Fatty Acid Oxidation by Skeletal Muscle Homogenates From Morbidly Obese Black and White American Women

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The purpose of this study was to determine if there were differences in the capacity of skeletal muscle from morbidly obese Black and White American women to oxidize fatty acids. The oxidation rates of ^{14}C-palmitate, ^{14}C-palmitoyl-CoA, and ^{14}C-palmitoyl-carnitine were measured in whole homogenates of rectus abdominus from Black and White women who were similar in age and body mass index (BMI). The activities of muscle citrate synthase (CS), β-hydroxy acyl-CoA dehydrogenase (β-HAD), and mitochondrial and microsomal acyl-CoA synthetase (ACS) were measured in the 2 groups. The results showed that the rate of ^{14}C-palmitate oxidation by muscle of Black women was 25% that of Whites (8.7 ± 1.5 *v* 34.4 ± 6.8 nmol $^{14}CO_2$ produced/gram tissue wet weight/ hour; $P < .05$), but the rates of ^{14}C-palmitoyl-CoA and ^{14}C-palmitoyl-carnitine oxidation were not different in the 2 groups. No differences were found in the activities of CS or β-HAD. However, the activities of both mitochondrial and microsomal ACS were lower in the Black women than the Whites (mitochondrial ACS 25.1 ± 3.9 *v* 36.4 ± 5.0 nmol/mg protein/min; $P < .05$; microsomal ACS 6.2 ± 0.5 *v* 8.5 ± 0.5; nmol/mg protein/min; $P < .005$). The lower rate of palmitate oxidation, and the lack of differences in the rates of palmitoyl-CoA and palmitoyl-carnitine oxidation indicate that there is a defect in the activation of the fatty acid in the muscle of the Black women. This was confirmed by the decrease in mitochondrial ACS activity in the Black women. The decreased fatty acid oxidation by skeletal muscle of obese Black women could result in shunting these fuels from muscle to adipose tissue for storage, which may contribute to the maintenance of obesity in the Black women.

IN THE UNITED STATES, the prevalence of obesity is greater in Black than in White women.[1,2] Black women gain weight at an earlier age and remain heavier than Whites of the same age.[3] They also lose less weight than White women do on similar weight loss regimens, such as exercise, caloric restriction, or gastric bypass surgery.[4-7] Obesity is a multifactorial metabolic disorder that involves environmental and genetic factors. The higher prevalence of obesity in Black women persists after elimination of environmental factors, including socioeconomic status, diet, and cultural perceptions of beauty.[8-10] These findings suggest that the increased prevalence of obesity in Black women may be partly due to inherent biochemical differences. Several studies have shown that Black women have a lower resting metabolic rate compared with White women.[11-15] Nicklas et al[16] reported that fatty acid oxidation was lower in postmenopausal Black women compared with similar Whites. In addition, other studies, including our own, have reported a decrease in whole-body fat oxidation by Black women during exercise.[17,18]

Fatty acids provide approximately 60% of the energy needs of skeletal muscle during rest in healthy individuals.[19] Because of its mass, skeletal muscle is important in oxidative lipid disposal. Kelley et al[19] have shown that fat oxidation measured by indirect calorimetry was lower in the legs of obese subjects than lean subjects. Kim et al[20] reported that the rate of palmitate oxidation by muscle homogenates was lower in obese subjects than lean controls, and that this decrease was partially associated with a decrease in the activity of carnitine-palmitoyl transferase I (CPT I). Thus, a decrease in the oxidation of fat by muscle appears to be a metabolic defect that is associated with obesity.

Because Black women gain weight at an earlier age and remain heavier than their White counterparts,[3-7] it is conceivable that a decrease in the rate of fatty acid oxidation by muscle would result in shunting fats towards adipose tissue for storage, thereby contributing to the obesity of the Black women. Thus, the purpose of this study was to determine if racial differences in fatty acid oxidation by muscle exist, and if they do, to determine the underlying causes. To that end, we determined the rates of palmitate oxidation by whole homogenates of skeletal muscle from obese Black and White women. We also measured the rates of oxidation of palmitoyl-CoA and palmitoyl-carnitine to determine if any differences in the capacity to oxidize palmitate still exist after fatty acid activation and the translocation of the activated fatty acids inside the mitochondria. In addition, we measured the activities of key enzymes that are directly involved in the oxidation of fatty acids. These included citrate synthase (CS), β-hydroxy acyl-CoA dehydrogenase (β-HAD), and acyl-CoA synthetase (ACS). Our goal was to gain insight on the potential mechanisms that underlie the maintenance of obesity in Black women.

MATERIALS AND METHODS

Subjects

A total of 26 (12 White, 14 Black) morbidly obese (body mass index [BMI] >37) subjects, free of vascular disease, diabetes, or cancer, and currently not taking medications that affect carbohydrate and lipid metabolism participated in this study. Body mass and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively, and BMI calculated. Rectus abdominus muscle biopsies were obtained from the participants undergoing gastric bypass surgery or total abdominal hysterectomy.[21] We have reported fiber type of rectus abdominus muscle to be 41% type I in gastric bypass patients and 55% in lean women undergoing hysterectomy surgery.[22] The fiber type of the vastus late-

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ralis is approximately 50% type I fibers in the general population. The type I fiber percentage is lower (approximately 45% type I fibers) in the obese population. This is consistent with the fact that the rectus abdominus is representative of mixed fiber type of skeletal muscle in general. Written consent was obtained from the participants after they were informed of the nature of the study. The Institutional Review Board of the University and Medical Center approved the protocols for these studies.

Plasma Analysis

Venous blood was drawn from each subject after a 12-hour fast into tubes containing EDTA (1 mg/mL) as an anticoagulant and aprotinin (10 KIU/mL) as a protease inhibitor. Plasma was separated from cells in a refrigerated centrifuge, and stored at −80°C until analyzed. Plasma glucose concentrations were measured using a Glucose and Lactate Analyzer model 2300 (YSI, Yellow Springs, OH). Plasma insulin concentrations were measured using a microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL).

Biochemical Methods

Rates of fatty acid oxidation were measured in a subset that included 8 White and 10 Black Americans, whereas enzyme activities were measured in tissue preparations from each subject.

The rate of fatty acid oxidation was determined as previously described[20] with a slight modification. Briefly, muscle was homogenized (1:19 wt:vol) in media containing 250 mmol/L sucrose, 1 mmol/L EDTA, 10 mmol/L Tris-HCl (pH 7.4) and 2 mmol/L adenosine triphosphate (ATP). Triplicate homogenate incubations were performed in media containing the following at final concentrations: sucrose 0.1 mol/L, Tris-HCl 10 mmol/L, K_2HPO_4 5 mmol/L, KCl 80 mmol/L, $MgCl_2$ 1 mmol/L, L-carnitine 2 mmol/L, malate 0.1 mmol/L, ATP 2 mmol/L, coenzyme A 0.05 mmol/L, dithiothreitol 1 mmol/L, EDTA 0.2 mmol/L, bovine serum albumin (BSA) 0.5%, and 1 of the following substrates: ^{14}C-1-palmitate (7.3 μCi/μmol/L), ^{14}C-1-palmitoyl-CoA (1.8 μCi/μmol/L), or ^{14}C-1-palmitoyl-carnitine (1.8 μCi/μmol/L). Incubations were terminated after 1 hour with the addition of 70% perchloric acid. One normal NaOH was used to trap $^{14}CO_2$ that evolved from the incubation. Data are expressed as nanomole of $^{14}CO_2$ produced/gram wet weight tissue/hour.

Enzyme Assays

CS activity was measured as previously described[23] with some modifications. Powdered frozen muscle was homogenized (1:9 wt:vol) in media containing 0.175 mmol/L KCl and 2.0 mmol/L EDTA (pH 7.4). CS activity was measured at 37.0°C in 0.1 mol/L Tris-HCl (pH 8.3) assay buffer containing 0.12 mmol/L 5,5′ dithiobis(2-nitrobenzoic acid) (DTNB) and 0.6 mmol/L oxaloacetate. After an initial absorbance reading taken at 412nm, the reaction was initiated with the addition of 3.0 mmol/L acetyl-CoA. The change in absorbance was measured every 15 seconds for 7 minutes. Enzyme activity is expressed as micromole/gram wet weight/minute.

β-HAD was assayed using a modification of the technique described by Chi et al.[24] Aliquots of homogenates that were used for the assay of CS were diluted with a buffer containing 50% glycerol, 20 mmol/L NaH_2PO_4, 5 mmol/L β-mercaptoethanol, 0.5 mmol/L EDTA, and 0.02% BSA (pH 7.4). β-HAD activity was determined in 100 μL assay buffer that contained 150 mmol/L imidazole-HCl, 200 μmol/L acetoacetyl-CoA, 100 μmol/L nicotinamide adenine dinucleotide (NADH), 1 mmol/L EDTA, and 0.05% BSA (pH 6.0). The rate of disappearance of NADH and the appearance of NAD was measured flourometrically. Enzyme activity is expressed as micromole/gram wet weight/minute.

ACS activity was measured essentially as previously described[25] with the following modifications: ^{3}H oleate was used instead of ^{3}H palmitate with a reaction time of 5 minutes. Total ACS activity was measured in whole homogenates used for the fatty acid oxidation experiments. Mitochondrial and microsomal fractions were obtained by differential centrifugation of fresh muscle. CS activity was measured in the mitochondrial and microsomal fraction to assess yield and the degree of mitochondrial ACS contamination, respectively. Contamination of mitochondria with the microsomal fraction was assessed by measuring the activity of diacylglycerol acyl transferase (DGAT), which is a microsomal enzyme. CS activity in the microsomal fraction was less than 10% that of the mitochondrial fraction, and no DGAT activity was detected in the mitochondrial preparations. ACS activity is expressed as nanomole enzyme activity/milligram protein/minute.

Table 1. Physical and Biochemical Characteristics of the Subjects

	White American (n = 12)	Black American (n = 14)
Age (yr)	41.8 ± 1.7	39.0 ± 1.9
BMI (kg/m^2)	45.9 ± 1.8	45.8 ± 1.7
Glucose (mg/dL)	96.4 ± 2.9	93.4 ± 3.9
Insulin (μU/mL)	11.4 ± 1.9	10.0 ± 1.1

NOTE. Data are expressed as mean ± SEM.

Statistics

Data are reported as mean ± SEM. The data were analyzed using independent *t* tests. Statistical significance was inferred at $P < .05$ (SPSS, Chicago, IL).

RESULTS

Table 1 shows the physical and biochemical characteristics of the subjects who participated in this study. The 2 groups of women did not differ from each other with respect to age or BMI. No differences were found in plasma glucose or insulin levels between the 2 groups.

Table 2 shows the rates of CO_2 production from the oxidation of ^{14}C-palmitate, ^{14}C-palmitoyl-CoA, and ^{14}C-palmitoyl-carnitine by whole homogenates of rectus abdominus from the 2 groups of women. The rate of palmitate oxidation by muscle homogenates of the Black women was only 25% ($P < .05$) that of the White women. In contrast to palmitate oxidation, the rate of CO_2 production from the oxidation of either ^{14}C-palmitoyl-CoA or ^{14}C-palmitoyl-carnitine was not different in the Black women compared with the rates from the White women.

Table 3 shows that there were no differences in the activities of either β-HAD and CS. However, total ACS activity in muscle homogenates of the Black women was approximately 60% ($P < .05$) of that in muscle from the White women. The activity of ACS associated with the mitochondria from muscle

Table 2. Rates of Substrate Oxidation in Muscle Homogenates

	White American (n = 8)	Black American (n = 10)
Palmitate	34.4 ± 6.8	8.7 ± 1.5*
Palmitoyl-CoA	177.1 ± 20.3	185.9 ± 15.4
Palmitoyl-carnitine	358.6 ± 32.0	375.2 ± 36.2

NOTE. Data are expressed as mean ± SEM. Substrate oxidation data are expressed as nanomole $^{14}CO_2$ produced/gram tissue wet weight/hour.

*Significantly different from White American women ($P < .05$).

Table 3. Enzymatic Activities in Muscle of the Two Groups

	White American (n = 12)	Black American (n = 14)
β-HAD	3.8 ± 0.5	4.1 ± 0.6
CS	2.6 ± 0.2	2.4 ± 0.1
Total ACS	10.5 ± 1.3	6.4 ± 0.8*
Mitochondrial ACS	36.4 ± 5.0	25.1 ± 3.9*
Microsomal ACS	8.5 ± 0.7	6.2 ± 0.5*

NOTE. Data are expressed as mean ± SEM. β-HAD, CS, and total ACS enzyme activities are expressed as μmol enzyme activity/gram tissue wet weight/minute. Mitochondrial and microsomal ACS activities are expressed as nanomole/mg protein/minute.

Abbreviations: β-HAD, β-hydroxy acyl-CoA dehydrogenase; CS, citrate synthase; ACS, acyl-CoA synthetase.

*Significantly different from White Americans ($P < .05$).

of Black women was 69% ($P < .05$) that of the Whites. Similarly, the activity of microsomal ACS from the Black women was 73% ($P < .05$) that of the White women.

DISCUSSION

The oxidation of fatty acids by mitochondria is a highly regulated process that involves the entry of fatty acids into the cell, their activation to acyl-CoA derivatives by ACS, and their entry into the mitochondrial matrix. Entry into the mitochondrial matrix is restricted by the impermeability of the inner mitochondrial membrane to long-chain acyl-CoAs, which is circumvented by the carnitine transport system. Fatty acyl-CoAs are converted to fatty acyl-carnitine by the enzyme CPT I and are translocated inside the mitochondrial matrix by the carnitine acyl carnitine translocase. This is followed by a conversion of the fatty acyl-carnitine back to the CoA derivative by CPT II, at which point the fatty acid is available to enter the oxidative pathway. Thus, alterations in any of these steps may lead to a decrease in the rate of oxidation of fatty acids. This is supported by findings of lower rates of fatty acid oxidation in obese individuals than lean controls due to a decrement in the activity of CPT I.[20]

In addition to the importance of activation and transport of the fatty acid, the number of mitochondria and the activities of the enzymes that are involved in the oxidation of fatty acids could influence the rate of fatty acid oxidation. These include the enzymes of the tricarboxylic acid (TCA) cycle and those of the β-oxidation spiral. Alterations in the number of mitochondria and/or the activities of any participating enzymes would be expected to lead to a less efficient oxidation of fatty acids. In this study, we addressed these issues by determining the rates of oxidation of palmitate and by examining the contributions of the transport system and mitochondrial function.

There were 4 novel findings from this study: (1) the rate of palmitate oxidation by muscle homogenates of obese Black women was lower than that of obese Whites; (2) the rates of oxidation of palmitoyl-CoA or palmitoyl-carnitine by homogenates from the 2 groups were not different; (3) the oxidative potential of mitochondria was not different in the 2 groups; and (4) the activity of ACS was lower in the muscles of Black women compared with that from Whites.

The decrease in palmitate oxidation in the muscle of the Black women could not be attributed to a decrease in the transport of the fatty acids inside the cell because the rate of palmitate oxidation was determined in muscle homogenates. Nor would this decrease be due to a decrease in CPT I function because the rates of palmitoyl-CoA and palmitoyl-carnitine oxidation by the same homogenates were not different in the Black women from the White women. It is important to note here that we cannot be sure that CPT I activity in the muscle of the obese Black women is not different than that in lean Black women until we make direct measurements. Furthermore, it should be realized that the decrease in palmitate oxidation in the homogenates may or may not be similarly affected in vivo.

The measurement of CS activity has been extensively used as an indication of oxidative capacity of mitochondria in tissues.[23] Our results showed that there were no differences in CS activity in the muscle homogenates from the Black and White subjects, indicating that the decrease in palmitate oxidation is not due to a decrease in the oxidative capacity of the mitochondria of the muscle from the Black women.

The activity of β-HAD has been used as a measure of the intactness of the β-oxidation spiral. Because there were no differences in the activity of β-HAD in the homogenates of muscle of the 2 groups, the decrease in palmitate oxidation in the homogenates from the Black women could not be attributed to an aberration in β-oxidation. Furthermore, the lack of change in the activity of β-HAD in the 2 groups is a confirmation of the findings with CS, namely, that the oxidative capacity of the mitochondria is not different in the 2 groups.

The significant finding from this study is the decrease in the activity of both the mitochondrial and microsomal ACS. The decrease in mitochondrial ACS activity may be directly related to the decrease in palmitate oxidation. All of the other evidence that was obtained in this study supports this conclusion. The underlying causes of this decrease, whether due to a decreased expression of the enzyme or other factors need to be explored further. An equally intriguing finding is the decrease in microsomal ACS activity. This suggests that activation of long-chain fatty acids that are used for glyceride synthesis would be decreased, which in turn, may decrease the cellular content of glycerides. If that were true, then accumulation of triglycerides in the muscle, which has been shown to be associated with insulin resistance,[26] will be decreased in the muscle of obese Black women. Our findings have to be viewed with caution, however. We measured the activity of microsomal ACS and expressed per milligram protein. However, we did not measure total ACS protein in the microsomal fraction, which may be higher in the Black women. This will suggest that total microsomal ACS activity may be increased in the muscle of the Black women.

The mean BMI for the obese individuals studied here was 46 kg/m^2. We have previously reported that, with respect to numerous metabolic properties in obese individuals, the overt cutoff for where the impaired metabolic profile is evident is at BMI = 29.7 kg/m^2. There is no further impairment beyond a BMI of 30 kg/m^2.[27,28] Therefore, it is likely that the severely obese individuals in the present study possessed a similar metabolic profile to moderately obese (BMI, 30 to 35) individuals.

The results of this study indicated that palmitate oxidation is decreased in muscle homogenates of obese Black women. This decrease was not the result of impairments in the transport of the fatty acids inside the mitochondria or the result of decreases in mitochondrial numbers or function, but rather because of a decrement in the activation of palmitate to the CoA derivative. These findings raise several issues that need to be investigated in the future. First, is fatty acid oxidation similarly affected in lean Black women? Second, are the changes in fatty acid oxidation that were observed in vitro in this study manifested in vivo? And finally, is the decrease in ACS activity due to a decrease in the expression of this protein? Answers to these questions might shed light on the cause and maintenance of obesity in Black women.

REFERENCES

1. Kuczmarski RJ, Flegel KM, Campbell SM, et al: The National Health and Nutrition Examination Survey. JAMA 272:205-211, 1994
2. Kumanyika SK: Obesity in minority populations: An epidemiologic assessment. Obes Res 2:166-182, 1994
3. Burke GL, Sprafka JM, Folsom AR, et al: Trends in coronary heart disease mortality, morbidity, and risk factors from 1960 to 1986: The Minnesota Heart Survey. Int J Epidmiol 18:S73-81, 1989 (suppl)
4. Striegel-Moore RH, Wilfly DE, Caldwell MB, et al: Weight related attitudes and behavior of women who diet to lose weight: A comparison of black dieters and white dieters. Obes Res 4:109-116, 1996
5. Pories WJ, MacDonald KG, Morgan EJ, et al. Surgical treatment of obesity and its effect on diabetes: 10-y follow-up. Am J Clin Nutr 55:582S-585S, 1992
6. Kumanyika SR, Obarrznek E, Stevens VJ, et al: Weight-loss experience of black and white participants in NHLBI-sponsored clinical trials. Am J Clin Nutr 53:1631S-1638S, 1991
7. Tyler DO, Allan JD, Alcozer FR: Weight loss methods used by African American and Euro-American women. Res Nurs Health 20: 413-423, 1997
8. Burke GL, Bild KE, Hilner JE, et al: Differences in weight gain in relation to race, gender, age and education in young adults: The CARDIA Study. Ethn Health 1:327-335, 1996
9. Kemper KA, Sargurt RG, Drane JW, et al: Black and white female's perceptions of ideal body size and social norms. Obes Res 2:117-126, 1994
10. Kumanyika SR, Wilson JF, Guilford-Davenport M: Weight related attitudes and behavior of black women. J Am Diet Assoc 93:416-422, 1993
11. Albu J, Shur M, Curi M, et al: Resting metabolic rate in obese, premenopausal black women. Am J Clin Nutr 66:531-538, 1997
12. Foreman J, Miller W, Szymanski L, et al: Differences in resting metabolic rates of inactive obese African American and Caucasian women. Int J Obes 22:215-221, 1998
13. Foster GD, Wadden TA, Vogt R: Resting energy expenditure in obese African-American women and Caucasian women. Obes Res 5:1-8, 1997
14. Jakicic J, Wing R: Differences in resting energy expenditure in African American vs Caucasian overweight females. Int J Obes 22: 236-242, 1998
15. Yanovski SZ, Reynolds JC, Boyle AJ, et al: Resting metabolic rate in African American and Caucasian girls. Obes Res 5:321-325, 1997
16. Nicklas BJ, Berman DM, Davis DC, et al: Racial differences in metabolic predictors of obesity among postmenopausal women. Obes Res 7:463-468, 1999
17. Chitwood LF, Brown SP, Lundy MJ, et al: Metabolic propensity toward obesity in black vs. white females: Responses during rest, exercise and recovery. Int J Obes 20:455-462, 1996
18. Hickner RC, Privette J, McIver K, et al: Fatty acid oxidation in African American and Caucasian women during physical activity. J Appl Physiol 90:2319-2324, 2001
19. Kelley DE, Goodpaster B, Wing RR, et al: Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 277: E1130-1141, 1999
20. Kim JY, Hickner RC, Cortright RL, et al: Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol 279: E1039-1044, 2000
21. Dohm GL, Tapscott EB, Pories WJ, et al: 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
22. Tanner CJ, Barakat HA, Dohm GL, et al: Muscle fiber type is associated with obesity and weight loss. Am J Physiol 282:E1191-1196, 2002
23. Srere PA: Citrate synthase. Methods Enzymol 13:5-26, 1969
24. Chi M, Hintz CS, Coyle EF, et al: Effects of detraining on enzymes of energy metabolism in individual human fibers. Am J Physiol 244:C276-287, 1983
25. Bar-Tana J, Rose G, Shapiro B: Long chain fatty acyl-CoA synthetase from rat liver microsomes. Methods Enzymol 35:117-122, 1969
26. He J, Watkins S, Kelly DE: Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. Diabetes 50:817-823, 2001
27. Barakat HA, Mooney N, O'Brien K, et al: Coronary heart disease risk factors in morbidly obese women with normal glucose tolerance. Diabetes Care 16:144-149, 1993
28. Elton CW, Tapscott, EB, Pories WJ, et al: Effect of moderate obesity on glucose transport in human muscle. Horm Metab Res 26:181-183, 1994