Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1542–1545

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Ketone body metabolism in lean and obese women

Esther Vice[a], Jonathan D. Privette[a], Robert C. Hickner[b], Hisham A. Barakat[a,*]

[a]Department of Internal Medicine, Brody School of Medicine, East Carolina University, Greenville, NC 27834, USA
[b]Department of Physiology, Brody School of Medicine, and Department of Exercise and Sport Science, East Carolina University, Greenville, NC 27834, USA

Received 4 March 2005; accepted 24 May 2005

Abstract

Previous studies have demonstrated decreases in whole-body and muscle fat oxidation in obese individuals. Because muscle also uses ketone bodies, and because the ketone body oxidation pathway differs from that of fatty acid oxidation, this study was initiated to determine whether there were differences in ketone body metabolism between obese and lean subjects. Plasma β-hydroxy-butyrate (β-OHB) concentration was measured in 47 lean and 47 age-matched obese women, and the rate of β-OHB oxidation by muscle homogenates was measured in a subset of 8 lean and 8 obese women. Plasma free fatty acid levels, which have been reported to correlate with ketone body production, were higher ($P < .05$) in the obese than in the lean women (662 $\pm$ 46 and 463 $\pm$ 44 nmol/L, respectively) as was plasma insulin level. However, the β-OHB concentration was lower in obese than in lean subjects (235 $\pm$ 17 and 323 $\pm$ 29 μmol/L, respectively; $P < .05$). The rate of β-OHB oxidation was also lower ($P < .05$) in muscle of the obese than that of the lean group (139.6 $\pm$ 12.6 vs 254.6 $\pm$ 30.0 nmol of CO_2 produced per gram of tissue per hour). These data illustrate that production and use of ketone bodies are lower in obese women than in lean controls. The decreased oxidation of ketone bodies by muscle is consistent with aberrations in muscle metabolism in the obese individuals that most likely relates to a decrease in mitochondrial numbers.

1. Introduction

Most of the energy needs (approximately 60%) of skeletal muscle during the postabsorptive state are derived from the oxidation of fatty acids [1]. In healthy individuals, skeletal muscle, by virtue of its mass, is important in oxidative lipid disposal. In obese individuals, on the other hand, a decrease in lipid oxidation by muscle has been reported. Kelley et al [1] have reported that fat oxidation, measured by indirect calorimetry, was lower in the legs of obese than those of lean subjects. Kim et al [2] showed that the rate of palmitate oxidation by muscle homogenates of obese individuals was lower than that of lean subjects, and that this decrease was partially associated with a decrease in the activity of carnitine-palmitoyl transferase I (CPT-I). This decreased capacity of muscle to oxidize fats would lead to accumulation of fats in the muscle. In fact, the intramuscular content of triglycerides in muscles of obese individuals has been shown to be higher than that of lean controls [3-5]. It has been postulated that the decrease in the capacity of muscle to oxidize fat and the concomitant accumulation of these compounds in the muscle might be associated with the resistance of muscle of obese individuals to the action of insulin [3,4,6]. Thus, aberrations in fat disposal by muscle appear to be metabolic defects that are associated with obesity.

Similar to fatty acids, ketone bodies serve as important energy sources to cardiac muscle, skeletal muscle, and nervous tissue during the fasting state. Three compounds are grouped together as ketone bodies: acetoacetate (AcAc), β-hydroxybutyrate (β-OHB), and acetone. In the fasted state, ketone bodies arise from the β-oxidation of long fatty acids that are primarily liberated from the lipolysis of the stored triglycerides in the adipose tissue. In liver, when the concentrations of circulating fatty acids are high, the production of acetyl-CoA can exceed cellular energy requirements [7]. During a high flux of fatty acids into the liver in the fasted state or upon catecholamine stimulation, the concentration of acetyl-CoA would exceed the capacity of the tricarboxylic acid cycle to oxidize it to CO_2 and water. Through a series of known reactions, the ketones are synthesized and exported out of the liver to the extrahepatic

* Corresponding author.
E-mail address: barakath@mail.ecu.edu (H.A. Barakat).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.05.023

tissues, where they are oxidized to CO_2 and water. Thus, the rate of production of ketone bodies is dependent on the abundance of plasma free fatty acid concentration.

The rate of oxidation of ketone bodies by extrahepatic tissues, mainly muscle, is proportional to their concentration in the plasma [8]. The pathway for the oxidation of ketone bodies by muscle differs from that of fatty acid oxidation. Ketones are small molecules that are readily soluble in the lipid bilayer and can pass through mitochondrial membranes without the requirement of transporting proteins. Thus, carnitine plamitoyl transferase and plamitoyl carnitine translocase, the 2 enzymes that are involved in the transport of long-chain fatty acids across the mitochondrial membrane, are not involved in ketone body oxidation. Furthermore, once inside the mitochondrial matrix, the coenzyme derivative of AcAc is formed, not through the action of acyl-CoA synthetase, but rather through the action of the mitochondrial enzyme acetoacetate: succinyl-CoA transferase. Because of these differences between the 2 pathways for the oxidation of fatty acids and ketone bodies, and because of the reported differences in the oxidation of fatty acids by muscle of obese compared to lean subjects, we initiated this study to determine whether there were differences in ketone body metabolism between obese and lean individuals. Toward that end, we determined whether there were differences in the concentrations of ketone bodies in the plasma of lean and obese subjects, and also whether there were differences in the rates of oxidation of β-OHB by muscle homogenates from the same 2 groups of subjects. We also measured citrate synthetase activity in muscle homogenates as an indicator of mitochondrial oxidative capacity.

2. Methods

2.1. Subjects

A total of 94 (47 lean and 47 obese individuals) subjects, free of vascular disease, diabetes, or cancer, and currently not taking medications that affect carbohydrate and lipid metabolism, participated in this study. A subset of 16 subjects (8 lean and 8 obese) were also studied for oxidation of β-OHB by muscle homogenates. Body mass index was calculated from body mass and height, which were recorded to the nearest 0.1 kg and 0.1 cm, respectively. Written consent was obtained from the participants after they were informed of the nature of the study. The University and Medical Center Institutional Review Board approved the protocols for these studies.

2.2. Blood analyses

Venous blood was drawn from an antecubital vein 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 prepared by centrifugation and stored at −80°C until analyzed. Glucose concentration was measured using a Glucose and Lactate Analyzer (model 2300, YSI Inc, Yellow Springs, Ohio). Insulin concentration was measured using a microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, Ill). β-OHB concentration was measured using a commercially available kit from Sigma (Sigma, St Louis, Mo), and nonesterified fatty acid levels were determined using a kit from Waco (Waco Chemicals, Richmond, Va).

2.3. Biochemical methods

Rectus abdominus muscle biopsies were obtained from the participants undergoing gastric bypass surgery [9,10] or total abdominal hysterectomy. The rate of β-OHB oxidation was determined as previously described [2] with a slight modification [11]. 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 ATP. Triplicate homogenate incubations were carried out in media containing the following at final concentrations: 0.1 M sucrose, 10 mmol/L Tris-HCl, 5 mmol/L K_2HPO_4, 80 mmol/L KCl, 1 mmol/L $MgCl_2$, 2 mmol/L L-carnitine, 0.1 mmol/L malate, 2 mmol/L ATP, 0.05 mmol/L coenzyme A, 1 mmol/L dithiothreitol, 0.2 mmol/L EDTA, 0.5% BSA, and 1.25 mmol/L [1 ^{14}C]-β-OHB (0.03 mCi/mL). 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 nanomoles of $^{14}CO_2$ produced per gram wet weight tissue per hour.

Citrate synthase activity was measured using a modified technique from Srere [12]. Muscles were thawed just before the start of the assay, refrozen in liquid nitrogen, then thawed to ensure freeze fracturing of the mitochondria. Muscle was homogenized (1:9 wt/vol) in media containing 0.175 mmol/L KCl and 2.0 mmol/L EDTA (pH 7.4). Citrate synthase activity was measured at 37°C in 0.1 mmol/L Tris-HCL (pH 8.3) assay buffer containing 0.12 mmol/L 5,5′-dithiobis(2-nitrobenzoic acid) and 0.6 mmol/L oxaloacetate. After an initial absorbance reading taken at 412 mm, 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 was expressed as micromoles per gram of tissue per minute.

Table 1
Physical and biochemical characteristics of all the subjects in the study

	Lean	Obese
N	47	47
Age (y)	43.4 ± 2.2	46.7 ± 2.8
BMI (kg/m^2)	24.5 ± 0.5	46.6 ± 1.7*
Glucose (mg/dL)	88.2 ± 3.5	96.1 ± 3.7
Insulin (μU/mL)	5.9 ± 0.8	15.8 ± 2.4*
Free fatty acids (nmol/L)	463 ± 44	662 ± 46*

Data are presented as mean ± SEM.

* Significantly different from the lean group ($P < .05$).

Table 2
Physical and biochemical characteristics of the subjects in the ketone-body oxidation arm of the study

	Lean	Obese
N	8	8
Age (y)	43.4 ± 2.2	45.9 ± 2.4
BMI (kg/m^2)	24.7 ± 0.7	43.6 ± 1.7*
Glucose (mg/dL)	87.8 ± 4.0	90.1 ± 5.1
Insulin (μU/mL)	3.8 ± 0.8	12.8 ± 1.9*

Data are presented as mean ± SEM.
* Significantly different from the lean group ($P < .05$).

2.4. Statistics

Data are reported as mean ± SEM. The data were analyzed using a 1-way ANOVA with Newman Keuls' post hoc testing when necessary. Statistical significance was inferred when $P < .05$ (SPSS Inc, Chicago, Ill).

3. Results

Table 1 shows the physical and biochemical characteristics of the subjects who participated in this study. There were no statistically significant differences in age or plasma glucose concentrations between the 2 groups, and none of the subjects was diabetic (fasting plasma glucose >125 mg/dL). As would be expected, the body mass index and plasma insulin concentrations were significantly higher in the obese than in the lean subjects. Free fatty acid concentration was significantly higher in the obese than in the lean women.

Table 2 shows that the physical and biochemical characteristics of the subset of subjects who participated in the arm of the study that examined ketone-body oxidation by muscle homogenates did not differ from the characteristics of all the subjects in the study.

The concentrations of β-OHB in the plasma of the 2 groups of subjects are shown in Fig. 1. Plasma β-OHB concentration in the obese subjects was lower by 37% ($P < .05$) than the concentration of β-OHB in the lean group.

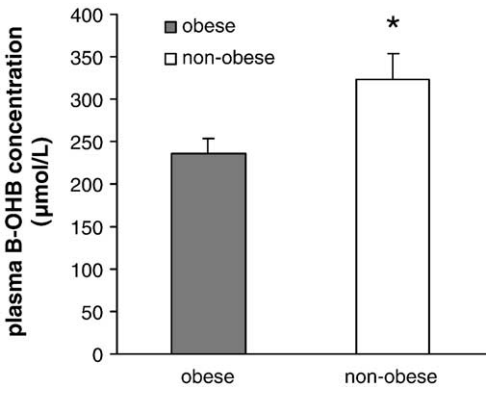

Fig. 1. Plasma β-hydroxybutyrate concentration in obese and lean women. β-Hydroxybutyrate concentration was determined using a commercial kit as described in Methods. β-Hydroxybutyrate levels were significantly lower in the plasma of the obese than in the plasma of the lean women. There were 47 subjects in each group.

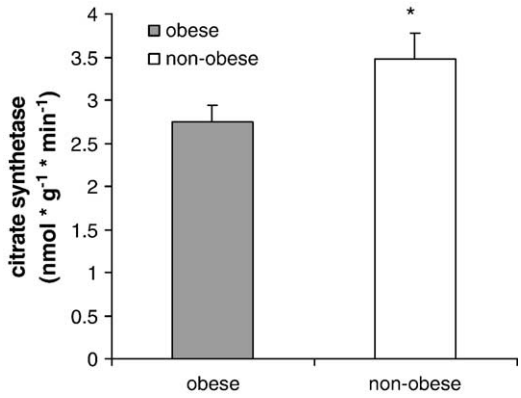

Fig. 2. Rates of oxidation of [1 ^{14}C]-β-hydroxybutyrate by muscle homogenates. The rate of β-hydroxybutyrate oxidation was determined by measuring the rate of CO_2 production from the complete oxidation of [1 ^{14}C]-β-hydroxybutyrate. Data are expressed as nanomoles of CO_2 produced per gram of wet tissue weight per hour. Details of the procedure that was used are described in Methods. The rate of β-hydroxybutyrate oxidation was significantly lower in the muscle homogenates of the 8 obese than those of the 8 lean women.

Fig. 2 shows the rates of oxidation of [1 ^{14}C]-β-OHB by muscle homogenates from subsets of the original lean and obese groups (8 subjects in each group). The rate of β-OHB oxidation, measured as the rate of $^{14}CO_2$ production from complete oxidation of ^{14}C-β-OHB by muscle homogenates of the obese group, was 45% lower ($P < .05$) than that of the nonobese group.

The activity of citrate synthetase in muscle whole homogenates from the lean and obese subjects who were included in β-OHB oxidation experiments (Table 2) is shown in Fig. 3. Consistent with previous results from our group, citrate synthetase activity in the obese was 22% lower than that in the lean subjects ($P < .05$)

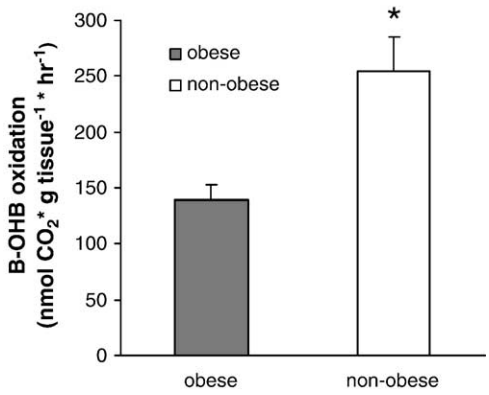

Fig. 3. Citrate synthetase activity in muscle homogenates. Citrate synthase activity was measured in muscle homogenates of the same subjects who were included in Fig. 2. Enzyme activity was significantly lower in the obese than in the lean women.

4. Discussion

The present study provided 2 novel findings with regard to the association of impairments in ketone body metabolism with obesity. The first is that circulating levels of ketone bodies are lower in obese than in lean women. Circulating levels of ketones are a function of the rate of their production by the liver and the rate of their use by extrahepatic tissues. The rate of ketone body production, in turn, is dependent on the abundance of plasma free fatty acids and the ability of the liver to oxidize these fatty acids. Our data show that plasma free fatty acid concentrations were higher in the obese than in the lean women (Table 1). These data suggest that despite elevated levels of plasma free fatty acids, the ability of the liver to produce ketone bodies might be lower in the obese than in the lean women. Although we did not determine the underlying causes of the lack of association between plasma free fatty acid concentration and plasma ketone body production by the liver, it is reasonable to assume that either the capacity of the liver to oxidize fatty acids or its capacity to synthesize ketone bodies is impaired in the obese subjects. The results of ketone body oxidation by muscle that we obtained provide clues on the metabolism of ketone bodies by liver, as further discussed below.

The second novel finding from this study is that the oxidation of ketone bodies was lower in muscle homogenates of the obese than of the lean women (Fig. 2). As mentioned earlier, as the rate of ketone body oxidation is related to plasma ketone body concentration, it is not surprising that the rate of oxidation of ketone bodies by muscle of the obese group was lower than that of the lean counterparts as plasma levels of ketone bodies were lower in the obese than in the lean group. Interestingly, this finding sheds some light on the relationship between obesity and ketone body metabolism by liver. Thyfault et al [13] have recently reported that whole-body fatty acid oxidation was lower in obese than in lean subjects. Because ketone body production by the liver is dependent on free fatty acid concentration in the plasma, and because plasma free fatty acid levels were higher in the obese than in the lean subjects, it could be safe to postulate that fatty acid oxidation by the liver is decreased in the obese women. The concomitant result is the decrease in ketone body concentration that we observed here.

The decreased rate of ketone body oxidation by muscle homogenates of obese subjects is further exacerbated by inherent differences in muscle metabolism in obese subjects. We have reported that the glycolytic muscle fibers are more abundant in obese than in lean subjects [14]. In addition, our finding in this study showing that citrate synthetase activity is lower in the muscle of obese than that of lean subjects, which is consistent with the previous findings from our group [2], suggests that mitochondrial function is impaired in obese subjects. Kelley et al [15] have reported that mitochondrial capacity and numbers are lower in muscle of obese individuals than that of lean subjects. Based on the data from the present investigation, we postulate that the decrement in ketone body oxidation by muscle of the obese subjects might be due to impairment in mitochondrial function and decreases in mitochondrial numbers. However, it is difficult from the present results to determine whether the abnormalities described are the result of obesity or predispose certain persons to obesity.

References

[1] 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 1999;277:E1130-41.
[2] Kim JY, Hickner RC, Cortright RL, et al. Lipid oxidation is reduced in obese human skeletal muscle. Am J Physiol 2000;279:E1039-44.
[3] Goodpaster BH, Kelley DE, Wing RR, et al. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. Diabetes 1999;48:839-47.
[4] Pan DA, Lillioja S, Kriketos AD, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 1997;46:983-8.
[5] Phillips DI, Caddy S, Ilic V, et al. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. Metabolism 1996;45:947-50.
[6] Simoneau J, Colberg S, Thaete F, et al. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. FASEB J 1995;9:273-8.
[7] Fukao T, Song XQ, Mitchell GA, et al. Enzymes of ketone body utilization in human tissues: protein and messenger RNA levels of succinyl-coenzyme A (CoA): 3-ketoacid CoA transferase and mitochondrial and cytosolic acetoacetyl-CoA thiolases. Pediatr Res 1997;42:498-502.
[8] Balasse EO, Fery F. Ketone body production and disposal: effects of fasting, diabetes, and exercise. Diabetes Metab Rev 1989;5:247-70.
[9] 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 1992;55:582S-5S.
[10] Pories WJ, Swanson MS, MacDonald KG, et al. Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. Ann Surg 1995;222:339-52.
[11] Privette JD, Hickner RC, Macdonald KG, et al. Fatty acid oxidation by skeletal muscle homogenates from morbidly obese black and white American women. Metabolism 2003;52:735-8.
[12] Srere PA. Citrate synthase. Methods Enzymol 1969;13:3-26.
[13] Thyfault JP, Kraus RM, Hickner RC, et al. Impaired plasma fatty acid oxidation in extremely obese women. Am J Physiol 2004;287:E1076-81.
[14] Tanner CJ, Barakat HA, Dohm GL, et al. Muscle fiber type is associated with obesity and weight loss. Am J Physiol Endocrinol Metab 2002;282:E1191-6.
[15] Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes 2002;51:2944-50.