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Intramuscular lipid oxidation and obesity

Running Head: lipid oxidation and obesity

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Abstract

There is an accumulating amount of evidence indicating that lipid oxidation is depressed in the skeletal muscle of obese individuals. Decrements in fatty acid oxidation (FAO) have been reported with obesity in models ranging from whole-body measurements to isolated skeletal muscle preparations as well as in myotubes raised in culture. This reduction appears to be associated with a depression in the activities of enzymes involved in various steps of lipid oxidation which subsequently partitions lipid entering the cell towards storage. The defect in FAO in skeletal muscle may be critical in relation to health, as a reduction in the capacity for lipid oxidation could directly or indirectly contribute to the insulin resistance commonly evident with obesity. Although less characterized, a decrement in FAO has also been linked with weight gain, which suggests that this characteristic may be an integral aspect leading to the obese state. In terms of intervention, weight loss does not seem to correct the defect in FAO with obesity. This review will provide evidence supporting a reduction in muscle FAO with obesity.

Key Words: Skeletal muscle, metabolism, insulin action

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Introduction

The prevalence of obesity is increasing dramatically in the United States and other industrialized nations (33,51). Obesity is associated with a variety of health-related risks such as coronary heart disease, hypertension, and type 2 diabetes all of which may center around insulin-resistance (i.e. the metabolic syndrome) (39). It is thus essential to identify characteristics that predispose individuals towards obesity or negative conditions that occur during the development of the obese state which subsequently alter metabolism and health status in a negative manner.

Skeletal muscle is a metabolically active tissue that is critical to maintaining whole-body homeostasis due to its energy needs as well as its relatively large mass. In terms of carbohydrate metabolism, a major portion of an ingested glucose load is either stored as glycogen or oxidized in skeletal muscle (14). Skeletal muscle also plays an important role in fatty acid oxidation (FAO). During the fasted state, FAO is the predominant metabolic activity of skeletal muscle (13,51). At rest, the oxidation of lipid contributes significantly to overall energy needs with most of the energy requirements of muscle being obtained via FAO (10,13). Such data indicates that skeletal muscle is quantitatively important in relation to the fate of lipid. Factors that elicit a decrement in the ability to oxidize lipid in skeletal muscle would thus be anticipated to evoke profound changes in whole-body homeostasis. The intent of this paper is to review some of the evidence indicating a decrement in skeletal muscle lipid oxidation with obesity.

Is there a decrement in fat oxidation with obesity?

Determining the mechanisms which are linked with obesity are problematic in that when studying the obese individual, it is difficult to discern if the findings obtained are due to 1) an inherent predisposition to obesity 2) conditions which developed during years of gaining body mass from positive energy balance or 3) both. In relation to the ability to utilize lipid, there are limited prospective data indicating

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that a propensity for weight gain is associated with substrate utilization which favors carbohydrate over lipid oxidation. When discussing whole-body substrate utilization the terms respiratory quotient (RQ) and respiratory exchange ratio (RER) are used interchangeably; regardless of terminology a higher RQ-RER is indicative of a reduced capacity to oxidize lipid. In nondiabetic Pima Indians, Zurlo et al. (54) reported a familial influence on RQ and that a low capacity for fat oxidation was associated with an increased risk for weight gain. Similar findings were obtained by Marra et al. (31) who reported that a higher whole-body RQ predicted weight gain in lean women. Other work has supported the premise that an elevated whole-body RQ-RER is predictive of weight gain in both lean and obese individuals (32,43) although this is not always the case (25). While these findings are somewhat sparse the data suggests that weight gain is linked with an inability to utilize lipid as measured at the whole-body level.

Another experimental method for discerning mechanisms potentially linked with obesity is to examine obese subjects after weight loss. This approach theoretically provides a glimpse of the “pre-obese” state as it has already been demonstrated that the subjects have a propensity towards obesity. A weight-loss model that our laboratory has utilized is studying extremely obese subjects (body mass index ≥ 40 kg/m²) before and approximately one-year or longer after gastric bypass surgery (36). This intervention induces a long-term weight loss of ~50 kg with patients becoming weight-stable at ~12 months after the surgery (36); subjects are thus tested in a relatively stable condition when confounding factors such as energy imbalance are minimized. Using this model, we observed that previously extremely obese women of mixed ethnicities after weight loss still exhibited a preference for carbohydrate over fat oxidation compared to weight-matched individuals who were never extremely obese; substrate utilization was determined at the whole-body level with indirect calorimetry during submaximal exercise (17). This observation was supported by tracking the oxidation of ¹³C-labeled palmitate at rest and during submaximal exercise in 1) lean, 2) extremely obese, and 3) extremely obese

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women after weight loss (48). The relative percentage of fat oxidation was similar in both the extremely obese and extremely obese women after weight loss and significantly reduced compared to the lean subjects (Figure 1); these findings suggest that a reduced capacity for lipid oxidation is linked with a propensity towards extreme obesity.

Other studies have examined the effect of weight loss on substrate utilization in obese individuals at the whole-body level and obtained similar results. Larson et al (30) studied previously obese individuals who lost a mean of 57 kg via energy restriction and found that fat oxidation (as determined with indirect calorimetry) was significantly depressed in the weight-loss (post-obese) group compared to weight-matched controls. Similarly, Kelley et al. (26) reported no change in resting RQ when determining substrate utilization across a skeletal muscle bed in the legs of obese individuals before and after weight loss. In post-obese individuals, a decrement in fat oxidation during submaximal exercise as well as at rest has also been found (for reviews see ref. 4-6).

Another approach to discerning if there is a defect in fat oxidation with obesity at the whole-body level has been to examine the responses to dietary lipid in obese or obese individuals after weight loss. Although the mechanistic link between a reduced capacity for fat oxidation and obesity has not been characterized, one possibility is that an inability to increase fat oxidation when faced with the consumption of dietary fat contributes to positive fat balance, the accumulation of adipose tissue, and eventually weight gain and obesity (16,38). In support of this hypothesis, obese individuals displayed an impaired capacity to oxidize lipid in response to an increase in dietary fat when compared to lean subjects (7,16,41). Individuals examined after weight loss also failed to increase the ratio of fat to carbohydrate oxidation when exposed to a high-fat diet which resulted in positive fat balance (1). Together, these findings imply that a reduced capacity for fat oxidation is evident at the whole-body level with obesity. There are conflicting data, however, which indicates either no difference (46,47,50)

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or an elevated (15,21,24) rate of lipid oxidation in obese compared to lean individuals. An explanation for this disparity may involve factors such as the degree of adiposity in the subjects examined, gender differences, nutritive status during the measurement of lipid oxidation (i.e. fasting or insulin-stimulated), how whole-body fatty acid oxidation is expressed (i.e. per kg body or fat mass), and in exercise studies the intensity of the workload selected (48); however, it is difficult to definitively explain why these differences exist between studies. The intent of the current review is to present and summarize findings indicating lipid oxidation in skeletal muscle is impaired with obesity and allow the reader to use these data to formulate their own hypotheses.

Is there a decrement in the ability of skeletal muscle to oxidize lipid with obesity?

The whole-body measurements discussed above are indicative of a decrement in FAO with obesity, which implies at least a partial involvement of skeletal muscle; in support, direct measurements made in this tissue indicate a decrement in the ability to oxidize lipid. In our laboratory we have observed a reduction in FAO in the skeletal muscle of extremely obese individuals under several different experimental conditions. In one of our first studies, muscle strip preparations were obtained from the rectus abdominus of lean, obese, and extremely obese individuals and incubated with labeled palmitate (22). In muscle strips from extremely obese individuals there was a significant decrease in FAO and increased partitioning of lipid towards storage (22). The finding of a reduction in FAO with obesity was validated in a subsequent study of another muscle group, the vastus lateralis, by obtaining needle biopsies from lean and extremely obese individuals and measuring labeled palmitate oxidation in muscle homogenates (28). The integration of these findings (22,28) provides relatively convincing evidence for a reduction in FAO in the skeletal muscle of extremely obese individuals, as FAO was reduced in two separate muscle groups in different anatomical areas. Validation in distinct muscle groups is critical as

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skeletal muscle is a heterogeneous tissue which can vary widely in respect to fiber composition and contractile activity.

Another approach to study metabolism in skeletal muscle is the use of primary cell cultures. Briefly, this method involves isolating satellite cells from a muscle biopsy and treating the cells so that they proliferate into myoblasts and ultimately differentiate into myotubes (3). The myotubes possess many of the qualities of intact muscle fibers and are suitable for examining substrate utilization. Using this human skeletal muscle cell culture method (HskMC), we compared the capacity for lipid oxidation in cells derived from lean and extremely obese donors and observed that FAO (measured with [1-¹⁴C] palmitate) was depressed in cells from obese subjects (23). The magnitude of the decrement in FAO was similar to our *in-vivo* (17,48) and *in-vitro* (22,28) findings comparing lean and extremely obese individuals (Figure 2). The HskMC technique involves culturing the cells *ex-vivo* for approximately 4-6 weeks; under these conditions it is believed that any expressed phenotype is largely the result of genetic or epigenetic origins (3). If this is the case, our HskMC data (23) implies that extremely obese individuals may have a predisposition towards a reduction in FAO in skeletal muscle. Another study reported that the ability to oxidize lipid in response to an increased lipid load in HskMC mirrored metabolic traits determined *in-vivo* in lean individuals, indicating the potential utility of HskMC in studying lipid oxidation (49).

Other work has indicated a defect in FAO specific to skeletal muscle with obesity. Colberg et al. (11) examined the ability of skeletal muscle to utilize free fatty acids (FFA) with the leg balance technique in lean and obese premenopausal women. These authors (11) reported that women with visceral obesity had a reduced rate of FFA uptake and oxidation in muscle under fasting conditions. Using similar methodology (leg balance), this group confirmed their earlier findings by reporting an elevated leg RQ in obese subjects (26). Utilizing an *in-vitro* muscle preparation, Kirkwood et al. (29) reported a

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statistically significant and negative relationship between maximally-stimulated muscle respiration and body fat content.

The reduction in FAO in the skeletal muscle of obese individuals may be linked, at least in part, with a decreased activity of enzymes involved in lipid oxidation. Although enzymatic activities or protein content may not necessarily reflect substrate flux, Simoneau et al. (45) reported that the activities of carnitine palmitoyl transferase (CPT), citrate synthase (CS), and cytochrome C oxidase (COX) were significantly depressed in skeletal muscle samples (vastus lateralis) obtained from obese compared to lean subjects. These data are significant in that they suggest defects at several key regulatory steps in FAO such as lipid transfer into the mitochondria (CPT), the Krebs Cycle (CS), and the electron transport system (COX). Several studies have confirmed reductions in CPT 1, CS, and COX in the skeletal muscle of obese individuals (19,26,28); similarly, others (27,40) have reported a reduction in the activity of the respiratory chain in skeletal muscle from obese individuals. Ratios of enzyme activities indicative of glycolytic to oxidative flux such as glycogen phosphorylase to CS (44) and lipid oxidation to lipid transfer (CPT to fatty acid binding protein) (45) are also supportive of a reduced capacity for lipid oxidation in skeletal muscle with obesity. Zurlo et al. (55) reported a negative relationship between 24 h RQ and β -hydroxyacyl-coenzyme A dehydrogenase (β -HAD) activity, which implies that variability exists in the process of β -oxidation which may influence the capacity for FAO. In support of a defect in β -oxidation, we reported a reduction in β -HAD activity in the skeletal muscle of extremely obese individuals (28). Together, these data provide evidence for a reduction in the activities of key oxidative enzymes involved in FAO in the skeletal muscle of obese individuals, which is unfortunately not corrected with weight loss (26,45). While the mechanism(s) responsible for the depression in enzyme activity is not evident, peroxisome-proliferator-activated receptor-gamma co-activator 1 alpha (PGC1 α)

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which is an important regulator of mitochondrial biogenesis, has been reported to be lower in the skeletal muscle of obese, insulin-resistant subjects compared to lean controls (19).

In addition to reductions in enzyme activities linked with FAO, there are also alterations in mitochondrial morphology with obesity. Kirwood et al. (29) demonstrated that the central distribution of body fat was associated with a lower mitochondrial density, as determined by electron microscopy (EM). Other work using EM (27) indicated that skeletal muscle mitochondria were smaller by ~35% in type 2 diabetic and obese compared to lean subjects. The mitochondria from the obese and diabetic subjects also had a less well-defined inner membrane structure including a narrower cristae (27). Similar alterations in mitochondrial morphology, such as reduced size, are evident in myopathies in which the function of this organelle is compromised (52). There may also be an alteration in the distribution of mitochondria within the skeletal muscle of obese individuals, as the number of subsarcolemmal mitochondria has been reported to be reduced with obesity and type 2 diabetes along with decreased electron transport chain activity (40). It is believed that one of the primary duties of subsarcolemmal mitochondria is to oxidize lipid (20), the reduction of mass in this fraction coupled with reduced enzymatic activity per unit of mitochondria with obesity (40) may provide an underlying mechanism for the decrement in FAO.

While the above findings indicate a defect in oxidative processes, there also appears to be a congruent increase in the capacity for lipid storage in the skeletal muscle of obese individuals, which would contribute to an accumulation of intramuscular lipid. For example, Simoneau et al. (45) reported elevated levels of fatty acid binding protein (FABP) in the plasma membrane fraction of muscle from obese individuals. Bonen et al. (8) studied giant sarcolemmal vesicles prepared from skeletal muscle biopsies and reported that long chain fatty acid transport into skeletal muscle was up-regulated by ~4-fold with obesity. This increase in fatty acid transport was associated with an elevated level of

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FAT/CD36 (fatty acid translocase) in the sarcolemmal fraction (8). We reported (23) that stearoyl-CoA desaturase 1 (SCD1), a lipogenic gene, was robustly up-regulated in the skeletal muscle of extremely obese individuals and that this characteristic was retained in culture (HskMC). Overexpression of SCD1 in myotubes from lean subjects increased fatty acid esterification and depressed FAO, suggesting a potential dual regulation of lipid-handling pathways by SCD1. Together, the findings of a depression in FAO coupled with an increased capacity for lipogenesis indicates a global organization of lipid metabolism in obese skeletal muscle that is biased towards storage and esterification (Figure 3).

Some insight into the mechanisms responsible for the decrement in FAO in skeletal muscle with obesity may be obtained by studying the effects of interventions. Weight loss does not seem to alter fat oxidation in the skeletal muscle of obese individuals (17,26,30,45,48). On the other hand, contractile activity, i.e. physical activity or exercise training has been reported to enhance the capacity for lipid oxidation in the skeletal muscle of obese subjects. We observed that a short-term endurance-oriented training model which did not induce weight loss (10 consecutive d, 60 min · day⁻¹ @ 75% VO_{2peak}) improved fatty acid oxidation in the skeletal muscle of morbidly obese individuals (2). Menshikova et al. (33) reported that in obese individuals moderate physical activity combined with weight loss increased oxidative enzyme activities without a significant increase in mitochondrial DNA, although there was evidence for an increase in mitochondrial cristae. This pattern differs from the classical mitochondrial biogenesis seen with physical activity in lean individuals (33). Together, these findings suggest that some aspect linked with contractile activity either directly overcomes or somehow compensates for the decrement in FAO seen in the skeletal muscle of obese individuals. The exact mechanism(s) responsible, however, remain to be discerned.

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Possible interactions between fatty acid oxidation and insulin action with obesity

A health concern with obesity is the presence of insulin resistance. The co-existence of insulin resistance, intramuscular lipid accumulation, and a decrement in FAO in skeletal muscle are often simultaneously evident in obese individuals (for reviews see 4,6,35). However, the mechanistic link between FAO and insulin resistance in the skeletal muscle of obese individuals is not yet apparent.

There is evidence suggesting that the accumulation of lipid within the skeletal muscle of obese individuals induces insulin resistance. Several studies suggest that fatty-acid derived metabolites such as long chain acyl CoA (LCACoA), diacylglycerol (DAG), and ceramide accumulate in the cytosol of the skeletal muscle of obese individuals. These intermediates then either directly or indirectly impair insulin signal transduction and/or the activity of enzymes involved in glucose utilization which in turn induces insulin resistance (for reviews see 12,18,36). The accumulation of these metabolically active lipid intermediates could be due, at least in part, to the disturbances in mitochondrial function and depression in FAO that may occur with obesity.

There are also findings which suggest that the capacity for FAO alone controls insulin action in skeletal muscle. When overexpressing CPT1 in L6 myotubes, Perdomo et al. (35) reported an increase in palmitate and oleate oxidation which provided a protective effect against fatty-acid induced insulin resistance. The intriguing finding of this study (35) was that CPT1 overexpression improved insulin action despite no decrease in intracellular DAG, LCACoA, or ceramide, compared to lipid-treated control cells that were insulin resistant; this observation prompted the hypothesis that accelerating FAO exerts an insulin-sensitizing effect that functions independently of changes in cytosolic lipid content. In support, *in-vivo* human findings indicate that the oxidative capacity of skeletal muscle is an effective predictor of whole-body insulin action (9). However, other work utilizing HSkMC reported that CPT1 overexpression was protective against the accumulation of DAG, ceramide and insulin resistance (42).

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While the cellular mechanism(s) responsible for lipid-induced insulin resistance remain to be discerned, the findings remain supportive of the integral role that FAO in skeletal muscle plays in controlling insulin action.

Perspectives and Significance

There is accumulating evidence indicating a defect in the capacity for FAO in the skeletal muscle of obese individuals. The broad implications of this finding is that such a defect may contribute to 1) the insulin resistance often evident with obesity and 2) the weight gain inherent with the obese state. The mechanisms linking the obesity-associated reduction in lipid oxidation with insulin-resistance and weight gain may be a fruitful area for future studies. For example, it is not evident why the skeletal muscle of obese individuals exhibits a depression in the activities of enzymes involved in various steps of lipid oxidation and how this alteration contributes to the partitioning of lipid entering the cell towards storage. It is also not apparent how interventions, such as physical activity, overcome or somehow compensate for the decrement in FAO seen in the skeletal muscle of obese individuals. In conclusion, a reduction in the capacity for lipid oxidation in the skeletal muscle of obese individuals may be an integral component of the obese state.

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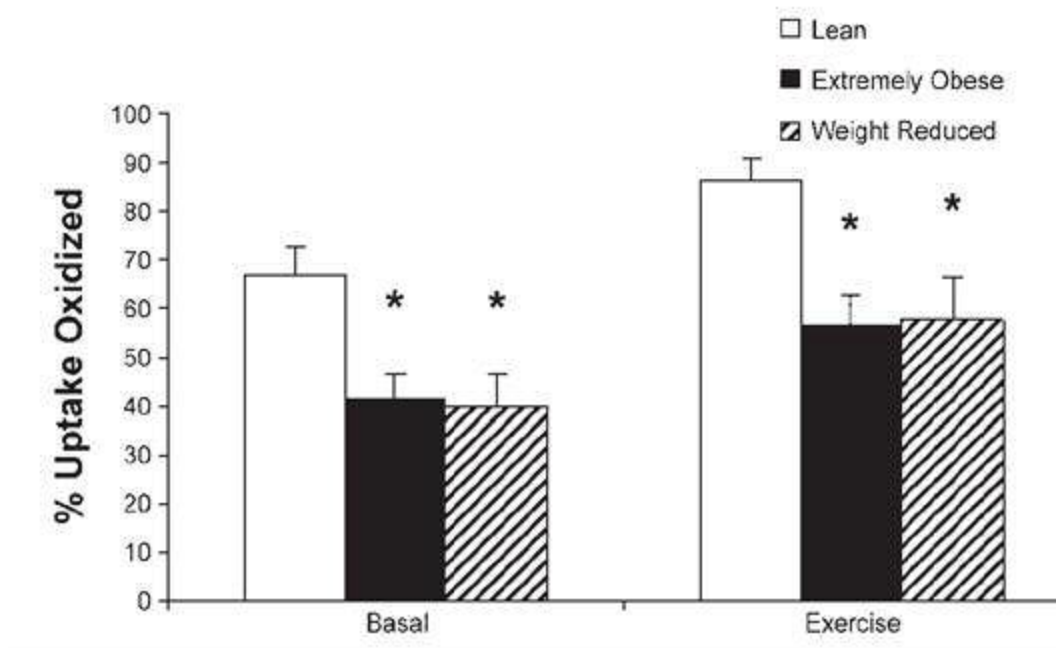
Figure Legends

Figure 1. Percentage of plasma free fatty acid (FFA) uptake oxidized during basal and exercise (50% maximum oxygen consumption). *Significantly decreased in extremely obese and extremely obese subjects after weight reduction (weight-reduced) compared with lean ($P < 0.05$). From Thyfault et al (48).

Figure 2. Decrement in fatty acid oxidation with obesity as indicated by various methodologies used in our laboratory. Data are redrawn from references 17,22,23,28,48.

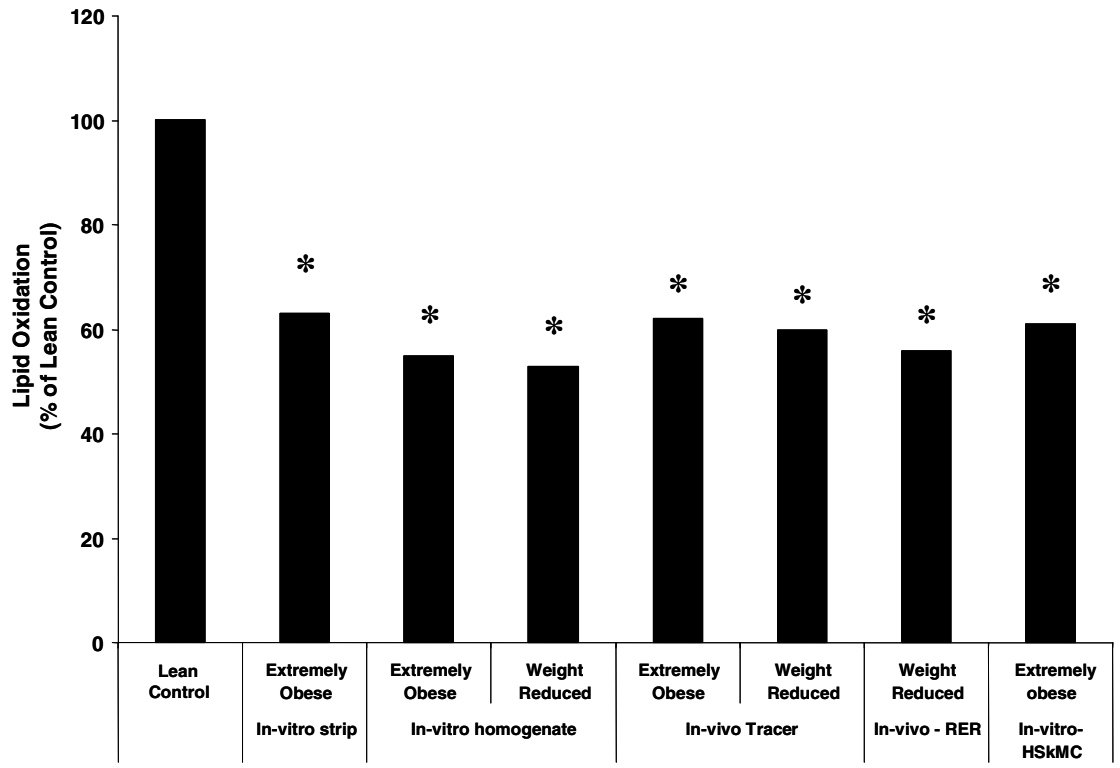
Figure 3. Fatty acid partitioning (labeled oleate incorporation into triacylglycerol (TAG) divided by fatty acid oxidation (FAO)) in myotubes cultured from skeletal muscle samples obtained from lean and obese donors (23). An increased fatty acid partitioning ratio indicates preferential partitioning towards storage within the muscle cell as opposed to oxidation. The positive relationship between the FAO partitioning index of the myotubes and the BMI of the donor was statistically significant ($r^2 = 0.66$, $p < 0.001$)

Revision II

Figure 1.

Revision II

Figure 2.



Revision II

Figure 3.