-Inflammatory Response and Metabolic Infeasibility in Skeletal Muscle)</td>
<td>Not required (Not federally funded)</td>
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* Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.