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Skeletal muscle lipid metabolism with obesity

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Hulver, Matthew W., Jason R. Berggren, Ronald N. Cortright, Ronald W. Dudek, R. Peter Thompson, Walter J. Pories, Kenneth G. MacDonald, Gary W. Cline, Gerald I. Shulman, G. Lynis Dohm, and Joseph A. Houmard. Skeletal muscle lipid metabolism with obesity. *Am J Physiol Endocrinol Metab* 284: E741–E747, 2003. First published December 27, 2002; 10.1152/ajpendo.00514.2002.—The objectives of this study were to 1) examine skeletal muscle fatty acid oxidation in individuals with varying degrees of adiposity and 2) determine the relationship between skeletal muscle fatty acid oxidation and the accumulation of long-chain fatty acyl-CoAs. Muscle was obtained from normal-weight ($n = 8$; body mass index (BMI) 23.8 ± 0.58 kg/m²), overweight/obese ($n = 8$; BMI 30.2 ± 0.81 kg/m²), and extremely obese ($n = 8$; BMI 53.8 ± 3.5 kg/m²) females undergoing abdominal surgery. Skeletal muscle fatty acid oxidation was assessed in intact muscle strips. Long-chain fatty acyl-CoA concentrations were measured in a separate portion of the same muscle tissue in which fatty acid oxidation was determined. Palmitate oxidation was 58 and 83% lower in skeletal muscle from extremely obese (44.9 ± 5.2 nmol·g⁻¹·h⁻¹) patients compared with normal-weight (71.0 ± 5.0 nmol·g⁻¹·h⁻¹) and overweight/obese (82.2 ± 8.7 nmol·g⁻¹·h⁻¹) patients, respectively. Palmitate oxidation was negatively ($R = -0.44$, $P = 0.003$) associated with BMI. Long-chain fatty acyl-CoA content was higher in both the overweight/obese and extremely obese patients compared with normal-weight patients, despite significantly lower fatty acid oxidation only in the extremely obese. No associations were observed between long-chain fatty acyl-CoA content and palmitate oxidation. These data suggest that there is a defect in skeletal muscle fatty acid oxidation with extreme obesity but not overweight/obesity and that the accumulation of intramyocellular long-chain fatty acyl-CoAs is not solely a result of reduced fatty acid oxidation.

long-chain fatty acyl-coenzyme A; intramyocellular triacylglycerol; fatty acids

THE PREVALENCE OF OVERWEIGHT/OBESITY and insulin resistance is continually increasing and is associated with increased risk for the development of non-insulin-dependent diabetes mellitus (NIDDM), hypertension, and cardiovascular disease (5, 11, 24). The cellular mechanisms responsible for insulin resistance with overweight and

obesity are not yet clear. Data have shown that intramyocellular triacylglycerols (IMTG) are increased with obesity and NIDDM (14, 19, 21). In addition, the accumulation of IMTG is associated with skeletal muscle insulin resistance (3, 13, 15, 19, 23, 28, 29, 31, 36, 39). It is believed, however, that the accumulation of IMTG is not the direct cause of the development of insulin resistance but that IMTG is an inert marker for the presence of other lipid intermediates (diacylglycerol, fatty acyl-CoAs, or ceramide, etc.), which have been directly linked to defects in insulin signaling (8, 17, 25, 32, 37).

To date, the mechanism(s) responsible for the accretion of IMTG and intermediates of lipid metabolism in intact skeletal muscle are not evident. Two possibilities include an increase in lipid synthesis and/or a reduction in fatty acid oxidation, both of which may result in the accumulation of IMTG and other intermediates of lipid metabolism. A previous study from our laboratory examining muscle homogenates from vastus lateralis muscle (22) demonstrated reduced fatty acid oxidation in obese skeletal muscle compared with lean controls. Although the homogenate technique provides valuable evidence indicating that mitochondrial fatty acid oxidative capacity is decreased, it does not allow us to determine several other rate-limiting steps in lipid metabolism, such as sarcolemmal and cytoplasmic fatty acid transport, and how these processes may have an impact on lipid partitioning to storage or oxidation. Thus, to more clearly characterize a defect in fatty acid metabolism with obesity, we have used intact human muscle strips (rectus abdominus) and compared fatty acid oxidation and lipid storage characteristics in normal-weight, overweight/obese, and extremely obese patients. In addition, skeletal muscle long-chain fatty acyl-CoA concentrations were measured, and the relationships among long-chain fatty acyl-CoA content, fatty acid oxidation, and fatty acid incorporation into intramyocellular lipid pools were examined. We hypothesized that a defect in skeletal muscle fatty acid oxidation with obesity would contribute to the accumulation of intramyocellular long-chain fatty acyl-CoAs.

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RESEARCH DESIGN AND METHODS

Human Subjects

Twenty-four females participated in this investigation, which consisted of eight normal-weight [age 45.1 ± 3.1 yr; body mass index (BMI) 23.8 ± 0.58 kg/m²], eight overweight/obese (age 44.0 ± 2.8 yr; BMI 30.2 ± 0.81 kg/m²), and eight extremely obese (age 37.9 ± 3.3 yr; BMI 53.8 ± 3.5 kg/m²) patients undergoing abdominal surgery, primarily gastric bypass and total abdominal hysterectomy. Research participants were categorized into their respective groups on the basis of BMI and the classifications of overweight and obesity set forth by the National Institutes of Health (26). BMI inclusionary criteria for the normal-weight, overweight/obese, and extremely obese subjects were ≤ 24.9 , 25.0–34.9, and ≥ 40 kg/m², respectively. The experimental protocol was approved by the East Carolina University Policy and Review Committee on Human Research, and informed consent was obtained from all patients. None of the subjects had any diseases or was taking any medications known to alter carbohydrate or lipid metabolism. All subjects had maintained a constant body mass during the year preceding surgery. After an overnight fast (12–18 h), general anesthesia was initiated with a short-acting barbiturate and maintained with fentanyl and a nitrous oxide-oxygen mixture.

Muscle Strip Incubation

Immediately after surgical removal, the rectus abdominus muscle sample was placed in a sealed container with oxygenated ice-cold Krebs-Henseleit buffer for transport to the laboratory. Muscle strips were incubated in a modified incubation system, as previously described (7). Briefly, muscle strips weighing ~25 mg were teased from the biopsy sample and clamped in lucite clips to maintain consistent resting muscle length and tension throughout the preparation. The clipped muscle strips were immediately placed in 3.0 ml of warmed (30°C) Krebs-Henseleit buffer gassed with 95% O₂-5% CO₂ (pH = 7.4) and containing 4% bovine serum albumin (fatty acid free, Sigma Chemical, St. Louis, MO), 5 mM glucose (Sigma Chemical), and 1 mM palmitate (Sigma Chemical). Palmitic acid was dissolved in ethanol, and a small volume (0.8% of total buffer volume) was added to the incubation buffer to achieve the final desired palmitate concentration.

After a 30-min preincubation period, muscle samples were incubated for 1 h at 30°C in the same incubation medium previously specified, with the addition of 0.75 μ Ci of [1-¹⁴C]palmitate (New England Nuclear, Boston, MA). This permitted monitoring of exogenous palmitate oxidation and incorporation of palmitate into endogenous lipid pools. In an effort to eliminate contamination of the analyses of endogenous lipid pools, all visible extramyocellular lipid was carefully teased from the muscle strips.

Palmitate Oxidation and Incorporation into Muscle Lipids

Oxidation. Total palmitate oxidation was determined by measuring and summing ¹⁴CO₂ production and ¹⁴C-labeled water-soluble metabolites. The measurement of ¹⁴C-water-soluble metabolites accounted for any ¹⁴C label that did not result in ¹⁴CO₂ due to isotopic exchange in the tricarboxylic acid cycle.

Gaseous ¹⁴CO₂ produced from the oxidation of [1-¹⁴C]palmitate during the incubation was measured by transferring 1.0 ml of the incubation medium to a 20-ml glass scintillation vial containing 1.0 ml of 1 M H₂SO₄ and a 0.5-ml Fisher

microcentrifuge tube containing 400 μ l of benzethonium hydroxide. Liberated ¹⁴CO₂ was trapped in the benzethonium hydroxide for 60 min, and the microcentrifuge tube containing trapped ¹⁴CO₂ was placed in a scintillation vial and counted. ¹⁴C-water-soluble metabolites were measured by sampling 0.5 ml of aqueous phase of the lipid extraction (explained in *Extraction of muscle lipids*), which was placed in a scintillation vial and counted.

Extraction of muscle lipids. After their incubation, muscles were blotted and weighed, placed in 13-ml plastic centrifuge tubes containing 1.5 ml of ice-cold 1:2 chloroform-methanol (vol/vol), and homogenized with a polytron (Brinkman Instruments, Mississauga, ON, Canada). An additional 0.5 ml of 100% chloroform was added, and the sample was rehomogenized. Samples were then centrifuged at 2,000 g (4°C) for 10 min, and the chloroform supernatant containing the lipids was transferred to a clean glass tube. Two milliliters of 1:1 chloroform-methanol (vol/vol) were added to the original pellet, which was vortexed and recentrifuged, and the supernatant was added to the previous aliquot. Two milliliters of deionized H₂O were added to the combined supernatants, which were then shaken for 10 min followed by 10 min of centrifugation. The aqueous phase was carefully sampled for the determination of ¹⁴C-water-soluble metabolites, and the remainder was aspirated without disturbing the lower chloroform phase. The chloroform phase was transferred to a clean glass tube, gently evaporated under a stream of 100% N₂, and redissolved in 100 μ l of 2:1 chloroform-methanol with the addition of phosphatidylcholine, palmitate, and mono-, di-, and tripalmitin (Sigma Chemical). Fifty microliters of each sample were spotted in respective lanes on an oven-dried silica gel plate (Silica Gel GF, 250 μ m; Analtech, Newark, DE), and the plates were placed in a sealed tank containing solvent (60:40:3, heptane-isopropyl ether-acetic acid) for ~40 min. Plates were air dried for 1 h and placed in a sealed tank containing iodide crystals, which permitted visualization of the bands depicting phospholipids (PL), monoacylglycerols (MG), diacylglycerols (DG), free fatty acids (FFA), and triacylglycerols (TG). The individual lipid bands were marked on the plate and scraped into vials for liquid scintillation counting. Eighty-five to ninety percent of the ¹⁴C label incorporated into the lipid pools of the muscle samples was recovered in the PL, MG, DG, FFA, and TG bands. Data are presented for palmitate incorporation into IMTG and the sum of palmitate incorporation into all lipid fractions (TG, DG, FA, MG, and PL), which is referred to as intramyocellular lipid content (IMLC).

Oil Red-O Analyses

Due to the incorporation pattern of [¹⁴C]palmitate into IMTG and IMLC, an additional experiment was performed to examine total IMLC (TLC) in a locomotive muscle in a separate set of female subjects. Oil Red-O analysis (30) was performed on vastus lateralis biopsy samples from normal-weight ($n = 6$, BMI 22.1 ± 0.8 kg/m²), overweight/obese ($n = 6$, BMI 32.9 ± 0.8 kg/m²), and extremely obese ($n = 7$, BMI 42.8 ± 0.9 kg/m²) females.

Muscle samples were obtained by biopsying the vastus lateralis muscle with a Bergstrom needle and a suction syringe, with subjects under local anesthesia. The biopsy samples were fractionated and prepared for bright-field microscopy and Oil Red-O analyses. Briefly, muscle samples were fixed in 10% buffered Formalin for 24 h followed by a 24-h incubation in 30% sucrose. Samples were mounted in an optimal cutting temperature compound (OCT)·tragacanth gum mixture and frozen in isopentane cooled over liquid N₂.

Muscle samples were sectioned in 10- μ m longitudinal slices and placed in a 60% isopropyl alcohol solution for 1 s followed by a 1-h incubation in Oil Red-O stain. Samples were then briefly (1–3 s) rinsed with 60% isopropyl alcohol and washed for 3 min under running tap water. A counterstain in Harris's hematoxylin was performed for 5 min, which was followed by a 3-min wash under running tap water, a 3-min wash with lithium chloride, and an additional 5-min tap water wash.

The slides were viewed by utilizing a Nikon Microphot FX microscope (Nikon, Tokyo, Japan) at a magnification of $\times 10$ and quantified with Spot Advanced 3.2.4 (Diagnostic Instruments, Sterling Heights, MI) and Image-Pro Plus 4.1 (Media Cybernetics LP, Silver Spring, MD). Spot Advanced 3.2.4 was used to capture and digitalize images from the light microscopy slides. The images were saved as a tagged image format file (TIFF-32 bit). Image-Pro Plus 4.1 was used to quantify the TLC in the digitalized images. Briefly, the image was magnified to enable viewing of the individual pixels that indicate areas of Oil Red-O staining. By use of a pointer tool, red-stained pixels were highlighted, which initiated an automated program response that highlighted other pixels in the image with same intensity of stain. After this procedure was repeated several times, the image was demagnified to its original size and inspected to see whether all areas of staining had been accounted for. At this time, the image was also inspected to make sure that no extramyocellular lipid or artifacts of staining had been erroneously highlighted. In addition, the total area of skeletal muscle was also determined by utilizing the same procedure.

Fatty Acyl-CoA Analyses

From the same rectus abdominus sample in which the incubated samples were obtained, a separate sample (50–100 mg) was fractionated, and all visible fat and/or connective tissue was removed and frozen in liquid N_2 for subsequent analyses. Long-chain fatty acyl-CoAs were extracted from the muscle sample by solid-phase extraction, and 17-carbon CoA was added as an internal standard, as previously described (2, 27). A tandem mass spectrometer API 3000 (Perkin-Elmer Sciex) interfaced with a TurboIonSpray ionization source was used for MS-MS analysis (27). Fatty acyl-CoAs were ionized in a negative electrospray mode, and the transition pairs $[M-^2H]^{2-}/[M-H-80]^-$ were monitored in multiple reaction monitoring mode. Doubly charged ions and corresponding product ions (precursor minus phosphate group) were chosen as a transition pair for multiple-reactions monitoring for quantitation (27). Total long-chain fatty acyl-CoA content was calculated as the sum of the long-chain fatty acyl-CoA species measured.

Calculations and Statistics

The absolute quantity of palmitate oxidized (nmol) was determined by measuring the radioactivity (dpm) in vials containing the trapped $^{14}CO_2$ and ^{14}C -water-soluble metabolites. On the basis of specific activity of labeled palmitate in the incubation medium [i.e., radiolabeled palmitate (dpm)/total palmitate (nmol)], dpm were converted to total palmitate oxidized. The amount of palmitate incorporated into endogenous lipid pools was calculated in the same manner after the radioactivity in the various lipid fractions was determined. A ratio of palmitate incorporation to palmitate oxidation was utilized to examine differences in skeletal muscle fatty acid partitioning between groups. All data are reported as means \pm SE. Results were analyzed using analysis of variance (ANOVA) procedures, and Tukey's post hoc test was used to test significant differences revealed by the

ANOVA. Pearson correlational analyses were used to examine associations among palmitate metabolism, subject characteristics, and fatty acyl-CoA content. Significance was accepted at $P < 0.05$.

RESULTS

Subject Characteristics

BMI was significantly different among the normal-weight, overweight/obese, and extremely obese subjects. Relative to the normal-weight group, the BMIs for the overweight/obese and extremely obese groups were 27 and 126% higher, respectively. The BMI of the extremely obese group was 78% higher than that of the overweight/obese group.

Palmitate Oxidation and Incorporation into Muscle Lipids

Palmitate oxidation and incorporation have been expressed relative to gram tissue wet weight. Previously, our laboratory (7) has shown that intact rectus abdominus muscle strips from normal-weight and extremely obese subjects did not differ in protein, fat, or water content. Thus we are confident that expressing our results relative to gram tissue wet weight provides the same result as would expressing our data relative to milligram protein. Palmitate oxidation (Fig. 1) was 58 ($P = 0.011$) and 83% ($P = 0.001$) lower in the extremely obese patients compared with the normal-weight and overweight/obese patients, respectively. No differences in palmitate oxidation were observed between the normal-weight and overweight/obese groups. Palmitate oxidation was negatively associated ($P = 0.03$, $R = -0.44$) with BMI.

No statistically significant differences were observed between groups for palmitate incorporation into IMTG or IMLC; however, palmitate incorporation into IMTG was 40 and 37% higher in the extremely obese compared with the normal-weight and overweight/obese patients, respectively. IMLC was 22% higher in the extremely obese compared with the normal-weight and overweight/obese patients, respectively. Palmitate incorporation into IMTG ($R = 0.54$, $P = 0.007$)

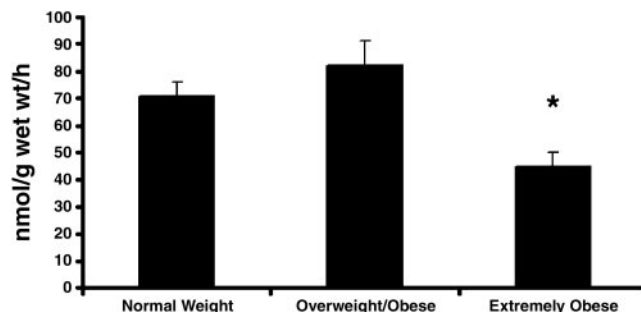


Fig. 1. ^{14}C palmitate oxidation in rectus abdominus strips from normal-weight, overweight/obese, and extremely obese females. Data are expressed as means \pm SE. *Extremely obese have statistically ($P < 0.05$) lower palmitate oxidation than normal weight and overweight/obese.

and IMLC ($R = 0.53$, $P = 0.009$) was significantly correlated with BMI.

The extremely obese patients exhibited a 94 and 105% higher fatty acid partitioning ratio (ratio between incorporation and oxidation) compared with the normal-weight and overweight/obese patients, respectively (Fig. 2). No differences in fatty acid partitioning were observed between the normal-weight and overweight/obese groups.

Oil Red-O Analyses

There were no differences in TLC (Fig. 3) between the normal-weight and overweight/obese obese patients. The skeletal muscle from the extremely obese patients contained 224 and 201% more TLC compared with the normal-weight and overweight/obese patients, respectively.

Long-Chain Fatty Acyl-CoA Analyses

Long-chain fatty acyl-CoA data are presented in Fig. 4. Concentrations of palmitoyl-CoA (C16:0), oleate-CoA (C18:1), linoleate-CoA (C18:2), and total fatty acyl-CoAs were significantly ($P < 0.05$) higher in both the overweight/obese and extremely obese skeletal muscle compared with normal-weight muscle. Compared with normal-weight skeletal muscle, palmitoleate-CoA (C16:1) was significantly higher only in the extremely obese, and stearoyl-CoA (C18:0) was significantly higher only in the overweight/obese. No significant differences were observed between overweight/obese and extremely obese skeletal muscle for total long-chain fatty acyl-CoAs or any long-chain fatty acyl-CoA subfraction. In addition, there were no associations between palmitate oxidation and incorporation with total long-chain fatty acyl-CoAs or any long-chain fatty acyl-CoA subfraction (data not shown).

DISCUSSION

There were two major findings from the present study. 1) *In vitro* palmitate oxidation is significantly reduced in the skeletal muscle of extremely obese patients; and 2) concentrations of total long-chain fatty acyl-CoAs and long-chain fatty acyl-CoA subfractions

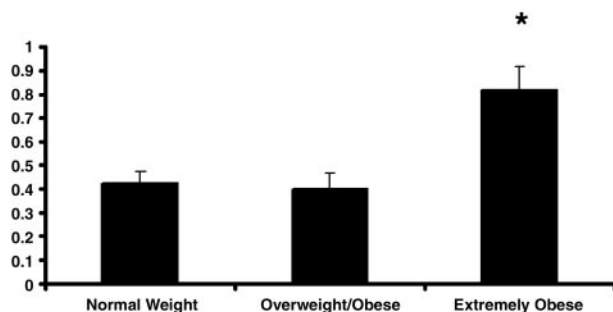


Fig. 2. Fatty acid partitioning (ratio of palmitate incorporation to palmitate oxidized) in rectus abdominus strips from normal-weight, overweight/obese, and extremely obese females. Data are expressed as mean ratios \pm SE. *Extremely obese have a significantly higher partitioning ratio ($P < 0.05$) compared with the normal weight and overweight/obese.

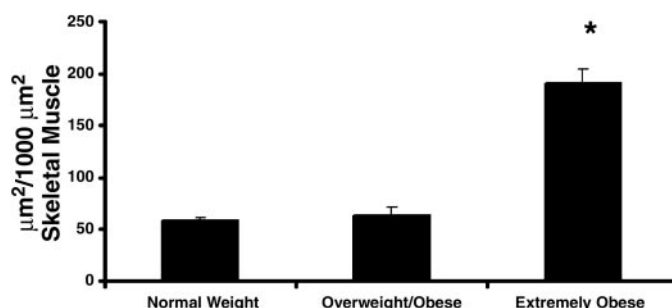


Fig. 3. Intramyocellular lipid content in vastus lateralis based on Oil Red-O analyses. Data are expressed as means \pm SE. *Extremely obese have statistically ($P < 0.05$) greater total intramyocellular lipid content than the normal weight and overweight/obese.

were significantly higher in overweight/obese and extremely obese patients compared with normal-weight patients despite a reduction in fatty acid oxidation only in the extremely obese.

The reduced palmitate oxidation observed with extreme obesity in the present study appears to be in conflict with previously reported data (10, 34, 35, 38). Steinberg et al. (34), using an *in vitro* model very similar to that used in the current investigation, reported no reduction in palmitate oxidation in intact muscle strips from obese women. Also, whole body calorimetry data indicate that the reliance on fat for energy is either greater (10, 38) or not different (35) in obese individuals compared with lean controls. The disparity between the results from the present study and those of others (10, 34, 35, 38) may possibly be explained by the degree of adiposity of the obese subjects investigated. The mean BMI for the overweight/obese and extremely obese groups from the current investigation were 30 and 54 kg/m^2 , respectively, compared with a BMI of 32 kg/m^2 for the obese group from Steinberg et al. Palmitate oxidation was not different between the overweight/obese and normal-weight skeletal muscle in the present study, which is in agreement with Steinberg et al. The obese groups examined in whole body calorimetry studies (10, 35, 38) were very similar, with respect to adiposity, to the obese group

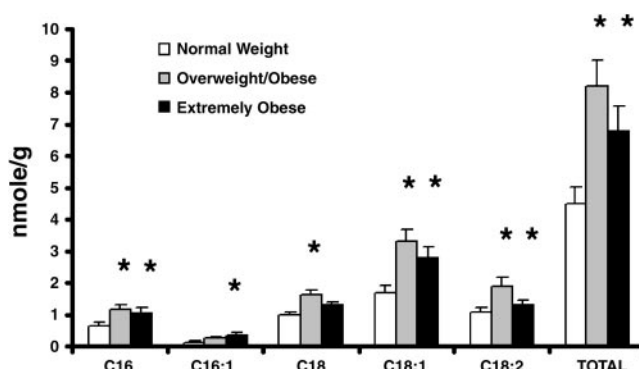


Fig. 4. Fatty Acyl-CoA concentrations: palmitoyl-CoA (C16:0), palmitoleate (C16:1), stearoyl-CoA (C18:0), oleate-CoA (C18:1), linoleate-CoA (C18:2). Data are expressed as means \pm SE. *Statistically different ($P < 0.05$) from normal weight. No differences were observed between overweight/obese and extremely obese.

from Steinberg et al. and the overweight/obese group from the present study. Thus it is not our intention to dispute the results of previous investigations (10, 34, 35, 38), as the data from the current study support the findings of no defect in fatty acid oxidation with moderate obesity; however, we have demonstrated that fatty acid oxidation is reduced with extreme obesity. A previous study from our laboratory (22) provides additional support for reduced fatty acid oxidation with extreme obesity but not moderate obesity. Kim et al. (22) examined fatty acid oxidation in vastus lateralis muscle homogenates in lean (BMI 23.8 kg/m²) and obese (BMI 38 kg/m²) women and observed an ~100% reduction ($P < 0.05$) in fatty acid oxidation with obesity. However, when the subjects (see Fig. 2A in Ref. 22) were divided into normal-weight (BMI <24.9 kg/m²), overweight/obese (BMI 25–35 kg/m²), and extremely obese (BMI >35 kg/m²) groups, fatty acid oxidation was not different between the normal-weight and overweight/obese groups but was 276% lower in the extremely obese. Together, our data (present study and Kim et al.) and those of others (10, 33, 35, 38) suggest that fatty acid oxidation is lower in skeletal muscle of extremely obese, but not moderately obese individuals.

A potential mechanism for the reduction in fatty acid oxidation with extreme obesity may be a blunted oxidative capacity. This notion is supported by previous work from our laboratory (22) in which two enzymes known to be rate-limiting steps in fatty acid oxidation (β -hydroxyacyl dehydrogenase and citrate synthase) were demonstrated to have significantly lower activity in rectus abdominus muscle tissue from extremely obese subjects compared with normal-weight controls. In addition, the activity of carnitine palmitoyltransferase I, which is a rate-limiting step for entry of fatty acyl-CoAs into the mitochondria for oxidation, was also significantly lower in extremely obese skeletal muscle compared with nonobese controls (21). Furthermore, Hickey et al. (18) demonstrated that the percentage of type I muscle fibers in rectus abdominus tissue was significantly lower in extremely obese compared with normal-weight subjects. Thus it appears that the reduction in fatty acid oxidation observed with extreme obesity may be due, at least in part, to a decrement in skeletal muscle oxidative capacity.

The accumulation of IMTG is associated with skeletal muscle insulin resistance (3, 13, 15, 19, 23, 28, 29, 31, 36, 39). Kelly and Goodpaster (20) have proposed that the accumulation of TG within obese skeletal muscle is due to a defect in fatty acid oxidation. The findings from the present investigation demonstrate that, in the presence of reduced skeletal muscle fatty acid oxidation in extremely obese patients, there was a trend for greater (37–40%) palmitate incorporation into IMTG compared with the normal-weight and overweight/obese patients. Furthermore, using Oil Red-O analyses in a separate set of female subjects, we observed significantly greater IMLC in vastus lateralis muscle tissue from extremely obese patients compared with normal-weight and overweight/obese patients. In

addition, we examined the ratio of palmitate incorporation to palmitate oxidation in an effort to determine whether extremely obese patients preferentially partition fatty acids toward storage and away from oxidation. The ratio was 95–105% higher in the extremely obese patients relative to the normal-weight and overweight/obese patients. Our data support the hypothesis that a defect in skeletal muscle fatty acid oxidation may result in the accumulation of IMTG. Little work has been done to examine TG synthesis and hydrolysis in skeletal muscle with human obesity. The possibility exists that not only is fatty acid oxidation reduced, but perhaps TG synthesis and hydrolysis are upregulated and downregulated, respectively, with extreme obesity.

The accumulation of IMTG is not believed to be the direct cause of the development of insulin resistance but more of a marker for the presence of other lipid intermediates, which have been directly linked to defects in insulin signaling (8, 17, 25, 32, 37). Intramyocellular long-chain fatty acyl-CoA accumulation has been implicated in the pathogenesis of insulin resistance in skeletal muscle (4, 8, 32, 37). A potential mechanism for the accumulation of long-chain fatty acyl-CoAs in skeletal muscle may be reduced fatty acid oxidation. In the current investigation, we examined the content of total long-chain fatty acyl-CoAs and long-chain fatty acyl-CoA subfractions in the same muscle tissue in which we examined palmitate oxidation. Our results do not support the notion that long-chain fatty acyl-CoA accumulation in skeletal muscle is due solely to a reduction in fatty acid oxidation. Long-chain fatty acyl-CoA content was higher in both the overweight/obese and the extremely obese patients compared with normal-weight patients despite significantly lower fatty acid oxidation only in the extremely obese. In addition, no associations were observed between long-chain fatty acyl-CoA content and palmitate oxidation. Thus the accumulation of intramyocellular long-chain fatty acyl-CoAs with obesity appears to be due to other, yet to be defined, mechanisms.

The present study is the first, to our knowledge, to report intramyocellular concentration of long-chain fatty acyl-CoAs in human subjects of varying degrees of adiposity. We observed higher long-chain fatty acyl-CoA accumulation in the skeletal muscle of overweight/obese and extremely obese subjects compared with normal-weight subjects. These findings match very nicely with previously reported data from our laboratory in which we established the presence of insulin resistance in moderately and extremely obese populations (1, 6, 7, 9). In addition, it appears that insulin resistance increases linearly with the progression of obesity up to a BMI of ~30 kg/m²; however, above this threshold there is no further progression of insulin resistance with increases in BMI (1, 9). As stated previously, intramyocellular long-chain fatty acyl-CoA accumulation has been implicated in the pathogenesis of insulin resistance in skeletal muscle (4, 8, 32, 37). More specifically, this intermediate of fatty acid metabolism can have direct effects on glucose utilization by altering enzyme activity and/or inhibit-

ing key components of the insulin-signaling pathway (4, 32). Thus our findings with long-chain fatty acyl-CoA concentrations may provide a mechanistic explanation as to why insulin resistance develops with the progression from normal weight to moderately obese but not with further increases in adiposity.

A decreased reliance on lipids as an energy source has previously been identified as a metabolic risk factor for weight gain (40). In addition, Kelley et al. (21) suggested that impairment in skeletal muscle fatty acid oxidation might be a predisposing factor contributing to the development of obesity as well as weight regain following weight loss. Furthermore, recent data from our laboratory (16) demonstrated the persistence of reduced fatty acid oxidation during exercise in formerly extremely obese patients after weight loss compared with controls matched for age, race, and BMI. These findings indicate that, even in a weight-reduced state, formerly extremely obese women demonstrate a defect in the ability to utilize fat for energy and thus provide additional support for the theory that defective lipid oxidation may predispose individuals to extreme obesity.

In summary, the findings from the current investigation indicate a defect in skeletal muscle fatty acid oxidation with extreme obesity but not with overweight/obese subjects. On the basis of these data, we hypothesize that extreme obesity may be due, in part, to inherent defects in skeletal muscle lipid metabolism. It is important to discern the cellular mechanisms that may contribute to extreme obesity, as extremely obese individuals comprise ~5% of the population and contribute significantly to health care costs (12). In addition, on the basis of the results of this study, the accumulation of intramyocellular long-chain fatty acyl-CoAs does not appear to be a result of defective lipid oxidation. Thus the accumulation of long-chain fatty acyl-CoAs implicated in the pathogenesis of insulin resistance in obese skeletal muscle must be due to some other, yet to be defined, mechanism.

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REFERENCES

- Barakat HA, Mooney N, O'Brien K, Long S, Khazani PG, Pories W, and Caro JF. Coronary heart disease risk factors in morbidly obese women with normal glucose tolerance. *Diabetes Care* 16: 144–149, 1993.
- Bligh EG and Dryer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem* 37: 911–917, 1959.
- Chen KS, Heydrick S, Kurowski T, and Ruderman NB. Diacylglycerol-protein kinase C signalling in skeletal muscle: a possible link to insulin resistance. *Trans Assoc Am Physicians* 104: 206–212, 1991.
- Cooney GJ, Thompson AL, Furler SM, Ye J, and Kraegen EW. Muscle long-chain acyl CoA esters and insulin resistance. *Ann NY Acad Sci* 967: 196–207, 2002.
- DeFronzo RA. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. *Neth J Med* 50: 191–197, 1997.
- Dohm GL, Elton CW, Raju MS, Mooney ND, DiMarchi R, Pories WJ, Flickinger EG, Atkinson SM Jr, and Caro JF. IGF-I-stimulated glucose transport in human skeletal muscle and IGF-I resistance in obesity and NIDDM. *Diabetes* 39: 1028–1032, 1990.
- Dohm GL, Tapscott EB, Pories WJ, Dabbs DJ, Flickinger EG, Meelheim D, Fushiki T, Atkinson SM, Elton CW, and Caro JF. An in vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J Clin Invest* 82: 486–494, 1988.
- Ellis BA, Poynten A, Lowy AJ, Furler SM, Chisholm DJ, Kraegen EW, and Cooney GJ. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. *Am J Physiol Endocrinol Metab* 279: E554–E560, 2000.
- Elton CW, Tapscott EB, Pories WJ, and Dohm GL. Effect of moderate obesity on glucose transport in human muscle. *Horm Metab Res* 26: 181–183, 1994.
- Felber JP, Ferrannini E, Golay A, Meyer H, Theibault B, Curchod B, Maeder E, Jequier E, and DeFronzo R. Role of lipid oxidation in the pathogenesis of insulin resistance of obesity and type II diabetes. *Diabetes* 36: 1341–1350, 1987.
- Flegal KM, Carroll MD, Kuczmarski RJ, and Johnson CL. Overweight and obesity in the United States: prevalence and trends, 1960–1994. *Int J Obes Relat Metab Disord* 22: 39–47, 1998.
- Flegal KM, Carroll MD, Ogden CL, and Johnson CL. Prevalence and trends in obesity among US adults, 1999–2000. *JAMA* 288: 1723–1727, 2002.
- Forouhi NG, Jenkinson G, Thomas EL, Mullick S, Mierisova S, Bhonsle U, McKeigue PM, and Bell JD. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. *Diabetologia* 42: 932–935, 1999.
- Goodpaster BH, Theriault R, Watkins SC, and Kelley DE. Intramuscular lipid content is increased in obesity and decreased by weight loss. *Metabolism* 49: 467–472, 2000.
- Grigsby RJ and Dobrowsky RT. Inhibition of ceramide production reverses TNF-induced insulin resistance. *Biochem Biophys Res Commun* 287: 1121–1124, 2001.
- Guesbeck NR, Hickey MS, MacDonald KG, Pories WJ, Harper I, Ravussin E, Dohm GL, and Houmard JA. Substrate utilization during exercise in formerly morbidly obese women. *J Appl Physiol* 90: 1007–1012, 2001.
- Hajdуч E, Balendran A, Batty IH, Litherland GJ, Blair AS, Downes CP, and Hundal HS. Ceramide impairs the insulin-dependent membrane recruitment of protein kinase B leading to a loss in downstream signalling in L6 skeletal muscle cells. *Diabetologia* 44: 173–183, 2001.
- Hickey MS, Carey JO, Azevedo JL, Houmard JA, Pories WJ, Israel RG, and Dohm GL. Skeletal muscle fiber composition is related to adiposity and in vitro glucose transport rate in humans. *Am J Physiol Endocrinol Metab* 268: E453–E457, 1995.
- Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, and Harig HU. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 48: 1113–1119, 1999.
- Kelley DE and Goodpaster BH. Skeletal muscle triglyceride. An aspect of regional adiposity and insulin resistance. *Diabetes Care* 24: 933–941, 2001.
- Kelley DE, Goodpaster BH, Wing RR, and Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. *Am J Physiol Endocrinol Metab* 277: E1130–E1141, 1999.
- Kim JY, Hickner RC, Cortright RL, Dohm GL, and Houmard JA. Lipid oxidation is reduced in obese human skel-

- etal muscle. *Am J Physiol Endocrinol Metab* 279: E1039–E1044, 2000.
23. **Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, and Shulman GI.** Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a ¹H NMR spectroscopy study. *Diabetologia* 42: 113–116, 1999.
 24. **Kuczmarski RJ, Flegal KM, Campbell SM, and Johnson CL.** Increasing prevalence of overweight among US adults. The National Health and Nutrition Examination Surveys, 1960 to 1991. *JAMA* 272: 205–211, 1994.
 25. **Montell E, Turini M, Marotta M, Roberts M, Noe V, Ciudad CJ, Mace K, and Gomez-Foix AM.** DAG accumulation from saturated fatty acids desensitizes insulin stimulation of glucose uptake in muscle cells. *Am J Physiol Endocrinol Metab* 280: E229–E237, 2001.
 26. **National Institutes of Health: National Heart, Blood, and Lung Institute.** *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults.* Bethesda, MD: National Institutes of Health, 1998, p. xiv.
 27. **Neschen S, Moore I, Regittng W, Yu CL, Wang Y, Pypaert M, Petersen KF, and Shulman GI.** Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. *Am J Physiol Endocrinol Metab* 282: E395–E401, 2002.
 28. **Pan DA, Lillioja S, Kriketos AD, Milner MR, Baur LA, Bogardus C, Jenkins AB, and Storlien LH.** Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46: 983–988, 1997.
 29. **Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, and Luzi L.** Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ¹H-¹³C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48: 1600–1606, 1999.
 30. **Phillips DI, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, and Taylor R.** Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. *Metabolism* 45: 947–950, 1996.
 31. **Shmueli E, Alberti KG, and Record CO.** Diacylglycerol/protein kinase C signalling: a mechanism for insulin resistance? *J Intern Med* 234: 397–400, 1993.
 32. **Shulman GI.** Cellular mechanisms of insulin resistance. *J Clin Invest* 106: 171–176, 2000.
 33. **Steinberg GR and Dyck DJ.** Development of leptin resistance in rat soleus muscle in response to high-fat diets. *Am J Physiol Endocrinol Metab* 279: E1374–E1382, 2000.
 34. **Steinberg GR, Parolin ML, Heigenhauser GJ, and Dyck DJ.** Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. *Am J Physiol Endocrinol Metab* 283: E187–E192, 2002.
 35. **Tataranni PA, Larson DE, and Ravussin E.** Body fat distribution and energy metabolism in obese men and women. *J Am Coll Nutr* 13: 569–574, 1994.
 36. **Teruel T, Hernandez R, and Lorenzo M.** Ceramide mediates insulin resistance by tumor necrosis factor- α in brown adipocytes by maintaining Akt in an inactive dephosphorylated state. *Diabetes* 50: 2563–2571, 2001.
 37. **Thompson AL and Cooney GJ.** Acyl-CoA inhibition of hexokinase in rat and human skeletal muscle is a potential mechanism of lipid-induced insulin resistance. *Diabetes* 49: 1761–1765, 2000.
 38. **Weyer C, Snitker S, Rising R, Bogardus C, and Ravussin E.** Determinants of energy expenditure and fuel utilization in man: effects of body composition, age, sex, ethnicity and glucose tolerance in 916 subjects. *Int J Obes Relat Metab Disord* 23: 715–722, 1999.
 39. **Yu C, Chen Y, Zong H, Wang Y, Bergeron R, Kim JK, Cline GW, Cushman SW, Cooney GJ, Atcheson B, White MF, Kraegen EW, and Shulman GI.** Mechanism by which fatty acids inhibit insulin activation of IRS-1 associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem* 277: 50230–50236, 2002.
 40. **Zurlo F, Lillioja S, Esposito-Del Puente A, Nyomba BL, Raz I, Saad MF, Swinburn BA, Bogardus C, and Ravussin E.** Low ratio of fat to carbohydrate oxidation as a predictor of weight gain: study of 24-h RQ. *Am J Physiol Endocrinol Metab* 259: E650–E657, 1990.