

Substrate Availability and Utilization During Sub-Maximal Exercise in the Elderly

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Aging is associated with a decline in physiological capacity and metabolic function, leading to increased risk for chronic conditions such as insulin resistance and diabetes. Reduced ability to utilize fatty acids in the elderly has been linked to increased fat deposition within skeletal muscle. However, intramuscular triglyceride (IMTG) content and its relation to substrate oxidation has not been clearly established in older individuals. The two main purposes of the present investigation were 1.) to compare IMTG stores in the elderly with a younger group of men and women and 2.) to examine the relationship between substrate content and utilization during an acute sub-maximal bout of exercise in the elderly. A total of thirteen sedentary, healthy older men (5) and women (8) aged 67 ± 3 yrs were matched for gender and percent body fat with a younger, control group (age= 39 ± 6 yrs) for IMTG comparison. Fat free mass (FFM), body mass index (BMI), weight, and height were not significantly different between the old and young and Oil Red O (ORO) staining from vastus lateralis muscle obtained by percutaneous needle biopsy revealed no statistically significant differences between the two groups. Sixteen sedentary elderly men (5) and women (11) were given constant infusions of ^{13}C palmitate and d_2 glucose during 60 minutes of cycle ergometry exercise at $\sim 58\%$ VO_2peak to assess substrate utilization during exercise. There was a significant negative correlation between IMTG content by ORO staining and total fatty acid oxidation ($\rho = -0.5989$; $p = 0.03$) and IMTG content and oxidative capacity of muscle ($\rho = -0.6485$; $p = 0.04$) induced by sub-maximal exercise.

Oxidative capacity of muscle was not associated with rates of NPFA oxidation during sub-maximal exercise. Fitness was marginally correlated with fasting insulin levels ($\rho = -0.4857$; $p = 0.056$) and HOMA IR ($\rho = -0.4441$; $p = 0.085$). In summary, IMTG content was not higher in this group of sedentary elderly compared to younger individuals, but higher IMTG content was associated with decreased fat oxidation during sub-maximal exercise in this group. In addition, higher fitness predicted more favorable trends for markers of insulin resistance.

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PREFACE

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One of the things I have learned from this experience is perseverance. With research, as with running, and life, there is not always instant gratification or gold medal success. It takes patience, time, and commitment. This pursuit lends credence to the saying:

"The race is not always to the swift... but to those who keep on running. "—Unknown

Life, like my profession, is a work-in-progress. I have learned the importance of and will continue to strive for balance in life. Without balance, there is regret.

Finally, I dedicate this work to my beautiful sons Andrew and Alex, who inspire me daily to become the best complete person I can be. Thank you boys, I love you.

1. Chapter One

1.1 Introduction

As humans age, there is a decline in physiological capacity and function, which can lead to increased risk for chronic disease and decreased quality of life. Exercise may decrease or reverse this age-related decline. It has been shown that both the quantity and quality of skeletal muscle decreases with aging. This has important implications with regard to oxidative metabolism in the elderly, as decreases in fatty acid metabolism have been linked to obesity and insulin resistance in younger adults.

Lipids constitute a major source of energy for skeletal muscle metabolism. Free fatty acids (FFA) account for a large portion of energy used during low to moderate exercise (Romijn 1993). The metabolism of FFA's for use at rest or during exercise is an intricate process that relies on FFA release from adipose tissue (lipolysis), transport of FFA in the plasma, diffusion across the cell membrane, transport within the muscle cell, and subsequent oxidation within the mitochondria. Perturbation of any number of these factors might alter fat oxidation.

An inability to utilize fatty acids for energy could have deleterious metabolic consequences. Reduced rates of fat metabolism have been associated with increased adiposity, increased insulin concentrations, and insulin resistance in middle-aged adults (Felber, Ferrannini et al. 1987; Zurlo, Lillioja et al. 1990; Groop, Saloranta et al. 1991; Mandarino, Consoli et al. 1996; Kelley, Goodpaster et al. 1999; Bavenholm, Pigon et al. 2000; Horowitz 2001).

Aging may also be associated with reduced ability to utilize fatty acids (Coggan, Spina et al. 1992; Proctor, Sinning et al. 1995; Sial, Coggan et al. 1996). Perhaps the increases in FM and reduction in muscle size and function seen in the elderly are associated with this decrease in oxidative metabolism.

Recent evidence suggests that intramuscular triglyceride (IMTG) may link impaired fatty acid metabolism and insulin sensitivity (Phillips, Green et al. 1996; Goodpaster, Thaete et al. 1997; Pan, Lillioja et al. 1997). Although it has been consistently shown that FM increases with age, whether IMTG stores are altered in the elderly has not been established. Since lower attenuation values within skeletal muscle have been linked to increased fatty deposition within the muscle (Goodpaster, Kelley et al. 2000), this suggests that increased lipid content within skeletal muscle may be associated with poor muscle function in the elderly.

Reduced rates of fatty acid metabolism have been linked to increased fat deposition within skeletal muscle (Kelley, Goodpaster et al. 1999). Increased IMTG stores in sedentary individuals have been associated with obesity and insulin resistance (Goodpaster, Thaete et al. 1997; Pan, Lillioja et al. 1997; Ebeling, Essen-Gustavsson et al. 1998; Goodpaster, Kelley et al. 1999; Krssak, Falk Petersen et al. 1999; Manco, Mingrone et al. 2000). Goodpaster et al (Goodpaster, Thaete et al. 1997) demonstrated in lean and obese individuals that increased lipid content in muscle by computed tomography (CT) was associated with obesity and decreased insulin sensitivity.

Although higher IMTG stores in sedentary individuals are associated with obesity and insulin resistance, it has been reported that highly trained endurance athletes have increased levels of IMTG, but are also very insulin-sensitive (Goodpaster, He et al. 2001). The reasons behind this inconsistency are not fully understood. However, exercise may mediate this increased insulin sensitivity with high IMTG content in trained individuals by repeated depletion and storage of triglyceride within the muscle.

It has recently been proposed that IMTG stores are utilized to fuel working muscles (Phillips, Green et al. 1996), especially with exercise training (Hurley, Nemeth et al. 1986;

Martin, Dalsky et al. 1993; Romijn 1993). An investigation by Hurley et al (Hurley, Nemeth et al. 1986) found that 12 weeks of endurance training increased caloric expenditure of fat derived from muscle triglyceride. Endurance training may also provide similar benefits in the elderly. Therefore, studies addressing the role of IMTG stores in the elderly are warranted.

Mechanisms by which exercise increase lipid metabolism include increases in lipolysis (Wahrenberg, Engfeldt et al. 1987), FFA transport across the cellular membrane (Kiens, Essen-Gustavsson et al. 1993), increased mitochondrial enzyme capacity (Gollnick and Saltin 1982), and increased utilization of IMTG (Martin, Dalsky et al. 1993; Maggs, Jacob et al. 1995).

Stable isotope infusion offers one mechanism by which fat metabolism can be indirectly measured (Wolfe 1998). Lipolysis results in the hydrolysis of a triglyceride molecule into glycerol and 3 fatty acids. Measures of the rate of appearance (Ra) and rate of disposal (Rd), or oxidation of labeled glycerol and palmitate can provide evidence of the contribution of fat utilized for energy at rest and during exercise.

A number of studies have used stable isotope infusion to quantify glycerol and FFA release and oxidation during rest and exercise (Martin, Dalsky et al. 1993; Romijn 1993; Bonadonna, Groop et al. 1994; Phillips, Green et al. 1996; Sidossis 1998; Kelley, Goodpaster et al. 1999). Glucose tracers are also used to allow the quantification of carbohydrate oxidation. Romijn et al. (Romijn 1993) utilized stable isotope methods to measure the contribution of fat versus carbohydrate oxidation at different exercise intensities and durations in trained cyclists.

Substrate metabolism can also be measured by indirect calorimetry. Indirect calorimetry permits quantification of fat and carbohydrate oxidation by measuring oxygen consumption and carbon dioxide production from expired air samples (Frayn, Lund et al. 1993). Many studies have utilized this non-invasive technique to quantify fat to carbohydrate oxidation during

exercise. Indirect calorimetry is especially useful in conjunction with isotope measurement to quantify the relative contributions of plasma and non-plasma sources of substrate oxidation.

The decline in physical function with aging has been well documented. Moreover, there are decreases in fat free mass (FFM), and oxidative metabolism (Coggan, Spina et al. 1992; Proctor, Sinning et al. 1995; Sial, Coggan et al. 1996; Imbeault, Prud'Homme et al. 2000; Tessari 2000), as well as increased fat mass (Meredith, Frontera et al. 1989; Sial, Coggan et al. 1996; Tremblay, Drapeau et al. 1998; Tessari 2000); including fat within the muscle (Goodpaster, Carlson et al. 2001) with aging. A study by Sial et al. (Sial, Coggan et al. 1996) found lower fat oxidation in the elderly when compared to young adults during aerobic exercise of the same relative or absolute exercise intensity. Mechanisms governing this decline in muscle function are of great importance in the prevention or reversal of impaired muscle function in the elderly.

Exercise could have the potential to ameliorate the decline in fat metabolism associated with aging, as seen in younger populations.

1.1.1. Rationale

It has been established that aging is associated with a decline in numerous physiological and metabolic parameters. These parameters may be dictated by physiological decline associated with aging per se or to the adoption of a more sedentary lifestyle in older age.

Changes in skeletal muscle are likely implicated as the culprit in the decline of fat oxidation in the elderly (Seals, Hagberg et al. 1984; Coggan, Spina et al. 1992; Proctor, Sinning et al. 1995; Sial, Coggan et al. 1998; Schunk, Pitton et al. 1999; Imbeault, Prud'Homme et al. 2000). It has been shown that increases in fat mass are associated with impaired fatty acid metabolism. It is possible that increased FM with aging can lead to changes in function of muscle oxidative potential, leading to the decrements in fatty acid metabolism seen in the elderly.

Increases in IMTG stores have been associated with impaired fatty acid metabolism and insulin resistance (Goodpaster, Thaete et al. 1997; Goodpaster, Carlson et al. 2001; Goodpaster, He et al. 2001). However, IMTG stores related to these parameters in the elderly have not been established.

1.1.2. Significance

Increased IMTG stores and reduced fatty acid metabolism are associated with risk factors identified in the development of metabolic diseases such as obesity and Type II diabetes. Identifying characteristics that are associated with these risks in elderly individuals are paramount to prevent or reduce the onset or severity of these conditions.

1.1.3. Statement of the Problem

The purpose of this investigation was to: 1) compare skeletal IMTG stores in the elderly and younger men and women 2) examine the association between substrate availability and utilization during an acute sub-maximal bout of exercise in the elderly 3) and determine if oxidative capacity of muscle in the elderly is associated with rates of non-plasma fatty acid (NPFA) utilization during exercise.

1.1.4. Hypotheses

It was hypothesized that:

1. Sedentary elderly men and women would have higher IMTG stores compared to sedentary younger adults of similar % body fat.
2. Among the elderly, higher oxidative capacity, but not IMTG stores in muscle would be associated with higher rates of non-plasma fatty acid (NPFA) oxidation during moderate intensity exercise.

2. Chapter Two

2.1 Physiological Alterations with Aging

Aging is associated with a decline in physiological capacity. Declines in muscle mass, muscular strength, muscular endurance, VO_2 peak, and cardiovascular function are associated with increased age as well as a sedentary lifestyle (Grimby 1995; Lexell 1995; Bembien 1998; Tremblay, Drapeau et al. 1998; Holloszy 2000). These declines lead to decreases in function and quality of life in the elderly.

2.1.1 Body Composition and Muscle Quality Changes with Aging

It is well documented that loss of skeletal muscle mass, or sarcopenia, occurs with aging (Lexell 1995; Evans 1997; Roubenoff 2000). It has been suggested that aging results in approximately 15-40% decrease in muscle cross-sectional area, depending on the muscle measured (Aniansson, Hedberg et al. 1986; Grimby 1995; Lexell 1995; Forbes 1999; Frontera W. 2000; Hebuterne 2001; Landers 2001). Decreases in skeletal muscle mass have been associated with muscle weakness and lower functional capacity. In addition to decreases in skeletal muscle mass, increases in fat mass occur with aging. It has been proposed that decreases in muscle mass lead to increased fat accumulation, which supports further muscle loss. According to Roubenoff (Roubenoff 2000), preliminary loss of muscle with aging results in a cascade of events which facilitates the further loss of muscle by 1) increasing fat mass, thereby 2) increasing the release of cytokines known to cause insulin resistance, thereby 3) facilitating additional fat mass accumulation.

A decreased percentage of FFM and an increased percentage of FM have been associated with functional disability in elderly women and men. Age-related sarcopenia may be caused not only by reduced muscle mass or quantity, but also a reduction in muscle quality in the elderly

(Broadwin 2001; Goodpaster, Carlson et al. 2001). Recent technological advancements, including computed tomography (CT), have allowed for more precise measurements of body composition (Szczepaniak 1999; Goodpaster, Thaete et al. 2000; Ross 2000; Goodpaster, Carlson et al. 2001) to allow not only quantitative information about fat versus fat-free mass, but also fat within the muscle, providing qualitative information about the muscle being studied. Several investigators have found increases in fat tissue accumulation in muscle of older individuals when compared to their younger counterparts (Rice 1989; Overend 1992; Goodpaster, Carlson et al. 2001) using CT. Although promising, methodology for these measurement tools is still being developed.

The importance of elucidating fat content in muscle is derived from the association of high fat content within skeletal muscle to a number of unfavorable conditions including insulin resistance, impaired fatty acid metabolism, and reduced strength (Goodpaster, Thaete et al. 1997; Goodpaster, Carlson et al. 2001; Goodpaster, He et al. 2001). These body composition changes have major implications for the elderly with respect to disease risk and quality of life. It is unclear whether these changes in muscle mass and composition are an inevitable result of the aging process, or are mediated by modifiable factors, such as physical activity.

2.1.2 Muscle Structure and Function Changes with Aging

Aging results in various skeletal muscle structural and functional changes. In relation to reduced cross-sectional area of muscle found in aging muscle, decreases in the number of muscle fibers per unit of muscle, altered muscle fiber ratios and genetic expression, reduced muscle capillarization and enzyme activity, and reduced skeletal muscle oxidative capacity in aging skeletal muscle has been shown (Essen-Gustavsson 1986; Coggan, Spina et al. 1992; Coggan 1993; Grimby 1995; Lexell 1995; Bembien 1998; Jozsi 2000; Kent-Braun and Ng 2000).

Using muscle morphology techniques from muscle biopsy samples, muscle fiber type and ratio, muscle lipid content, muscle capillarization and enzyme activity, and oxidative activity can be measured. Muscle biopsy samples are taken primarily from the vastus lateralis of the quadriceps muscle, but other muscles such as the gastrocnemius or biceps brachii have been studied. It is important to note that skeletal muscle fiber type and ratio varies depending on the muscle studied, so caution should be exercised when comparisons are made between muscle samples from different locations in the body (J. Keul 1972; Squire 1986).

A study by Coggan et al. (Coggan, Spina et al. 1992) found decreased muscle fiber area in gastrocnemius muscle of elderly sedentary men (64 ± 1 yrs) and women (63 ± 1 yrs) when compared to the younger men (26 ± 1 yrs) and women (23 ± 1 yrs) studied. Histochemical comparisons of the two groups studied revealed significantly smaller type IIa and type IIb fiber areas in the elderly group when compared to the younger group, with no differences in type I fiber area. These reductions in both type II fibers reduced the type IIa/type I and type IIb/type I ratios for this group. No differences in the proportions of the fibers types were seen between the young and old groups.

The literature is relatively coherent with respect to fiber size reduction in the elderly. On average, type II fiber size is reduced an average of 20-50%, whereas type I fibers average a 1-25% loss (Coggan, Spina et al. 1992; Grimby 1995; Lexell 1995; Doherty 2001). Recent investigations measuring entire cross-sections of cadaver vastus lateralis muscle have shown, in addition to reduction of fiber size, an approximately 50% reduction in number of both type I and type II fibers of 90 year olds, when compared to 20 year olds. These alterations in aging muscle lead to further declines in physiological function and quality of life in the elderly population.

2.1.3 Substrate Metabolism with Aging

When compared to their younger counterparts, elderly individuals have been shown to exhibit lower rates of fat oxidation during exercise in several studies (Essen-Gustavsson 1986; Coggan 1993; Sial, Coggan et al. 1996; Kent-Braun and Ng 2000). A study by Sial et al. (Sial, Coggan et al. 1996) found lower (25-35%) fat oxidation in the elderly when compared to young adults during aerobic exercise of the same relative or absolute exercise intensity. In addition to lower fat oxidation, carbohydrate oxidation was increased in this group. The authors attributed the switch in substrate metabolism seen in the elderly to age-related changes in skeletal muscle mitochondrial respiratory capacity. These results are in support of other studies reporting similar decline (Meredith, Frontera et al. 1989; Coggan, Spina et al. 1992; Coggan, Spina et al. 1993; Proctor, Sinning et al. 1995; Kent-Braun and Ng 2000) in muscle mitochondrial respiratory capacity. The ability of older individuals to utilize IMTG is not known. Although the subjects in this study were said to be physically active (although not participating in any organized exercise program) and matched for gender, height, weight, and lean body mass, the younger subjects had a significantly higher fitness level than the older subjects.

Reduced endurance capacity in the aged is supported by reductions in oxidative capacity and blood flow in muscle as seen in several investigations. Enzymatic analysis of muscle biopsy samples taken from sedentary older man and women (Coggan, Spina et al. 1992) revealed an average 25% reduction in the mitochondrial marker enzymes succinate dehydrogenase (SDH), citrate synthase (CS), and β -hydroxyacyl-CoA dehydrogenase. There were a few sex differences in enzymatic activities of older muscle in this study. Older women exhibited lower succinate dehydrogenase and citrate synthase activities than older men. Glycolytic enzyme activity was not reduced in the elderly individuals in this study. In addition, capillary density, number, and capillary-to-fiber ratios were also reduced in this group of older individuals when compared to

the younger group studied. This investigation provides evidence that older sedentary individuals have reduced mitochondrial enzyme activity and muscle capillarization.

Animal studies substantiate decreases in endurance capacity in older versus younger rats. A review by Cartee et al. (Cartee 1994) proposed that the reduction in muscle endurance seen in older rats due to reduced blood flow and muscle oxidative capacity is the result of increased reliance of glucose metabolism during exercise.

A few studies have found no differences in skeletal muscle oxidative capacity in the elderly (Grimby, Danneskiold-Samsoe et al. 1982; Aniansson, Hedberg et al. 1986; Grimby 1995). It has been reported that the differences seen with these investigations on Swedish elderly populations may be due to the lack of control of physical activity patterns (they were possibly quite physically active) and lack of a young control group, making comparisons difficult.

Age-associated changes in body composition, muscle structure and function, and substrate metabolism have been associated with muscle weakness, reduced endurance capacity, and diminished quality of life in this population. It is unknown whether the aging process itself, physical inactivity, or a combination of the two is responsible for these changes seen in the elderly.

2.2 Muscle Fat, Obesity, and Diabetes

Obesity and aging are risk factors for the development of type 2 diabetes. It is well known that insulin has powerful effects in the regulation of free fatty acid (FFA) release from adipose tissue. It is also known that obese individuals exhibit insulin resistance, particularly those with visceral obesity or larger quantities of adipose tissue within skeletal muscle (Pan, Lillioja et al. 1997; Belfiore 1998; Kelley, Goodpaster et al. 1999; Goodpaster 2000; Kelley 2001; Kelley 2002).

Recent research has addressed the effects of fat accumulation in skeletal muscle in the development of metabolic disturbances associated with obesity and diabetes. Kelley et al. (Kelley, Goodpaster et al. 1999) found that obese subjects had lower fatty acid oxidation rates during fasting conditions than lean subjects. This disturbance was caused by a decreased oxidative enzyme capacity of skeletal muscle. The obese individuals in this study also had lower attenuation values for skeletal muscle when measured by CT. The lower attenuation values (low-density muscle) observed in this study were negatively correlated with the suppression of fat oxidation by insulin infusion. Therefore, it appears that the fatty deposits within muscle are responsible, in part, for the metabolic disturbances in fatty acid metabolism seen in obesity. How muscle fat influences fatty acid metabolism with aging has not been studied.

Another population with large quantities of fat within skeletal muscle is endurance athletes. Although endurance athletes have high levels of intramuscular fats, they are not insulin-resistant. Goodpaster et al. (Goodpaster, He et al. 2001) found that exercise-trained individuals had a higher oxidative capacity of muscle, despite the high lipid content within the muscle when compared to sedentary lean individuals, obese individuals, or obese type 2 diabetics.

Therefore, the muscle's ability to oxidize fat may be a pivotal factor in relation to the accumulation of lipid in muscle, obesity, insulin resistance, and the development of type 2 diabetes. Similar questions related to metabolic dysregulation in aging have not been addressed.

2.3 Substrate Metabolism During Exercise

During exercise, the two main substrates that provide fuel for exercising muscles are fat and carbohydrate (CHO). The intensity and duration of an activity, as well as the training status of the individual, will dictate which metabolic pathways are utilized.

2.3.1 Fat Oxidation

As mentioned previously, lipids provide a substantial source of energy during low to moderate exercise. Fats in the form of stored adipose tissue or free fatty acids (FFA) are utilized by exercising muscles. Several factors contribute to the utilization of fatty acids as the predominant energy source during exercise, which is influenced with endurance exercise training (Kiens, Essen-Gustavsson et al. 1993; Martin, Dalsky et al. 1993; Turner 1997; Hawley, Brouns et al. 1998).

FFA oxidation has been shown to be highest with low-intensity exercise (25% -65% VO_2max). Research suggests that concomitant changes in FFA availability and reductions in long-chain fatty acid (LCFA) oxidation are responsible for the decreased reliance of FFA as a fuel with increasing intensity (Kanaley 1995; Sidossis, Gastaldelli et al. 1997; Hawley, Brouns et al. 1998; Sidossis, Wolfe et al. 1998). LCFA depend on an enzyme-mediated transport (CPT-1) across the mitochondrial membrane, whereas medium-chain fatty acids can diffuse freely. An investigation by Sidossis et al (Sidossis, Gastaldelli et al. 1997) examined the regulation of fatty acid oxidation during exercise at 40% and 80% VO_2peak using isotopic tracers [$1\text{-}^{13}\text{C}$] oleate (LCFA) and [$1\text{-}^{14}\text{C}$] octanoate (MCFA). Results showed decreased oleate oxidation and increased octanoate oxidation during exercise at 80% VO_2peak , when compared to 40% VO_2peak , suggesting that fat oxidation was diminished due to inhibition of LCFA into the mitochondria. This inhibition of LCFA into the mitochondria appears to be mediated by the actions of glucose and insulin (Sidossis 1996; Sidossis 1998; Sidossis, Wolfe et al. 1998).

IMTG oxidation appears to be low during low-intensity exercise and highest with moderate (65% VO_2peak) or high-intensity (85% VO_2peak) exercise (Romijn 1993).

In addition to intensity, the duration of exercise has an impact on substrate utilization. In another study using stable isotope tracers, a reduction in glucose release and oxidation and an

increase in total fat oxidation was seen with prolonged endurance training in previously active, but untrained males during 120 minutes of cycle ergometry at 60% VO_2peak at 31 days of training. The shift to fat oxidation with increasing exercise duration may also be related to depletion of blood glucose and glycogen stores in the muscle.

When measured with isotopic tracers, it is evident that although total body fat metabolism is increased following training, free fatty acid metabolism is actually lower. Due to this discrepancy, it has been suggested that the increased fat metabolism seen with training is a result of oxidation of non-plasma fatty acids (NPFA), most likely in the form of IMTG (Hurley, Nemeth et al. 1986; Martin, Dalsky et al. 1993; Kanaley 1995; Andersson 2000).

2.3.2 Role of IMTG

IMTG oxidation is greater in trained individuals than untrained individuals, and appears to increase significantly with exercise training (Hurley, Nemeth et al. 1986; Martin, Dalsky et al. 1993; Romijn 1993; Kanaley 1995; Sial, Coggan et al. 1998; Andersson 2000). Martin et al. (Martin, Dalsky et al. 1993) found that non-plasma fatty acid (NPFA) depletion increased from 23-47% after aerobic exercise training in previously sedentary, healthy men and women. The authors speculated that the source of these NPFA was IMTG. Another study found that fatty acid oxidation exceeded FFA availability during running at, above, and below the lactate threshold (Kanaley 1995). Here again, the authors suggested that the additional fatty acids oxidized during exercise came from IMTG.

Although a number of studies have found depletion of IMTG stores during exercise, a few studies have shown no differences (Kiens, Essen-Gustavsson et al. 1993; Rico-Sanz 2000). One study (Kiens, Essen-Gustavsson et al. 1993) did not find depletion of IMTG during exercise, but found a significant decrease in IMTG during the post-exercise recovery period by muscle biopsy. Trained subjects cycled at varying intensities until exhaustion (50-90% VO_2peak , for an

average of 110 min). Muscle biopsy samples were taken before exercise, following exercise, and at 3, 6, 30, and 42 hours post exercise. No significant differences were seen immediately after exercise, but significant reductions in IMTG were found at 3,6, and 30 hours post exercise. Another study (Rico-Sanz 2000), which also employed exercise at varying intensities until exhaustion, found no differences in pre and post-exercise IMTG with magnetic resonance spectroscopy. This study did not measure post exercise recovery period. It is unclear why this discrepancy exists, but it may be due to exercise protocol.

Romijn et al (Romijn 1993) utilized stable isotope methods to measure the contribution of fat versus carbohydrate oxidation at different exercise intensities and durations in trained cyclists. Tracer methods were then compared to indirect calorimetry data to derive the relative contribution of IMTG and glycogen stores to total fat and carbohydrate oxidation. During low-intensity exercise (25% VO_2 peak) peripheral lipolysis was high, with no further increases with increasing intensity, and IMTG lipolysis was low. During exercise at 65% VO_2 max, IMTG accounted for a considerable percentage of fat utilized. Both muscle glycogen and glucose oxidation increased in relation to increased exercise intensity and changes in fat metabolism reflected changes in CHO metabolism.

IMTG can be measured directly by muscle biopsy, CT, MRI, or MRS; or indirectly by total fat oxidation measurements obtained with isotopic tracers, labeled breath samples, and indirect calorimetry.

2.3.3 Carbohydrate Oxidation

Whereas during rest and low-intensity activities most of the fuel utilized is from fat, exercise at higher intensities of 70-80% VO_2 peak or above result in the preferential shift from fat to carbohydrate (CHO) oxidation (Hawley, Brouns et al. 1998). After the first few minutes of exercise, glucose in the form of muscle glycogen or plasma glucose, is utilized for energy.

Glucose oxidation is increased with increased exercise intensity and duration (Romijn 1993). During prolonged exercise when muscle glycogen and blood glucose levels are depleted, the body can also produce glucose via gluconeogenesis.

As seen with fat metabolism, endurance training alters glucose utilization in muscle. Endurance training causes a decreased muscle glucose uptake for a given absolute workload. In addition, increased gluconeogenesis and increased lactate removal with training have been reported.

Substrate utilization during exercise is a complex process where the human body utilizes both glucose and fatty acids to supply energy to working muscles. The energy demands for a particular task will dictate to what extent each metabolic pathway is used. The specific energy demands of exercise in the elderly warrant further investigation.

2.4 Conclusion

Aging is associated with reductions in physiological capacity and function. Loss of muscle mass and increases in fat mass lead to a number of metabolic disturbances associated with obesity, insulin resistance, and impaired fatty acid metabolism. Although information exists regarding muscle mass loss in the elderly, quantification of fat in muscle of the elderly has not been established. In addition, little information exists regarding the role IMTG plays in substrate oxidation during exercise in the elderly. Elucidating the amount of fat within muscle of the elderly as well as its contribution to substrate oxidation during exercise would lead to a better understanding of the metabolic and functional implications these factors impose on this population. In addition, this information would be useful in the management of at risk individuals through exercise prescription.

Physical inactivity has been associated with many chronic disease states such as obesity, diabetes, heart disease, osteoporosis, and certain cancers. Physical inactivity may play a large role in the time of onset and continuance of the physiological declines seen with aging. Increasing physical activity in the elderly would be a particularly useful strategy to reduce the age-associated physiological and metabolic changes seen in this group.

Chapter 3

3.1 Methods

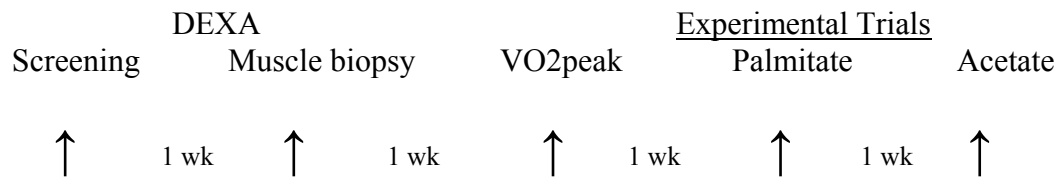
3.1.1 Subjects

Sixteen sedentary men and women between the ages of 65-75 served as subjects for this investigation. Subjects were recruited by flyers and newspaper ads. Exclusion criteria for participation in this study are listed below. Informed, written consent was obtained from all potential volunteers at the screening. After data collection of the elderly subjects was complete, muscle biopsy and DEXA data from a younger, control population was obtained from a database of previously studied individuals for comparison with the elderly subjects. The elderly subjects had the same exclusionary criteria and same sampling protocol for the biopsy and DEXA as the younger, control group.

3.1.2 Experimental Design

This study combined both an observational design (IMTG content) and a cross-sectional experimental design (exercise bout). For comparison of IMTG content between the elderly and younger control groups, the independent variable was age group and the dependent variable was IMTG content. The independent variables for the cross-sectional exercise bout in the elderly were IMTG content, time and intensity. The dependent variable was fat oxidation during exercise. The study design flow is depicted in Table 1.

Table 1: Study Design Flow



After an initial screening to determine eligibility, body composition by DEXA and a muscle biopsy was performed. Each subject then completed an initial VO₂max test, followed by the experimental trial (palmitate) and the acetate recovery trial, both of which consisted of 60 minutes of cycling at 50% VO₂ peak, as determined by the VO₂peak test.

3.1.3 Screening

A thorough medical examination was performed at the screening visit. Exclusionary criteria for this study were primarily related to chronic diseases or medications which may have altered substrate metabolism or otherwise confounded the results of the study. These are as followed:

- A history of diabetes, myocardial infarction or peripheral vascular disease, proteinuria, liver disease, alcohol or drug abuse, malignancy or neuromuscular disease.
- Taking chronic medications known to adversely affect glucose homeostasis (thiazide diuretics, oral glucocorticoids, nicotinic acid, beta blockers).
- Gaining or losing more than 3 kg during the past 6 months.
- A clinically positive VO₂peak test revealing > 2mm ST-segment depression or evidence of CV compensation or a VO₂peak ≥ 55 ml/kg/min fat free mass.
- Participation in any continuous activity lasting >20 minutes more than two times per week.
- Smoking
- A blood pressure of > 150 mm Hg systolic, or > 95 mm Hg diastolic.
- Screening blood laboratory results of anemia (Hct < 34%), elevated liver enzyme (25% above normal), proteinuria, or hypothyroidism (sTSH > 8).

-Findings on physical examination which include cardiac murmurs, diminished pulses or the presence of bruits in the lower extremities, previous history of lower extremity thrombophlebitis, evidence of peripheral neuropathy or muscle wasting, paresis, or edema.

Screening laboratory evaluation included a blood count, general chemistry profile, sTSH, fasting lipid profile and urinalysis. In addition to medical screening, volunteers completed the Modifiable Activity Questionnaire (Pereira 1997) to assess current and past year physical activity patterns.

After the screening visit, subjects were scheduled for an admission to the University of Pittsburgh GCRC to assess initial fitness level, skeletal muscle composition and FFA utilization during exercise. Volunteers were scheduled for a graded exercise test at the Obesity Nutrition Research Center (ONRC) to assess physical fitness (VO_{2peak}) and to determine sub-maximal workloads for the subsequent sub-maximal exercise trials. After the exercise test, subjects were scheduled for an outpatient visit to the GCRC for a DEXA scan to measure total body composition and a muscle biopsy. Approximately one week following this outpatient visit, volunteers were asked to perform two one-hour sub-maximal exercise bouts to assess fat metabolism during exercise.

3.1.4 Physiological Determinants

3.1.4.1 Dual Energy X-Ray Absorptiometry (DEXA):

DEXA was performed to assess whole body composition including total body fat mass and fat free mass. The DEXA divides the body into 3 compartments: lean tissue, fat tissue, and total body mineral. Subjects laid on the DEXA machine, where a full-body X-ray is used to pass high and low photon energy levels through the tissues of the body, which is divided into a 'grid' of pixels. The reduced energy, or attenuation, is measured as it passes through the tissue. Attenuation is dependent of the element composition of the tissue being measured (Gotfredsen

1986). Once the values are obtained, a computer program is used to separate the body into compartments to obtain bone mineral, FFM, and FM for specific sites such as the arms, legs, and trunk.

3.1.4.2 Muscle biopsy:

A needle biopsy of the vastus lateralis muscle was performed using the Bergstrom technique (1), using suction to increase sample size (3). The typical yield is ~ 150 mg wet weight. This method has been utilized in prior studies (11, 12, 23), without complications in > 500 volunteers. This procedure was performed on an outpatient basis approximately one week prior to the first sub-maximal exercise bout. Skeletal muscle lipid content was assessed by histochemical and biochemical methods.

3.1.4.3 Peak Aerobic Power (VO_{2peak}):

Subjects performed a VO_{2peak} test at the ONRC to determine the target work rate (50% of VO_{2peak}) used for the subsequent exercise studies. This test, lasting approximately 8 to 10 min, was conducted using an incremental protocol on an electronically-braked cycle ergometer (SensorMedics Ergoline 800S). The cycle ergometry protocol consisted of a graded exercise test, which began at either 20 (women) or 40 (men) Watts for the first two min, and then increased 10 to 25 Watt every two min thereafter until volitional exhaustion or one of the established criteria for VO_{2peak} had been reached: an RER > 1.15, HRmax \geq age-predicted HRmax (220 - age), or a plateau in the VO_2 -work rate curve (Medicine 2000). Heart rate, blood pressure and ECG measures were recorded prior to, during and immediately following this test. Subjects breathed through a mouthpiece connected to a two-way breathing valve (Hans Rudolph, Kansas City, MO) during the test, and expired air was collected via open-circuit spirometry. Expired air was collected into a mixing chamber interfaced to a computerized metabolic cart (SensorMedics

2900) to measure expiratory flow and expired air for CO₂ and O₂ fractions. The metabolic cart analyzed and integrated signals for the determination of oxygen consumption (VO₂) every 20 seconds. None of the subjects showed any signs or symptoms of cardiovascular (CV) compensation, e.g. hypotensive response to exercise. These graded exercise tests were supervised and interpreted by a physician at the University of Pittsburgh Medical Center.

3.1.4.4 Experimental Trials:

Palmitate:

At least one week following the VO_{2peak} test, subjects were admitted to the General Clinical Research Center (GCRC) the evening prior to the exercise study where they were fed a standard dinner consisting of 10 kcal/kg; 50% carbohydrate, 30% fat, 20% protein and then fasted until completion of the study. Additionally, they were instructed to avoid strenuous physical activity for two days prior to the study and to eat at least 200 g of carbohydrate for the three days preceding the study to ensure adequate glycogen stores for the exercise bout, since this may significantly influence substrate utilization during exercise (Karlsson 1971). A 12-hour, overnight urine collection was obtained to measure nitrogen excretion rates to correct rates of lipid and carbohydrate oxidation for protein oxidation. At ~ 7:00 A.M. a teflon catheter was placed in a forearm vein for tracer infusions. Another catheter, used for blood sampling only, was placed in the dorsal hand vein of the contralateral arm in the retrograde direction, using a heating pad to obtain arterialized blood samples. Palmitate trial blood and breath collection times are depicted in Table 2 (pg. 27). Additional blood collection and exercise protocol details for the palmitate trial are provided in Appendix C (pg. 43). Blood and breath samples were collected before tracer infusion for basal determination of plasma glucose, lactate, FFA, insulin, epinephrine, norepinephrine, glycerol, and background isotope enrichment. Background ¹³CO₂ enrichment was used to calculate rates of ¹³C-palmitate oxidation. After obtaining basal blood,

primed constant infusions of [6, 6-2H₂] glucose (0.22 $\mu\text{mol}\cdot\text{kg}^{-1}$, 17.6 $\mu\text{mol}\cdot\text{kg}^{-1}$ prime) and [2H₂] glycerol (0.1 $\mu\text{mol}\cdot\text{kg}^{-1}$, prime 1.5 $\mu\text{mol}\cdot\text{kg}^{-1}$) both 99% enriched (Isotech, Inc., Miamisburg, OH) were started with a calibrated syringe pump (Harvard Apparatus, Natick, MA), allowing 2 hours for isotopic equilibration before basal measurements of rates of glucose and glycerol rates of appearance (Ra) and disappearance (Rd). After baseline measurement, blood samples were drawn at 105,110,115, and 120 minutes to measure basal rates of plasma glucose and FFA oxidation. To determine rates of FFA Ra and Rd, a continuous infusion of 0.08 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of [1-13C] palmitate (99% enriched, Isotech, Inc., Miamisburg, OH) bound to 5% human albumin was begun at the onset of exercise to achieve isotopic equilibrium. A priming dose of 18 $\mu\text{mol}\cdot\text{kg}^{-1}$ NaH¹³CO₃ was given prior to [1-13C] palmitate infusion to shorten the time required to achieve equilibration of recovered expired ¹³CO₂. Exact infusion rates were calculated by measuring the concentrations of the isotopes in each infusate.

Exercise was initiated at ~ 9:30 A.M., and the rates of isotopic administration were doubled for glucose and doubled for glycerol (Wolfe 1992) to minimize changes in isotopic enrichment. To measure substrate utilization during exercise, subjects cycled for 60 minutes on a bicycle ergometer (Bosch ERG 601, Germany) at a work rate corresponding to 50% of their predetermined $\text{VO}_{2\text{peak}}$. This was determined using the work rate- VO_2 regression obtained during the incremental $\text{VO}_{2\text{peak}}$ test. VO_2 during exercise was precisely determined during the first 15 minutes of exercise, with work rate being monitored and either increased or decreased to precisely maintain 50% of $\text{VO}_{2\text{peak}}$. Thereafter, the determined work rate was held constant for the remaining 45 minutes of exercise. In addition, since we had a separate heart rate (HR)-work rate regression from the $\text{VO}_{2\text{max}}$ test, HR during exercise was recorded continuously during

exercise with a telemetry unit and monitor (Polar, Finland) as another measure of exercise intensity.

Indirect calorimetry was performed intermittently at 0, 10, 25, 40 and 55 minutes of exercise to measure oxygen consumption (VO₂) and carbon dioxide production (VCO₂) and to determine rates of total lipid and carbohydrate oxidation. To obtain these measurements, subjects breathed through a mouthpiece connected to a two-way breathing valve at 15 minute intervals for a duration of 5 minutes to allow sufficient time for gas exchange equilibrium with the dead space in the tubing, using only the average of the last 2 minutes of VO₂ and VCO₂ data. The gas analyzer was calibrated prior to each test. Water was provided ad libitum during exercise, and subjects were cooled by a fan. Blood samples were obtained in 10-ml vacutainers containing EDTA at 0, 15, 30, 45, and 60 minutes of exercise to determine plasma glucose, FFA, insulin, glycerol, and isotope enrichment. Samples for epinephrine and norepinephrine were collected in iced tubes containing heparin, reduced glutathione, and ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid. Insulin is a powerful inhibitor of lipolysis, whereas catecholamines stimulate both lipolysis and glycogenolysis during exercise.

Table 2: Palmitate Trial Blood and Breath Collection Flow

	Rest				'0'	Exercise			
Baseline	(0)	105min	110min	115min	120min	15EX	30EX	45EX	60EX
Blood Samples:	↑	↑	↑	↑	↑	↑	↑	↑	↑
Breath Samples:	↓				↓	↓	↓	↓	↓

Acetate:

Approximately one week later, the subjects came back to the GCRC to repeat the overnight procedure. It has recently been proposed that measurement of breath fatty acid

oxidation using tracers is underestimated due to its loss into the TCA cycle. Recent studies have shown that measuring acetate recovery during a separate infusion trial could correct for this loss (Schrauwen 1998; Schrauwen 2000). Therefore, we measured acetate recovery during a separate infusion of non-radioactive 1-¹³ C acetate (0.08 μmol•kg⁻¹) continued during one hour of exercise at 50% VO_{2peak} (Table 3, pg.29). During this trial 1-¹³ C acetate was measured in plasma and in breath. Since glucose and glycerol were not measured, only five 5ml blood samples were required during this additional trial. Additional blood collection and exercise protocol details for the acetate trial are provided in Appendix D (pg. 45).

Table 3: Acetate Trial Blood and Breath Collection Flow

	Rest		Exercise		
Baseline	'0'	15EX	30EX	45EX	60EX
Blood Samples:	↑	↑	↑	↑	↑
Breath Samples:	↓	↓	↓	↓	↓

3.1.5 Sample Analyses

3.1.5.1 Blood Sample Analysis

Plasma glucose was measured with a glucose analyzer (YSI glucose analyzer, Yellow Springs, OH). FFA were extracted from plasma, isolated by thin-layer chromatography, derivatized to their methyl esters, and measured using gas chromatography-mass spectrometry (GC-MS). Glycerol was analyzed from plasma samples using the glycerol kinase/glycerol phosphate oxidase method (Sigma Chemical Co. No. 337-UV). Plasma insulin concentrations were determined using a commercially available radioimmunoassay kit (Coat-A-Count, Diagnostics

Products Corporation) and an automated gamma counter (Wallac, Terku, Finland). Plasma epinephrine and norepinephrine were measured by HPLC using a reference laboratory at UPMC arranged through the GCRC. HOMA IR, a marker of insulin resistance, was calculated by multiplying fasting glucose by fasting insulin divided by 22.5.

Enrichment of plasma [6,6 ²H] glucose was determined by GC-MS (GC, 6890 Series; MS, 5973N electron impact ,) analysis of the glucose pentaacetate derivatives, selectively monitoring ions at mass-to-charge (m/e) 200 and 202. Plasma [1-¹³C] palmitate enrichment was determined by GC-MS analysis on the methyl ester derivatives, selectively monitoring ions of m/e 270 and 272. Isotopic enrichment of glycerol was measured by GC-MS, selectively monitoring ions of m/e 253,254, and 257. The ¹³C/¹²C ratio in breath samples were analyzed by Dr. Robert Wolfe at the University of Texas Medical Branch in Galveston, TX using gas chromatography continuous flow isotope ratio mass spectrometry (GC-IRMS).

3.1.5.2 Microscopy for fat content of muscle

Neutral fat of skeletal muscle was determined on light micrographs of 8 μm-thick transverse cryostat sections stained with Oil Red O, using the method of Lillie (Lillie 1943). The Oil Red O solution was prepared as a stock solution (300 mg Oil Red O in 100 mL isopropanol 99%). Prior to each staining session, a working solution (12 mL Oil Red O stock + 8 mL DH₂O) was filtered (Whatman #42; Whatman, Maidstone, UK) to remove crystallized Oil Red. Slides were rinsed in DH₂O for 40 seconds (2 x 20 seconds) and then rinsed for another 10 minutes with running tap water. After air-drying, stained sections were covered with a cover slip using pure glycerol (Sigma, St Louis, MO) as an organic mounting medium, and then sealed around the edges of the cover slip using a butyl acetate-based acrylic polymer (Pavion, Nyack, NY) to prevent cover slip movement during microscopy. Fields were examined at high power (40X),

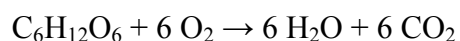
using a light microscope (LEITZ) connected to a CCD camera (Sony, Tokyo, Japan), with an analog to digital conversion system for generation of grayscale images (Tagged image File Format). Grayscale images were computer analyzed (NIH Image software) for fiber area and area of lipid droplets. Oil Red staining was quantified by establishing thresholds for the intensity of staining using the image analysis software. The full range of grayscale imaging was 0 to 255 [arbitrary units (AU)], where 255 represents white, or no intensity (staining), and 0 represents black, or complete staining. Staining intensities between these two extremes represent a continuum from white to black when converted to grayscale. The area occupied by at least 80 muscle fibers per subject was measured with the NIH Image software.

3.1.6 Substrate Metabolism Measurement Techniques

3.1.6.1 Indirect Calorimetry

Indirect calorimetry (IC) is used to estimate rates of whole body lipid and CHO oxidation (energy expenditure) by measuring oxygen consumption (VO_2) and carbon dioxide production (VCO_2). The end result of the metabolism of fat and CHO is complete oxidation into carbon dioxide and water. One assumption with indirect calorimetry is that all oxygen consumed to oxidize the substrates and all CO_2 produced is recovered. Therefore, it is possible to calculate total energy production. Calculations for the oxidation of glucose and an average fat is as follows (Ferrannini 1988; Frayn, Lund et al. 1993):

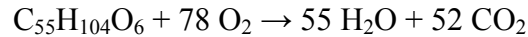
Glucose:



For each mole of glucose oxidized, 6 mol of O_2 is consumed and 6 mol of CO_2 is produced.

Therefore, the respiratory quotient (RQ), or the ratio of CO_2 to O_2 , is 1.0.

Triglyceride:



For each mole of a typical triglyceride oxidized (assumed to be palmitoyl-stearoyl-oleoyl-glycerol) 78 mol of O₂ is consumed and 55 mol of CO₂ is produced. The RQ for fats is 0.70. The RQ for protein is 0.80. Urinary nitrogen excretion can be used to estimate the relative contribution of protein metabolism to reduce error in total substrate oxidation using indirect calorimetry and tracers.

Volumes of O₂ consumed and CO₂ produced in glucose, fat, and protein oxidation can be converted to liters produced per gm or mmol of substrate oxidized. One mole of gas (O₂ or CO₂) occupies 22.4 liters. Calculations of rates of substrate oxidation in mmol are useful when indirect calorimetry is used in conjunction with tracer methodology [57].

O₂ and CO₂ gas samples are obtained by expired air collected into a mixing chamber interfaced to a computerized metabolic cart (SensorMedics 2900). The metabolic cart measures and records the expiratory flow and expired air for CO₂ and O₂ fractions every 30 seconds. These numbers are then averaged to obtain information about substrate oxidized.

Indirect calorimetry provides information about whole body substrate utilization, however, does not have the ability to distinguish the source of oxidized substrate (ie. plasma glucose or glycogen) or the ability to assess rates of substrate release to oxidation.

3.1.6.2 Tracers

Stable isotope tracers have gained recent popularity in the assessment of whole body substrate metabolism during exercise. Tracers are beneficial to studying substrate metabolism because they allow for the measurement of substrate rate of appearance (R_a), or release and rate of disappearance (R_d), or oxidation. Stable isotopes are not radioactive, and thereby safer to use.

By using constant infusions of labeled glucose, FFA, and glycerol tracers, it is possible to measure total and percentage of contribution of each substrate during rest or exercise.

Tracers are designed to be chemically identical to the substance to be measured (tracee), but altered in a way that makes it distinguishable from the tracee for measurement purposes. A typical stable isotope is ^{13}C , or the stable isotope of carbon. Whereas ^{12}C represents the most abundant naturally occurring mass (98.98%) of carbon, ^{13}C occurs with only a 1.11% frequency. In this fashion, identification of the tracer/tracee ratio is possible when measuring Ra and Rd when the small amount of naturally-occurring ^{13}C is accounted for. Tracers are labeled by numbers, which correspond to 1) their position on the molecule 2) their molecular weight, and 3) the number of atoms labeled on the carbon. For example, the stable FFA isotope [1- ^{13}C] palmitate refers to a molecule of palmitate that is labeled at the 1 position with carbon enriched with the stable isotope of mass 13 (Wolfe 1992).

Since fatty acids are not water-soluble, albumin must be added to the palmitate tracer infusate for entry into the bloodstream. The tracer is then infused at a constant rate before exercise (until equilibration is reached) and during exercise. Frequent samples are taken to ensure proper measurement. Plasma Ra and Rd are calculated during exercise using non-steady state equations (Wolfe 1992) for stable isotopes as follows (Goodpaster 2002):

$$Ra = \frac{F \cdot Vd \cdot [(C2 + C1)/2] \cdot [(E2 - E1)/(t2 - t1)]}{[(E2 + E1)/2]}$$

$$Rd = Ra \times Vd \times [(C2 - C1)/(t2 - t1)]$$

F = infusion rate Vd = palmitate volume of distribution

$C_2; C_1$ = substrate concentrations at times $t_2; t_1$, respectively.

When measuring the Ra of FFA in plasma with stable isotopes, it is assumed that all the FFA released from triglyceride is oxidized. However, it is possible that FFAs are re-esterified, causing an underestimation of oxidation. Because glycerol cannot be turned back into triglyceride, measurement of glycerol turnover can provide evidence about lipolysis (Wolfe 1992).

When FFA and glycerol tracers are used in conjunction with indirect calorimetry, quantification of substrate oxidation can be established. Labeled CO_2 is used in conjunction with labeled substrate to determine the enrichment of CO_2 in expired air. Due to the lengthy period of time it would take for labeled breath to equilibrate, a primer bolus of bicarbonate is infused into the subject at the same time as the primed bolus of the tracers. The equation used to calculate the oxidation of plasma FFA is:

$$V^{13}CO_2(\text{mmol/kg/min}) = [ECO_2 - Ebg] \times VCO_2$$

ECO_2 = mean breath $^{13}C/^{12}C$ ratio

Ebg = breath $^{13}C/^{12}C$ ratio at rest before tracer infusion

Moles of gas obtained from IC can be converted to liters of O_2 consumed and CO_2 produced per mmol/min, as 1 mole of gas = 22.4 liters. Therefore, direct comparisons of IC and tracer results can be made.

Since it is possible to have tracer loss in the TCA cycle from labeled expired air, it has been suggested that the acetate recovery factor (Sidossis 1995; Schrauwen 1998; Schrauwen

2000) be determined for each subject to correct for this loss (Schrauwen 2000; Borghouts 2001). This requires a separate exercise trial to establish the acetate correction factor if only stable isotopes are utilized.

Although direct measures of nonplasma fatty acid oxidation and glycogen oxidation cannot be made with tracers and IC, calculations for these substrate pools can be indirectly ascertained. Nonplasma fatty acid oxidation (presumably IMTG) can be calculated as the difference between the total fatty acid oxidation derived from IC and total plasma FFA oxidation derived from the [1-¹³C] palmitate tracer. Stable isotope enrichments are measured by gas chromatography/mass spectrometry (GS/MS).

3.1.7 DATA ANALYSIS:

Independent t-tests were used to test for significant differences between IMTG of elderly versus younger individuals. Subjects were matched for gender and percent body fat. Muscle biopsy samples from the younger control population were obtained from a database of previously studied individuals, with the younger subjects having the same exclusionary criteria and sampling protocol for the biopsy as the elderly.

Repeated measures ANOVA was used to test for differences between plasma metabolites and hormonal values at baseline and during exercise. Spearman's Rho correlation coefficients were used to determine the relation between substrate content and rates of substrate utilization during exercise averaged over the 60- minute bout of exercise. Spearman's Rho correlation coefficients were also used to examine the relationship between oxidative capacity of muscle and rates of NPFA utilization during exercise averaged over the 60-minute bout of exercise. A significance level of $p < .05$ was considered significant. Due to technical difficulties, the glycerol samples were not available at the present time, and were not included.

4. Chapter 4

4.1 Results

4.1.1 IMTG Comparison

A primary objective of this study was to determine differences in IMTG content between old and young individuals. There was no difference in IMTG content ($p= 0.791$) between thirteen healthy elderly subjects aged 67 ± 2 and younger individuals aged 39 ± 6 years (Table 4). The older subjects were those who were recruited to participate specifically in these studies of substrate metabolism, while the younger subjects were accessed by database of participants of previous studies in the laboratories of Drs. Kelley and Goodpaster. Older subjects were matched with younger subjects according to gender and % body fat. Subject descriptive characteristics are shown in Table 1. Independent t-tests showed no significant differences between the elderly and younger group for % body fat, FFM, FM, BMI, weight, or height.

Table 4: Subject Descriptive Characteristics for IMTG Comparison

	Elderly Group	Younger Group
<hr/>		
N (8 women, 5 men)		
IMTG (AU)	22.90 ± 7.07	23.11 ± 13.38
Weight (kg)	77.0 ± 9.45	81.2 ± 14.48
Height (m)	1.66 ± 0.09	1.69 ± 0.10
Body fat (%)	31.8 ± 7.37	31.6 ± 8.04
BMI (kg/m^2)	28.0 ± 3.72	27.7 ± 4.32
FM (kg)	24.6 ± 6.94	24.7 ± 7.10
FFM (kg)	52.4 ± 8.17	54.6 ± 13.22

Values are means \pm SD; N=13 in each group; BMI, body mass index; FM, total body fat; FFM, fat free mass; IMTG, intramuscular triglyceride; AU units, arbitrary units of density of Oil Red O staining.

4.1.2 Baseline Parameters

Fasting blood measures for the elderly subjects at screening, prior to the sub-maximal exercise bout, are reported in Table 5. These subjects were sedentary by self-reported structured physical activity of less than two days per week at screening. Physical fitness determined by rates of peak oxygen consumption during a graded cycle ergometer exercise test was 1.59 ± 0.11 L/min and subjects reported expending roughly 278 kcals/week in lifestyle physical activity (Table 6).

Table 5: Subject Fasting Blood Measures at Screening

Plasma glucose (mg/dl)	91 ± 3
Insulin (uU/ml)	11.1 ± 1.6
FFA (umol/l)	602 ± 55
Total Cholesterol (mg/dl)	204 ± 6
Triglycerides (mg/dl)	143 ± 84
HDL (mg/dl)	59 ± 4
LDL (mg/dl)	123 ± 8

Values are means \pm SE; N=16; FFA, free fatty acids; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 6: Physical Fitness, Self-reported Physical Activity and Sub-maximal Exercise Responses of Subjects

VO ₂ peak (L/min)	1.59 ± 0.11
MAQ (kcal/wk)	278 ± 84
Fat Oxidation (μmol/kgFFM/min)	18.39 ± 1.35
Fat Oxidation (proportion of energy, %)	53.95 ± 4.57
Relative Exercise Intensity (%VO ₂ max)	57.6 ± 0.02
RER	0.84 ± 0.01

Values are means ± SE; N=16; MAQ, Modifiable Activity Questionnaire; Fat oxidation reported as mean rates of fat oxidation during 30-60 min of sub-maximal exercise; RER, respiratory exchange ratio.

4.1.3 Plasma Metabolite Responses to Exercise

Free fatty acids remained relatively constant throughout the 60-minute exercise bout (Figure 1). Blood glucose ranged from 5.1 ± 0.09 mmol/l at rest to 4.9 ± 0.10 mol/l at 60 minutes of exercise, as depicted in Figure 2. Blood lactate levels increased from baseline to 60 minutes of exercise (1.0 ± 0.09 to 2.2 ± 0.30 mmol/l), but remained relatively constant throughout the exercise session (Figure 3). No significant differences were found for values of FFA or glucose, but there was a significant time effect for lactate (p= 0.002) by repeated measures ANOVA.

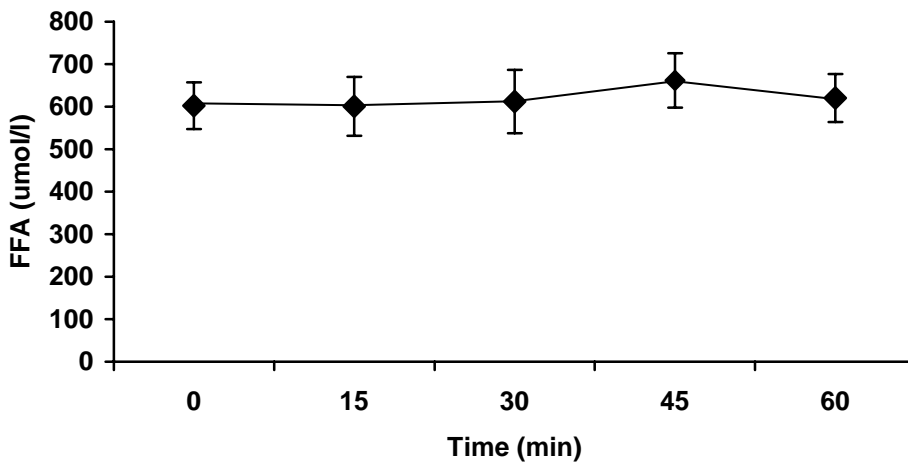


Figure 1: Free Fatty Acids (FFA) (mean + SE) at rest, 15, 30, 45, and 60 minutes of exercise.

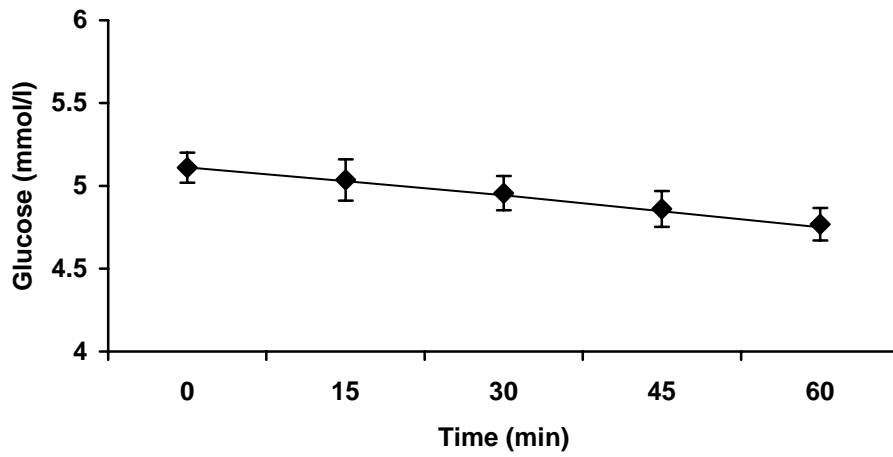


Figure 2: Blood glucose (mean \pm SE) at rest, 15, 30, 45, and 60 minutes of exercise

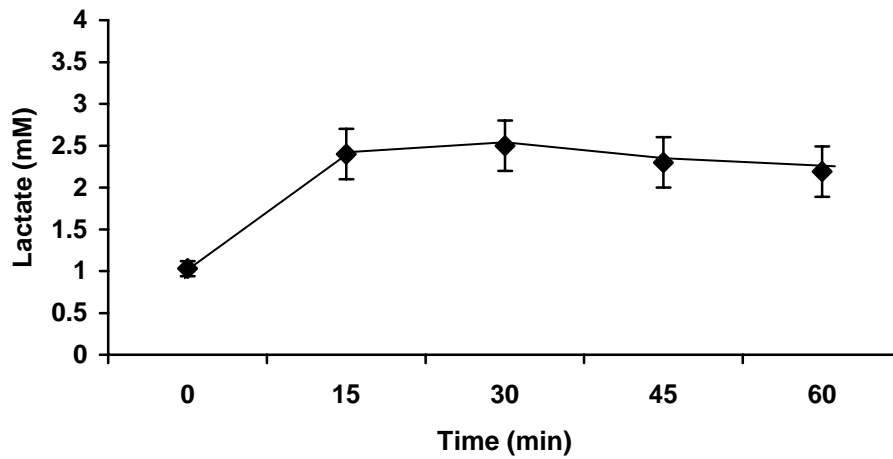


Figure 3: Plasma Lactate concentrations (mean \pm SE) at rest, 15, 30, 45, and 60 minutes of exercise.

4.1.4 Hormonal Response to Exercise

Insulin levels decreased slightly from baseline ($11.1 \pm 1.6 \mu\text{U/mL}$) to 60 minutes of exercise ($9.3 \pm 1.3 \mu\text{U/mL}$) (Figure 4), but they were not statistically significant. Norepinephrine levels rose from a baseline value of $361 \pm 67 \text{ pg/mL}$ to $582 \pm 66 \text{ pg/mL}$ at 60 minutes of exercise (Figure 5). Repeated measures ANOVA revealed a main effect of time for norepinephrine levels ($p=.0195$).

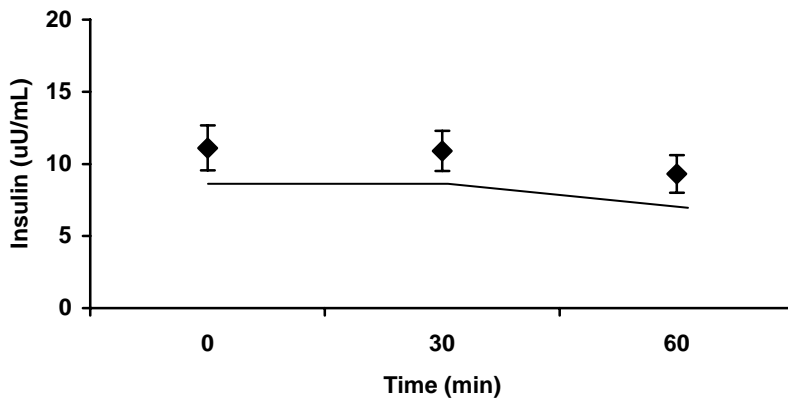


Figure 4: Plasma Insulin concentrations (mean \pm SE) at rest, 30, and 60 minutes of exercise.

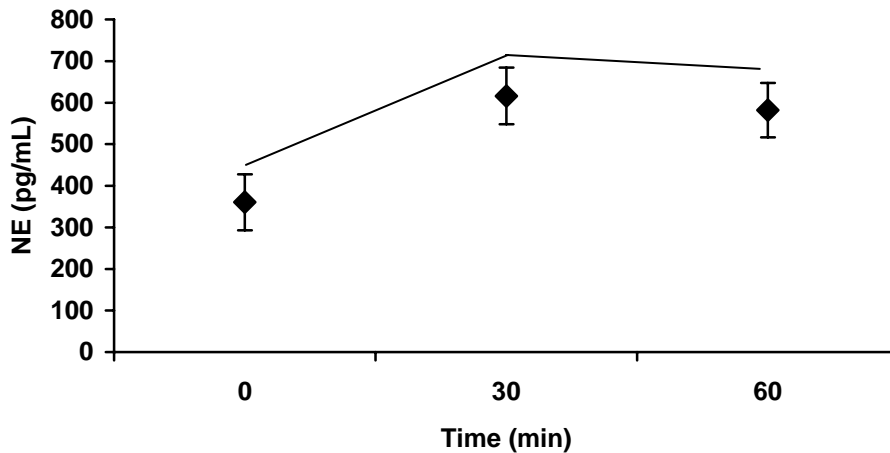


Figure 5: Plasma norepinephrine (NE) concentrations (mean \pm SE) at rest, 30, and 60 minutes of exercise.

4.1.5 Substrate Oxidation

RER obtained by indirect calorimetry was 0.77 at rest and averaged 0.84 throughout the duration of the 60-minute exercise bout (Figure 6). Fat oxidation during the sub-maximal exercise bout was 18.39 $\mu\text{mol}\cdot\text{kgFFM}\cdot\text{min}$, providing fifty-four percent of the energy utilized for the 60-minute exercise bout (Figure 7). Exercise was performed at 58% of VO_2peak .

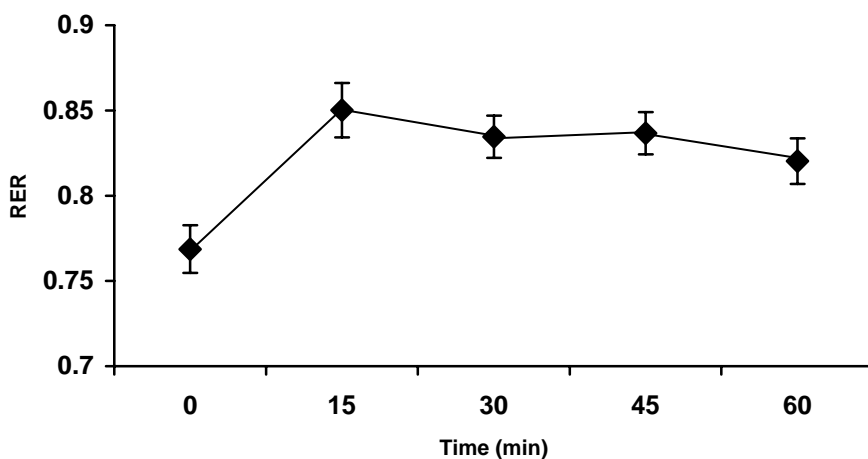


Figure 6: RER concentrations (mean + SE) at rest, 15, 30, 45, and 60 minutes of exercise.

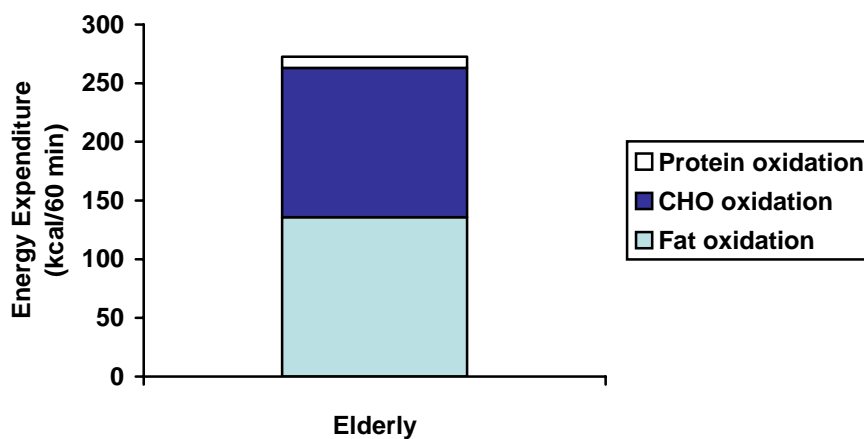


Figure 7: Total energy expenditure during 60 minutes of sub-maximal exercise.

4.1.6 Fatty Acid Metabolism Determined by Stable Isotope Turnover

Isotopic enrichment of palmitate remained relatively constant throughout the sub-maximal exercise bout (Figure 8). Substrate kinetics were calculated from the last 3 time points; 30, 45 and 60 minutes of exercise. Plasma fatty acid oxidation derived from the recovery of labeled C13 in breath is depicted in Figure 9. Values increased from baseline to exercise at 15 minutes, but remained relatively constant through 30, 45, and 60 minutes of exercise, respectively. Palmitate Ra (Figure 11) and FFA Ra (Figure 12) showed similar patterns throughout the 60-minute exercise bout.

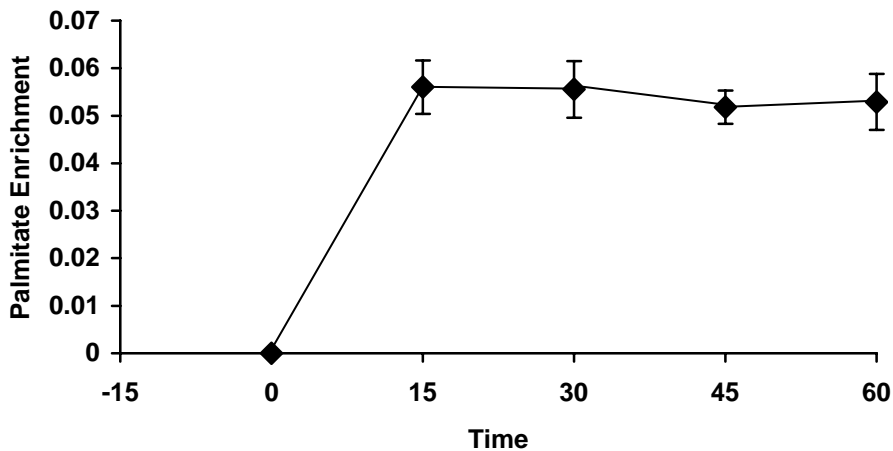


Figure 8: Isotopic enrichment of palmitate in plasma (mean \pm SE) at rest, 15, 30, 45, and 60 minutes of exercise.

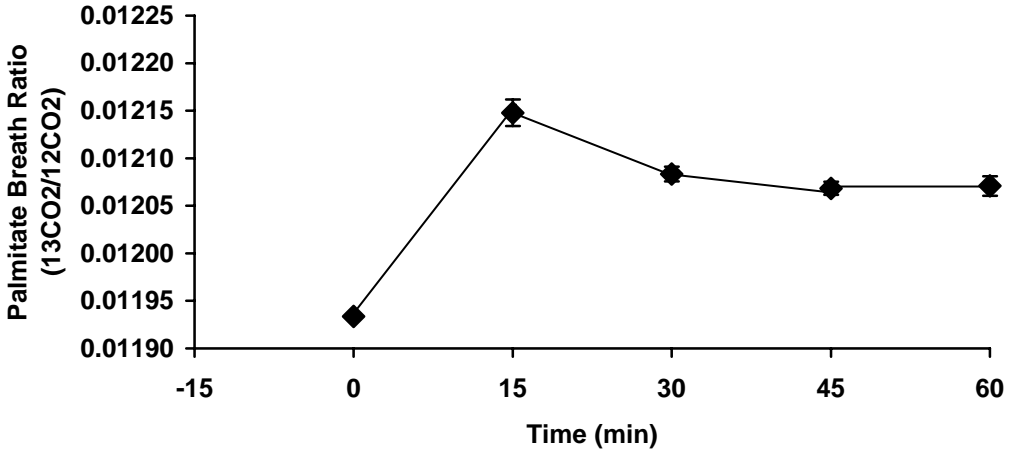


Figure 9: $^{13}\text{C}/^{12}\text{C}$ palmitate in breath ratio (mean \pm SE) at rest, 15, 30, 45, and 60 minutes of exercise.

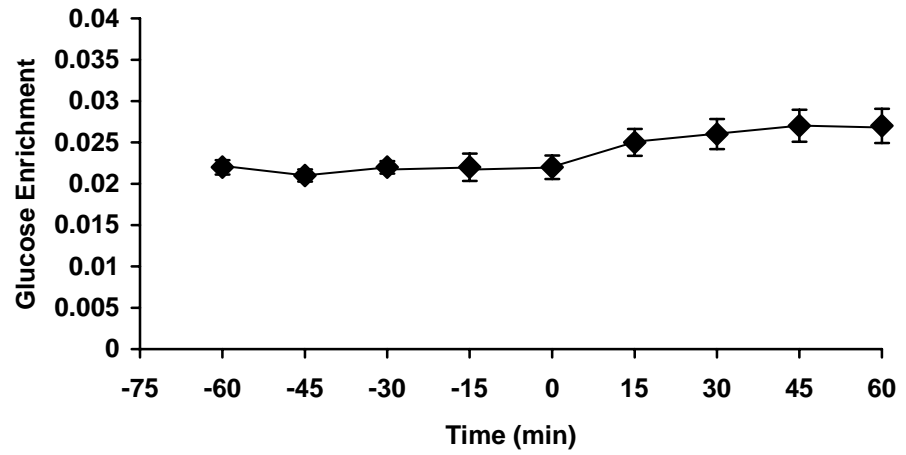


Figure 10: Isotopic enrichment of glucose in plasma (mean \pm SE) at rest (-60, -45, -30, -15, 0) and during 15, 30, 45, and 60 minutes of exercise.

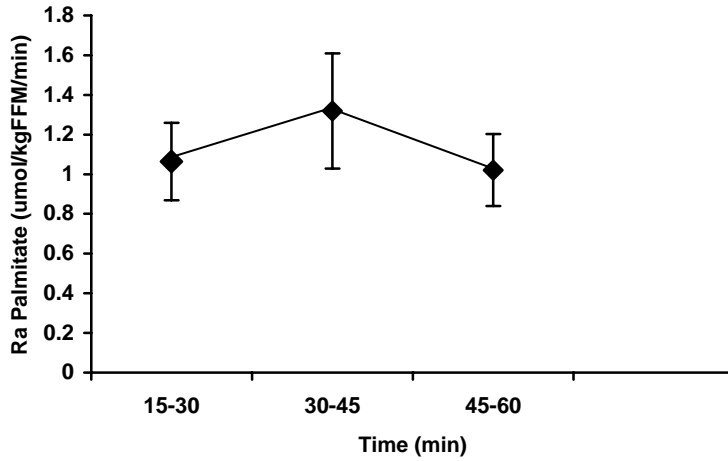


Figure 11: Fatty acid of appearance (Ra) (mean \pm SE) in $\mu\text{mol/kgFFM/min}$ at 15-30, 30-45, and 45-60 minutes of exercise.

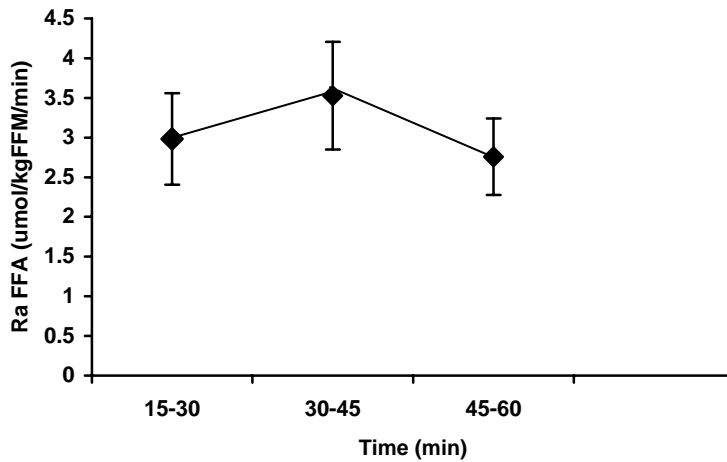


Figure 12: Free fatty acid (FFA) rate of appearance (Ra) (mean \pm SE) in $\mu\text{mol/kgFFM/min}$ at 15-30, 30-45, and 45-60 minutes of exercise.

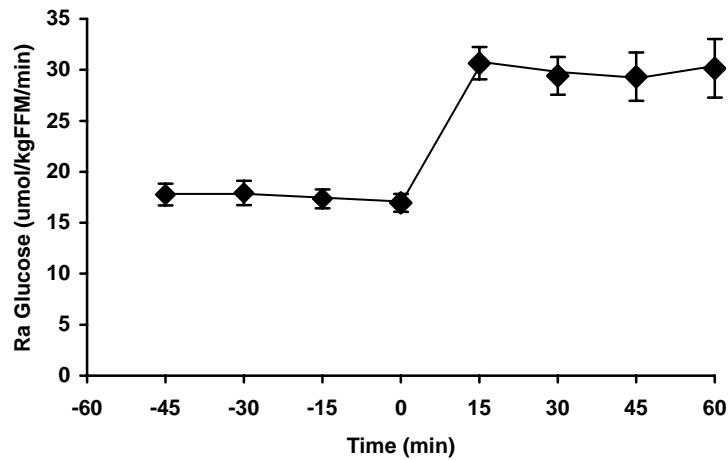


Figure 13: Glucose rate of appearance (Ra) (mean \pm SE) in $\mu\text{mol/kgFFM/min}$ at -45,-30, -15, 0, 15, 30, 45, and 60 minutes of exercise.

Glucose enrichment increased from baseline (measured at 15 minute increments 60 minutes prior to the exercise bout) to exercise, but also remained relatively stable during the 60-minute exercise bout (Figure 10). Glucose Ra remained constant throughout the baseline measurements and exercise measurements. However, there was an increase in glucose Ra from baseline to exercise (Figure 13).

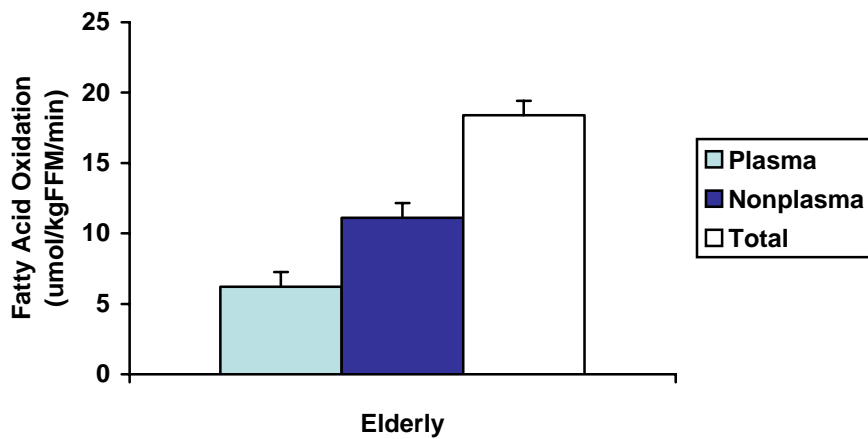


Figure 14: Rates of plasma, non-plasma, and total fatty acid oxidation (mean \pm SE) during exercise.

The older subjects oxidized a significantly higher amount of non-plasma fatty acids ($11.12 \pm \mu\text{mol/kgFFM/min}$) than plasma fatty acids ($6.21 \pm 1.15 \mu\text{mol/kgFFM/min}$) during the exercise bout (Figure 14). Similarly, glycogen oxidation was higher than plasma glucose oxidation during exercise, as shown in Figure 15.

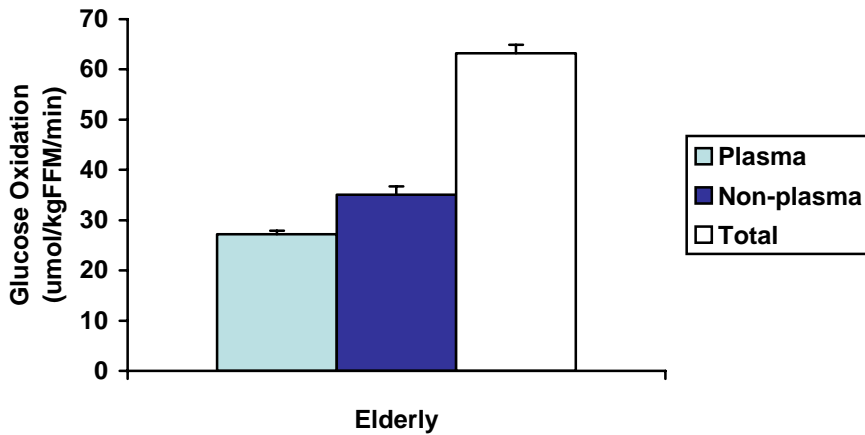


Figure 15: Rates of plasma glucose, estimated glycogen, and total glucose oxidation (mean \pm SE) during exercise.

Percent contribution of the various substrates is depicted in Table 16. NPFA accounted for roughly 33% of total energy expenditure during exercise, whereas plasma fatty acids accounted for about 19%. Plasma glucose and muscle glycogen accounted for about 22% and 26% of total energy expenditure during exercise, respectively.

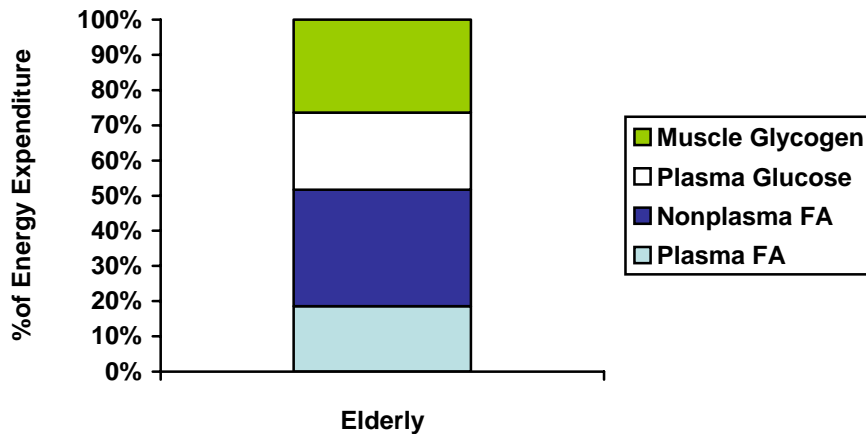


Figure 16: Relative contribution of the various substrates to total energy expenditure in the elderly (mean \pm SE) during exercise.

4.1.7 Correlations between substrate content, oxidative capacity, and fat oxidation

There was a significant negative association between IMTG content by Oil Red O staining and fat oxidation ($\rho = -0.5989$; $p = 0.03$) induced by sub-maximal exercise in the older subjects. A negative association also existed between IMTG content and oxidative capacity of muscle (SDH staining) ($\rho = -0.6485$; $p = 0.04$). No significant association between oxidative capacity and rates of NPFA oxidation in the elderly during exercise existed, however, there was a trend toward significance between oxidative capacity and plasma fat oxidation ($\rho = 0.5245$; $p = 0.08$).

VO₂peak was marginally correlated with fasting insulin levels ($\rho = -0.4857$; $p = 0.056$) and HOMA IR ($\rho = -0.4441$; $p = 0.0848$). However, no significant differences were found for IMTG content and fasting insulin or HOMA IR or fat oxidation and fasting insulin or HOMA IR.

5. Chapter Five

5.1 Discussion

5.1.1 IMTG and Aging

A primary objective of this study was to determine whether older individuals have greater IMTG compared to younger subjects. Contrary to our hypothesis, IMTG stores were not significantly different between older and younger subjects who were matched for generalized body composition, including body fat and lean mass. This was an important finding given the recent evidence suggesting that higher IMTG is linked to age-associated insulin resistance (Petersen 2003).

Although it has been suggested that increased IMTG stores occur with aging in both humans (Cree 2004) and rodents (Tucker 2003), the available evidence that higher IMTG is directly related to aging is not clear. In aging rats, fatty acid uptake under insulin-stimulated conditions was significantly increased and correlated with rates of triglyceride synthesis (Tucker 2003). The authors speculated that the increase in fatty acid uptake could be the preliminary factor in the accumulation of triglyceride within skeletal muscle. Other factors associated with increased IMTG levels in humans include obesity (Goodpaster 2000) and paradoxically, chronic exercise training (Martin, Dalsky et al. 1993). We matched our older and younger subjects in this comparison for level of obesity, and both groups were sedentary by self-reported lack of participation in regular exercise. However, we were not able to match these subjects according to levels of physical fitness, which may have confounded any age-related differences. This is in contrast to Petersen and colleagues who found higher IMTG levels in older subjects, although they did not obtain objective measures of physical activity or physical fitness (Petersen 2003). This contrast could be related to several methodological differences between the two studies. In

the current study, IMTG content was assessed by microscopy, with Oil Red O staining of a muscle biopsy sample, whereas Petersen et al. (Petersen 2003) used NMR ^1H spectroscopy to obtain intramyocellular lipid content (IMCL).

It is possible that the NMR ^1H spectroscopy methods can be confounded by the technical challenges of quantifying IMTG defined as IMCL in older subjects who typically have a considerable amount of extracellular lipid in muscle. The necessity to use relatively large voxel sizes for these spectroscopic measurements makes the IMCL quantification difficult. It is also possible that biochemical determinations of IMTG may also be confounded by small amounts of extracellular lipid in the muscle specimen (Guo 1999), which again, could be more problematic in older subjects. Thus, the direct quantification approach of lipid staining of IMTG in the current study is perhaps the first direct comparison of IMTG levels in older versus younger humans suggesting that higher IMTG is not a function of age per se, at least in these relatively healthy older men and women.

In addition, caution must be used when comparisons are made between muscle samples from different locations in the body, as skeletal muscle fiber types and ratios vary, depending on the specific muscle studied (J. Keul 1972; Squire 1986). In the current study, IMTG content of the vastus lateralis muscle was measured, whereas IMCL content of the soleus muscle was measured in the study by Petersen et al. (Petersen 2003). Therefore, specific muscles with specific fiber type ratios may be more susceptible to the accumulation of IMTG.

5.1.2 IMTG Content and Insulin Resistance

High levels of IMTG have been associated with insulin resistance and Type 2 Diabetes in younger, sedentary populations (Goodpaster, Thaete et al. 1997; Pan, Lillioja et al. 1997; Ebeling, Essen-Gustavsson et al. 1998; Goodpaster, Kelley et al. 1999; Krssak, Falk Petersen et al. 1999; Manco, Mingrone et al. 2000; Kelley 2001; Perseghin 2003). It has also been

suggested that higher IMTG in the elderly characterizes age-related insulin resistance (Petersen 2003). Therefore, high IMTG levels in the elderly could predispose this population to these metabolic derangements. In a study by Cree et al., increases in IMTG seen in the elderly were significantly correlated with insulin sensitivity (Cree 2004), even after controlling for body fat. Although insulin sensitivity was not measured in the current study, higher fasting insulin levels and HOMA IR were not significantly associated with IMTG content.

5.1.3 IMTG Content and Fat Oxidation

Another primary objective of this study was to examine whether fat oxidation during sub-maximal exercise was associated with substrate, i.e. IMTG availability. Increased levels of IMTG by ORO staining were associated with lower fat oxidation during exercise in the present study. This is in accord with other studies suggesting that IMTG may link impaired fatty acid metabolism and insulin sensitivity (Phillips, Green et al. 1996; Goodpaster, Thaete et al. 1997; Pan, Lillioja et al. 1997; Kelley 2001). Thus, these data would indicate that a reduced capacity for fat oxidation may contribute to lipid accumulation within the muscle in sedentary older adults. It is possible that an increased ability to oxidize fat would protect older subjects against insulin resistance and age-associated metabolic diseases such as type 2 diabetes. In support of this concept, Petersen et al. (Petersen 2003) demonstrated that older subjects who were insulin resistant and had higher IMTG levels also had a lower capacity for oxidative metabolism compared to younger subjects. These data alone would suggest that a low capacity for fat oxidation leads to IMTG accumulation.

However, in a recent review, Kelley and Goodpaster (Kelley 2001) suggested that IMTG may not result in adverse metabolic conditions in the presence of efficient fat oxidation by muscle. This is evidenced in highly trained endurance athletes, who possess greater muscle fat stores, but are highly insulin sensitive (Goodpaster, He et al. 2001). Further, a sub-group of

subjects in the current study who went on to participate in a 12-week aerobic exercise program had a significant increase in IMTG (Pruchnic et al), providing additional evidence that exercise training may increase IMTG, even in older subjects. This is consistent with the notion that triglycerides themselves do not confer insulin resistance (Yu 2002), but rather diacylglycerol and ceramides may have direct effects on insulin signaling (Schmitz-Peiffer 2000). This is also consistent with studies showing that IMTG is a readily available energy source during physical exercise (Hurley, Nemeth et al. 1986; Romijn 1993). Therefore, increased IMTG stores in the presence of enhanced muscle oxidative capacity may represent an increased ability of the muscle to oxidize fat.

5.1.4 Aging and Fat Oxidation

Fat oxidation rates induced by sub-maximal exercise in the present study are consistent with those reported by Sial et al. In that study, elderly men and women had lower fat oxidation during 60 minutes of moderate intensity ($55.7 \pm 3.1\%$ VO_{2peak}) cycle ergometry exercise (Sial, Coggan et al. 1996) when compared to a younger, control group matched for gender and FFM. It has been suggested that aging is associated with a reduced ability to utilize fatty acids (Coggan, Spina et al. 1992; Calles-Escandon 1995; Proctor, Sinning et al. 1995; Sial, Coggan et al. 1996; Petersen 2003). Perhaps the reduction in muscle size and metabolic function seen in the elderly are associated with this decrease in oxidative metabolism. Calles-Escandon et al. (Calles-Escandon 1995) found a reduction in basal fat oxidation with increasing age in a group of women. The single best predictor of the decline in basal fat oxidation in their study (Calles-Escandon 1995) was a reduction in FFM. Preservation of FFM with exercise training may reduce the decrease in fat oxidation seen in the elderly.

It has been suggested by Coggan et al. (Coggan 1993) that the lower capacity for fat oxidation in older adults can partly be explained by the physical inactivity that accompanies

aging. In support of this, this same group has also demonstrated increases in exercise-induced fat oxidation with exercise training. This is also consistent with a recent study from our group showing that older subjects have a significantly greater reliance on fat as an energy source during exercise following 12 weeks of exercise training (Pruchnic 2004). Therefore, it may not be the aging process itself, but a sedentary lifestyle that contributes to these metabolic insufficiencies associated with the elderly. To further support this idea, Rimbart et al. found that impairment of muscle fat oxidative capacity was not correlated with age, but with physical inactivity and insulin sensitivity in a group of elderly (64 ± 3 yrs) sedentary subjects (Rimbart 2004). In the current study, higher fasting insulin levels and HOMA IR were marginally associated with lower fitness levels. This association may have been greater with a greater sample size.

5.1.5 Obesity and Fat Metabolism

Substrate availability and capacity for oxidative metabolism are two primary factors that could influence fat oxidation during exercise. Our finding that lower fat oxidation during exercise was associated with higher IMTG content is in apparent contrast to other investigations demonstrating that obese individuals, who usually exhibit higher IMTG content, oxidize more fat during exercise than lean controls (Horowitz 2000; Goodpaster 2002). One could surmise from these studies that during exercise increased IMTG oxidation is associated with higher IMTG availability. This phenomenon may represent an adaptation to avoid insulin resistance by utilizing available IMTG's during exercise. This, however, does not appear to be the case for the older subjects in this study. In the present study, oxidative capacity of muscle was not related to fat oxidation during sub-maximal exercise in the elderly, however, lower oxidative capacity of muscle was associated with higher IMTG stores.

The increase in fat oxidation seen in obese individuals during exercise is in contrast to reports of lower fasting rates of fat oxidation in the presence of high IMTG content with obesity

(Kelley, Goodpaster et al. 1999). This is also consistent with lower oxidative enzyme capacity in obesity (Kelley 2001) and obesity-associated insulin resistance (Kelley 2001). In another study of overweight women, normal insulin sensitivity and lower IMTG content was associated with higher fasting lipid oxidation (Perseghin 2002). Taken together, these data suggest a complex association between substrate availability and capacity for oxidative metabolism in obesity as well as in older adults. Future studies are needed to explore this further.

5.1.6 Aging and Carbohydrate Oxidation

Increased skeletal muscle glycogen utilization during exercise in the aging has been demonstrated in both human (Sial, Coggan et al. 1996) and rat studies (Cartee 1994). This is consistent with the present study, in which carbohydrates accounted for about half of the total substrate utilized during the 60-minute sub-maximal exercise bout, with muscle glycogen accounting for roughly 22% of this oxidation. The increased oxidation of carbohydrate in the elderly may reflect the decline in skeletal muscle respiratory capacity, and subsequent decrease in fat oxidation seen in this group (Meredith, Frontera et al. 1989; Coggan, Spina et al. 1992; Sial, Coggan et al. 1996). Lower muscle glycogen levels have also been found in the elderly, and may be linked with decreased insulin sensitivity (Meredith, Frontera et al. 1989). If the elderly possess less muscle glycogen stores at the start of exercise, depletion could occur sooner, creating earlier fatigue.

5.1.7 Conclusion

The results of this study indicate that IMTG content is similar between older and younger individuals when matched for percent body fat and gender, instead of being higher in the elderly as originally hypothesized. As predicted, IMTG content was not associated with higher NPFA oxidation, although higher IMTG was associated with lower total fat oxidation during sub-maximal exercise. Oxidative capacity of muscle was not associated with NPFA oxidation as

originally hypothesized. Low capacity for fat oxidation was associated with higher IMTG content, suggesting that reduced capacity for fat oxidation in muscle leads to IMTG accumulation. Caution must be taken when interpreting these results due to the small sample size of 16 subjects. We saw several trends that may have reached statistical significance had our sample size been larger.

Other limitations to the present study included the lack of fitness data in the younger, comparison group, which may have influenced IMTG content in this group, as well as the lack of a control group for the sub-maximal exercise bout. Finally, the current investigation was a preliminary, cross-sectional study designed to determine the relative content of IMTG in elderly and its relation to substrate metabolism during sub-maximal exercise. Training studies are needed to gain a better understanding of the relationship between IMTG content and substrate metabolism, where each subject can act as their own control.

5.1.8 Future Research Recommendations

Based upon the literature, a relationship exists between IMTG, insulin resistance and decreased fat oxidation. However, it is unclear what role IMTG content plays in this metabolic dysregulation. Specifically, does IMTG content precipitate or result in the decrements in fat oxidation and is this phenomenon consistent in different populations (ie. elderly/obese/diabetic)?

Exercise training studies have shown improvement in muscle fat oxidative capacity in the elderly, but the relationship to IMTG content and NPFA utilization deserve further examination. Finally, due to the small sample size in the present investigation, gender comparisons of IMTG content and substrate utilization in the elderly were not possible, and should be accounted for in future studies.

APPENDIX A- INFORMED CONSENT

CONSENT TO ACT AS A SUBJECT IN AN EXPERIMENTAL STUDY

TITLE: THE EFFECTS OF EXERCISE TRAINING ON SKELETAL MUSCLE FFA UTILIZATION DURING EXERCISE IN THE ELDERLY

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SOURCE OF SUPPORT: NIH K01 (BG)

DESCRIPTION:

Why is this research being done?

I understand that I have been asked to participate in a research study that will assess how my body burns fat during exercise, which may be important in determining the relationship of aging and physical fitness, and may provide important information about how to design effective programs to help prevent the effects of aging on the loss of muscle function.

Subject's initials _____

Who is being asked to take part in this research study?

I am being asked to participate in this study because I am a normal, healthy volunteer between the ages of 65-75. I will have an examination to determine if I am in good general health for participating in this study, and these results will be given to me for my records. If I qualify I will be one of 20 subjects in this study.

What procedures will be performed for research purposes?

If I participate in the study, I will be asked to participate in the following procedures: (1) A scan (called a DEXA scan which is similar to an x-ray) to measure total fat content of my body; (2) an exercise test to determine my level of fitness; (3) and two exercise tests lasting one hour which will determine how my body burns fat; (4) a needle biopsy to obtain a small piece of muscle tissue from my thigh to evaluate the amount of fat contained in my muscle; and (5) a Magnetic Resonance Imaging (MRI) scan (like an x-ray but with no radiation) to measure the amount of fat and muscle in the leg. The purpose of these tests is to determine how aging and the amount of fat and muscle in the leg affect the body's ability to burn fat during exercise. Following these tests, I will participate in a 12-week exercise program to determine how exercise may help prevent some of the negative effects of aging on the body's metabolism.

On one morning I will be scheduled for a screening at the General Clinical Research Center (GCRC) to see if I qualify for this study. This will include a physical exam, blood draw and a graded exercise test (stress test) to assess physical fitness. This test will take approximately one hour. On a separate day I will be scheduled for an outpatient visit to the GCRC at the University Hospital for an MRI scan of the mid thigh, a DEXA scan to measure body fat and the muscle biopsy. The MRI, DEXA and muscle biopsy will each take about one hour (three hours total for that day). Approximately one week following the outpatient visit, I will be admitted for an overnight visit to the GCRC and the following morning asked to perform a one hour, relatively easy exercise bout to assess fat metabolism during exercise. Approximately one week following this, I will come back to the GCRC for another overnight visit and relatively easy exercise bout to assess fat metabolism during exercise. This week I will also begin participation in a 12-week exercise training program. Following the training program, I will be asked to undergo the exercise tests, DEXA, MRI and muscle biopsy in the same sequence and in the same manner as prior to exercise training.

The MRI scan involves lying on a table for about 45 minutes so that the fat in my thigh muscle can be measured.

The DEXA scan is performed similar to an x-ray study and I will lie on an examination table in a room with the DEXA scanner for approximately 30 minutes. The test is painless except for any discomfort I may experience because of lying on my back on the firm examination table.

Subject's initials _____

On the same morning as the DEXA and MRI scans, a small sample of muscle tissue (the size of pencil tip) from the thigh will be obtained and evaluated. On the outer surface on one thigh an area the size of a quarter will be numbed by an injected anesthetic and a biopsy needle will be passed into the muscle in order to obtain a small piece of muscle.

At the screening examination to measure my fitness level, I will undergo an exercise test on a stationary bicycle. My heart rate will be monitored by adhesive pads attached to my chest that are connected to a heart monitor. I will be asked to breathe through a mouthpiece in order to measure how much oxygen my body is using as I pedal the bike. Every two minutes the bike will be adjusted to make pedaling more difficult, and I will be asked to keep working until I am too tired to continue. If I develop a pain in my chest or have particular patterns on the heart monitor, I will be asked to stop pedaling. This test will take approximately one hour.

Participation will also involve four overnight admissions to the University of Pittsburgh General Clinical Research Center (GCRC). Prior to each overnight admission, I will be instructed to avoid strenuous physical activity for two days prior to this study and to eat at least 200 g of carbohydrate per day for the three days preceding the study to make sure that my energy levels are high for the exercise bout. I will be admitted in the evening and receive a normal dinner that evening and then not eat until after the exercise study is completed the following day, although I can have water. On that evening in the GCRC I will not perform any type of rigorous physical activity. The next morning I will perform the exercise test. At about 6:30 A.M., I will be transported to the Obesity Nutrition Research Center Laboratory on the 8th floor Montefiore University Hospital where an intravenous catheter (plastic needle in my vein) will be placed in my arm to introduce glucose and fatty acid metabolic tracers into my blood. These tracers, containing absolutely no radioactivity, are used to measure my body's use of glucose and fat. Another catheter will be placed in the vein of my hand to periodically sample blood. For the entire study including rest and exercise, I will have 8 blood samples taken, each being about 5 ml or about 3 tablespoons total.

After starting the tracers at about 7:30 A.M., I will rest in a chair for approximately two hours before I get on a stationary bike at ~ 9:30 A.M. for exactly one hour of cycling. The exercise on the bike will be relatively easy in the beginning but may become more difficult as my muscles tire. During the exercise, I will be wearing a belt around my chest, which contains a transmitter to measure my heart rate. I will be asked to breathe through a mouthpiece connected to a breathing valve for 5 minutes at four different times during the exercise test in order to collect my expired air for the measurement of my exercising metabolism. During this period I will be breathing in normal room air. A nurse will take a 5 ml blood sample (about 1 teaspoon) from me every 15 minutes during exercise. I will be allowed to drink water at any time during exercise and cooled by a fan if I request.

Subject's initials _____

RISKS AND BENEFITS:

What are the possible risks, side effects, and discomforts of this research study?

Participation may entail some risks. These are related to exercise, blood sampling and exposure to radiation from the DEXA scans: 1) The risks of inserting a catheter into a vein for blood sampling and the muscle biopsy procedure are bleeding, bruising and discomfort.

In our experience using similar protocols, subjects have not experienced any adverse effects from these procedures other than a small amount of residual localized soreness at the blood sampling or biopsy sites. 2) Participation in this research study involves a minimal radiation exposure from the DEXA. The amount of radiation exposure that you will receive from this procedure is about 0.06 mRAD to your whole body. For comparison, radiation workers are permitted, by federal regulation, a maximum whole body radiation exposure of 5 RADS per year. There is no minimum amount of radiation exposure that is recognized as being totally free of the risk of causing genetic mutations (abnormal cells) or cancer. However, the risk associated with the amount of radiation exposure that I will receive from taking part in this study is felt to be low and comparable to everyday risks. 3) MR imaging has certain conditions that would exclude me from participating in this study. These include cardiac pacer, shrapnel, or other metal devices. Metal objects present in the body could be moved by the large magnet involved in the MRI, and such movement could cause serious injury. Pregnancy and fear of closed spaces are also reasons to be excluded from the study. No serious biological effects have been reported from being in a magnet. If I experience a fear of the confined space while in the magnet, I can terminate the study. More severe reactions such as respiratory distress or severe drop in blood pressure are rare. Trained medical personnel are always in attendance during these studies; 4) The exercise test (VO₂max) and 60 minute exercise bout may cause muscle soreness or fatigue, but in adults without a known history of heart disease, the risk of heart attack or death from these tests is extremely low. American College of Sports Medicine (ACSM) criteria will be used to halt maximal exercise testing should adverse cardiovascular responses develop. I will be encouraged to finish the 60 minute exercise bout, but if I fail to keep a pedaling rate of at least 60 revolutions/minute, the test will be stopped. In the case of an adverse event during either of the exercise tests my primary care physician will be notified.

What are possible benefits from taking part in this study?

I will not benefit personally from participation in this study except to the extent that I may benefit from participation in these exercise studies because of the health benefits associated with regular exercise. Information on exercise capacity, fitness and energy metabolism during exercise will be shared with me if I desire. This will include a copy of the maximal exercise test and body composition results.

I understand that I should inform the investigators if I have participated in any other research study during the previous year, and I have done so. Prior participation in another research study does not necessarily exclude me from this study. However, this information may be

Subject's initials _____

used to determine whether or not any prior participation in research studies may affect the results of this study. I understand that this study is a research study and may not be of direct benefit to me. If requested, a report will be generated for my medical record that will include any information important for my medical care.

NEW INFORMATION:

If I agree to take part in this research study, will I be told of any new risks that may be found during the course of the study?

If new information, either good or bad, about this study comes to the attention of the investigator during the course of this study which may relate to my willingness to participate, it will be provided to me or my representative. A copy of this consent form will be given to me to keep for careful rereading.

COSTS AND PAYMENTS:

Will I or my insurance provider be charged for the costs of any procedures performed as part of this research study?

I understand that neither I, nor my insurance provider will be required to pay for any procedures associated with this research study.

Will I be paid if I take part in this research study?

I will receive a payment of \$200 for the completion of the first series of procedures, including the exercise trials, muscle biopsy, DEXA, MRI and VO2max procedures. After completing the exercise training regime, I will be asked to repeat these procedures, and if successful, I will receive an additional \$300. Parking costs or transports, in an amount not to exceed \$10 will be given to volunteers.

COMPENSATION FOR ILLNESS OR INJURY:

Who will pay if I am injured as a result of taking part in this study?

University of Pittsburgh investigators and their associates who provide services at the UPMC Health System (UPMC HS) recognize the importance of my voluntary participation to their research studies. These individuals and their staffs will make reasonable efforts to minimize, control, and treat any injuries that may arise as a result of this research. If I believe that I am injured as a result of the research procedures being performed, please contact the Principle Investigator listed on the cover sheet of this form or the University of Pittsburgh Institutional Review Board (412-578-3424). Emergency medical treatment for injuries solely and directly relating to my participation in this research will be provided to me by hospitals of UPMC HS. It is possible that the UPMC HS may bill my insurance provider for the costs of this emergency treatment, but none of these costs will be charged directly to me. If my research-related injury requires medical care beyond this emergency treatment, I will be responsible for the cost of this follow-up care unless otherwise specifically stated below. I will not receive monetary payment for, or associated with, any injury that I suffer in relation to this research.

Subject's initials _____

CONFIDENTIALITY:

Who will know about my participation in this research study?

Any information about me will be handled in a confidential manner consistent with other medical records. Information obtained about me from this research project will be part of my research record. Research records will be kept in locked files in the office of Dr. Goodpaster. All data obtained will be maintained by Dr. Goodpaster's laboratory for a period of at least five years upon termination of this study with access restricted to authorized personnel only. However, in unusual circumstances, my research records may be inspected by appropriate government agencies, or released in response to an order from a court of competent jurisdiction. I will not be identified by name in any publications but consent to publication of any information for scientific purposes so long as my identity is not revealed. In signing this form I consent to the publication of the results of the study for scientific purposes.

RIGHT TO WITHDRAW:

Is my participation in this research study voluntary?

I understand that I do not have to take part in this research study. Should I change my mind I can withdraw from the study at any time. Other care and benefits will be the same whether I participate in this research study or not.

If I agree to take part in this research study, can I be removed from the study without my consent?

I should also understand that I may be withdrawn from the study by the investigators if they feel my health is in danger, if any untoward findings develop during the course of the study, or if I have failed to follow their instructions.

VOLUNTARY CONSENT:

I certify that I have read the preceding, or it has been read to me, and I understand its contents. Any questions I have pertaining to the research have been, and will continue to be answered by the investigators listed at the beginning of this consent form at the phone numbers given. Any questions I have concerning my rights as a research subject will be answered by the Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (412-578-8570). A copy of this consent form will be given to me. My signature below means that I have freely agreed to participate in this project.

Subject's signature _____ Date and time _____

Witness _____ Date and time _____

INVESTIGATOR'S CERTIFICATION: I declare that I have personally explained the above information to the patient or the patient's legal representative.

Investigator's signature _____ Date _____

APPENDIX B- FAME STUDY GENERAL HEALTH HISTORY

FAME STUDY GENERAL HEALTH HISTORY

Please fill out this questionnaire carefully:

Name _____ Date _____
 Last First M.I.

Street Address _____ Home Phone (____)

City _____ State _____ Zip Code _____ Work Phone (____)

1. Sex M _____ F _____ 2. Age _____ 3. Birthdate ____/____/____
 month day year

4. Current Weight _____ kg. 5. Height _____ cm

6. Race
 1) Caucasian
 2) African American
 3) Hispanic
 4) Asian
 5) Other (please specify)

7. Name and phone number of friend or family member not living with you:

Name _____ Phone Number (____)

8. Please list all medications that you are currently taking:

Medication	Dose	Reason for taking
a. _____	_____	_____
b. _____	_____	_____
c. _____	_____	_____
d. _____	_____	_____
e. _____	_____	_____
f. _____	_____	_____
g. _____	_____	_____

General Health History

9. Do you have or have you ever had any of the following medical conditions?

Condition	Check if yes	Date of Diagnosis (approximately)	Describe the Problem
a. Heart attack			
b. Angina (chest pain on exertion)			
c. Irregular heart rhythm			
d. Heart failure			
e. Fainting spells			
f. Other heart problems			
g. Liver disease			
h. Kidney disease			
i. Chronic diarrhea			
j. Stroke			
k. Substance abuse			
l. Thyroid problems			
m. Kidney stones			
n. Gallstones			
o. Gout			
p. Emotional/psychiatric problems			
q. Diabetes			
r. Cancer			
s. High blood pressure			
t. Circulation Problems			
u. Any other medical problems			

ALCOHOL INTAKE

1. On how many weekdays (Monday through Friday) do you usually drink wine, beer, or liquor?

- | | | | |
|----------------------|-----------|-----------------|-----------|
| Never | _____ (0) | Two days/week | _____ (4) |
| Less than once/month | _____ (1) | Three days/week | _____ (5) |
| 1-2 times/month | _____ (2) | Four days/week | _____ (6) |
| One day/week | _____ (3) | Five days/week | _____ (7) |

2. On those weekdays you drink wine, beer, or liquor how many drinks do you have?

3. On how many weekend days (Saturday & Sunday) do you usually drink wine, beer, or liquor?

- | | | | |
|----------------------|-----------|-----------------------|-----------|
| Never | _____ (0) | One weekend day/week | _____ (3) |
| Less than once/month | _____ (1) | Two weekend days/week | _____ (4) |
| 1-2 times/month | _____ (2) | | |

4. On those weekend days you drink wine, beer, or liquor how many drinks do you have?

FOR WOMEN ONLY

1. Are you currently pregnant? Yes _____ No

2. Do you plan to become pregnant in the next two years? Yes _____ No

3. Have you gone through menopause or the change of life? Yes _____ No

4. Have you had a hysterectomy? Yes _____ No _____ Date of surgery _____ / _____ /
month day year

5. If yes, was the hysterectomy partial (uterus only) or complete (uterus & ovaries)?
partial _____ complete

6. When was your last menstrual period? _____ / _____ /
month day year

7. Do you take: Birth control pills? Yes _____ No

Estrogens (e.g., premarin)? Yes _____ No

Progesterone (e.g., provera)? Yes _____ No

Current Physician:

Name _____

Address _____

City _____ State _____ Zip

Phone Number (____) _____

APPENDIX C-MODIFIABLE ACTIVITY QUESTIONNAIRE

MODIFIABLE ACTIVITY QUESTIONNAIRE

Name _____

Date _____

ID# _____

1. Please circle all activities listed below that you have done more than 10 times in the past year:

- | | | |
|--|---------------------------------------|-----------------------------------|
| Jogging (outdoor, treadmill) 1 | Football/Soccer 14 | Stair Master 27 |
| Swimming (laps, snorkeling) 2 | Racquetball/Handball/Squash . . 15 | Fencing 28 |
| Bicycling (indoor, outdoor) 3 | Horseback riding 16 | Hiking 29 |
| Softball/Baseball 4 | Hunting 17 | Tennis 30 |
| Volleyball 5 | Fishing 18 | Golf (cart) 31 |
| Bowling 6 | Aerobic Dance 19 | Canoeing/Rowing/Kayaking . . 32 |
| Basketball 7 | Water Aerobics 20 | Water Skiing 33 |
| Skating (roller, ice, blading) 8 | Dancing (Square, line, ballrm) . 21 | Jumping rope 34 |
| Martial Arts (karate, judo) 9 | Gardening or Yardwork 22 | Snow Skiing (X-C/Nordic trk) . 35 |
| Tai Chi 10 | Badminton 23 | (downhill) 36 |
| Calisthenics/ Toning exercises 11 | Strength/weight training 24 | Snow shoeing 37 |
| Wood Chopping 12 | Rock climbing 25 | Yoga 38 |
| Water/coal hauling 13 | Scuba diving 26 | Other 39 |
| Walking for exercise (outdoor, indoor at the mall or fitness center, treadmill) 40 | | |

List each activity that you circled in the "Activity" box below. Check the months you did each activity over the past year (12 month) and then estimate the average amount of time spent in that activity.

Activity	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Average # of Times per month	Average # of Minutes each time

2. In general, how many HOURS per DAY do you usually spend watching television? ____ hrs
3. Over this past year, have you spent more than one week confined to a bed or chair as a result of an injury, illness, or surgery? Yes _____ No _____
4. Do you have difficulty doing any of the following activities?
- | | | |
|---|-----------|----------|
| a. getting in or out of a bed or chair? | Yes _____ | No _____ |
| b. walking across a small room without resting? | Yes _____ | No _____ |
| c. walking for 10 minutes without resting? | Yes _____ | No _____ |
5. Did you ever compete in an individual or team sport (not including any time spent in sports performed during school physical education classes)?
If yes, how many total years did you participate in competitive sports? _____
6. Have you had a job for more than one month over this past year, from last _____ to this _____?

List all JOBS that the individual held over the past year for more than one month. Account for all 12 months of the past year. If unemployed/disabled/retired/homemaker/student during all or part of the past year, list as such and probe for job activities of a normal 8 hour day, 5 day week.

Job Name	Job Code	Walk or bicycle to/from work Min/Day	Ave Job Schedule			Hrs. spent sitting at work Hrs Sitting	A	B	C
			Mos/Yr	Day/Wk	Hrs/Day				

Category A
(includes all sitting activities)

Category B
(includes most indoor activities)

Category C
(heavy industrial work, outdoor construction, farming)

Sitting
Standing still w/o heavy lifting
Light cleaning-ironing,
Cooking, washing, dusting
Driving a bus, taxi, tractor
Jewelry making/ weaving
General office work
Occasional/short distance walking

Carrying light loads
Continuous walking
Heavy cleaning-mopping, sweeping
scrubbing, vacuuming
Gardening- planting, weeding
Painting/ Plastering
Plumbing/ Welding
Electrical work
Sheep herding

Carrying moderate to heavy loads
Heavy construction
Farming-hoeing, digging
mowing, raking
Digging ditches, shoveling
Chopping (ax), sawing wood
Tree/ pole climbing
Water/coal/wood hauling

JOB CODES

Not employed outside of the home:

1. Student
2. Home Maker
3. Retired
4. Disabled
5. Unemployed

Employed (or volunteer):

6. Armed Services
7. Office worker
8. Non-office worker

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APPENDIX D-EXERCISE TEST

EXERCISE TEST

_____Study

Name_____ ID#_____ Age_____

Wt._____ Ht._____ APM_____

Resting HR_____ Resting BP_____

<u>Time</u>	<u>Women</u>	<u>HR</u>	<u>BP</u>	<u>RPE</u>	<u>Men</u>
0-2	50 W				100 W
2-4	75 W				125 W
4-6	100 W				150 W
6-8	125 W				175 W
8-10	150 W				200 W
10-12	175 W				225 W
12-14	200 W				250 W
14-16	225 W				275 W

Termination Time_____

Reason for Termination_____

Peak: HR_____ BP_____

Recovery:

_____min HR_____ BP_____

_____min HR_____ BP_____

_____min HR_____ BP_____

NOTES:

APPENDIX E- PALMITATE BLOOD COLLECTION SHEET

FAME STUDY BLOOD COLLECTION SHEET: PALMITATE

IRB# 980276 The Effects of Exercise Training on Skeletal Muscle FFA Utilization During Exercise in the Elderly

Investigators: Bret Goodpaster, PhD (PI) ph: 692-2437 pg: 958-6186
David E. Kelley, MD ph: 692-2158 pg: 958-5200
Silva Arslanian, MD ph: 692-5174
Fernando Boada, PhD ph: 647-9712
Carena Winters-Hart, MS ph: 692-2158 pg: 958-7925
(coordinator)

Subject Name _____ Date _____
Subject ID _____ Visit # _____
Height _____ cm Weight _____ kg BSA _____ m²
Heart Rate (rest) _____ Blood Pressure (rest) _____

*6,6 d2 Glucose infusate 1 ml ALIQUOT saved? _____
6,6 d2 Glucose prime dose of _____ given @ _____
6,6 d2 Glucose infusion started @ _____ stopped @ _____ total vol infused _____ ml
*[d-5] Glycerol infusate 1 ml ALIQUOT saved? _____
[d-5] Glycerol prime dose of _____ given @ _____
[d-5] Glycerol infusion started @ _____ stopped @ _____ total vol infused _____ ml
H₂CO₃ prime dose of _____ given @ _____
*Palmitate infusate 1 ml ALIQUOT saved? _____
Palmitate infusion of _____
started @ _____ stopped @ _____ total vol infused _____ ml

*Make Draw-off Tubes for ¹³C Palmitate, ²H glucose, and ²H glycerol.
Double glucose and glycerol at the start of palmitate infusion

EXERCISE

H₂CO₂ Bolus _____ **Watts**
15 min
30 min
45 min
60 min

Acetate Start: _____
Bike Start: _____ VO₂max= _____ l/min
50%= _____ l/min

BLOOD COLLECTION SHEET

ID# _____

Visit # _____

Sample	Time Due /Drawn	Catechol amines	d ² Glucose	FFA/ Palmitate	Insulin	BreathX2	Glycerol d-5 Glycer Enrichmt	Plasma Glucose/ Lactate
Volume/ Tube		5 ml green top	2.5ml grey top	(1) 5 ml purple	3 ml purple	(2) 10ml red	(1) 5 ml purple	0.3 micro- tube
Background ***See below for background blood draw instructions***								
Bkg								
0 min	Investigators to start 6,6 d2 glucose infusion after verifying that all baseline/background was drawn							
0 min	Investigators to start [d-5] glycerol infusion							
+105 min								
+110 min								
+115 min								
+120 min								
Time reset to "0". Exercise Test begins. PI to start palmitate infusion/administer H2CO3 bolus/double glucose/glycerol								
15EX								
30EX								
45EX								
60EX								

Signature _____ Initials _____

Signature _____ Initials _____

Signature _____ Initials _____

Signature _____ Initials _____

Background Blood Draw Instructions

At time of antecubital line placement (and BEFORE investigators start the 6,6 d2 glucose) all of the following blood samples must be drawn after the Abg has been ok'd by the P.I.

Two 2.5 ml grey tops (as baseline for deuterated glucose) Keep on ice Put small amount in microtube for plasma glucose/lactate analysis

Three 5 ml purple tops (as baseline for FFA/glycerol enrichment, insulin and glycerol) Keep on ice

Additional Info:

All 3 isotopes run until study is over

APPENDIX F- ACETATE BLOOD COLLECTION SHEET

FAME STUDY BLOOD COLLECTION SHEET: ACETATE

IRB# 980276 The Effects of Exercise Training on Skeletal Muscle FFA Utilization During

Exercise in the Elderly

Investigators: Bret Goodpaster, PhD (PI) ph: 692-2437 pg: 958-6186
David E. Kelley, MD ph: 692-2158 pg: 958-5200
Silva Arslanian, MD ph: 692-5174
Fernando Boada, PhD ph: 647-9712
Carena Winters-Hart, MS ph: 692-2158 pg: 958-7925
(coordinator)

Subject Name _____ Date _____
Subject ID _____ Visit # _____
Height _____ cm Weight _____ kg BSA _____ m²
Heart Rate (rest) _____ Blood Pressure (rest) _____

*Acetate infusate 1 ml ALIQUOT saved? _____
Acetate infusion of _____
started @ _____ stopped @ _____ total vol infused _____ ml

*Make Draw-off Tubes for ¹³C Acetate.

EXERCISE

H₂CO₂ Bolus _____ **Watts**
15 min
30 min
45 min
60 min

Acetate Start: _____
Bike Start: _____
VO₂max= _____ l/min
50%= _____ l/min

BLOOD COLLECTION SHEET

ID# _____

Visit # _____

Sample	Time Due	Time Drawn	Acetate	BreathX2	Plasma glucose/lactate
Volume/ Tube			(1) 5ml purple	(2) 10ml red	0.3 ml micro-tube
Background					
0 Exercise begins. PI to administer H2CO3 bolus/ start acetate infusion					
15EX					
30EX					
45EX					
60EX					

Signature _____ Initials _____

Signature _____ Initials _____

Signature _____ Initials _____

Signature _____ Initials _____

Background Blood Draw Instructions

After PI ok's Abg draw two 5 ml purple tops (as baseline for acetate) Keep on ice

Additional Info:

Acetate isotope runs until study is over

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