In Vivo Assessment of the Mitochondrial Response to Caloric Restriction in Obese Women by the 2-Keto[1-^{13}C]Isocaproate Breath Test

Dolores Parra, Álvaro González, J. Alfredo Martínez, Idoia Labayen, and Nieves Díez

The 2-keto[1-^{13}C]isocaproate breath test has been proposed as a tool to detect mitochondrial dysfunction in alcoholic liver disease. The aim of this study was to evaluate if the 2-keto[1-^{13}C]isocaproate breath test could detect in vivo dynamic changes on mitochondrial activity due to caloric restriction in obese women. Fifteen obese women (body mass index [BMI] > 30 kg/m^2) participated in the study at baseline. Ten of these women agreed to participate on a diet program to induce body weight loss. Fifteen lean women (BMI < 25 kg/m^2) were included as a control group. The breath test was performed by the oral administration of the tracer measuring $^{13}CO_2$ enrichment in breath before and after ingestion using isotope ratio mass spectrometry. Body composition, resting energy expenditure, and plasma levels of insulin and leptin were measured. There were no relationships observed between the 2-keto[1-^{13}C]isocaproate breath test and the plasma insulin (before diet: *P* = .863; after diet: *P* = .879), or leptin (before diet: *P* = .500; after diet: *P* = .637). In obese women before treatment, kilograms of fat free mass (*P* = .108), resting energy expenditure adjusted for body composition (*P* = .312), and the 2-keto[1-^{13}C]isocaproate breath test (*P* = .205) were similar in comparison to lean women. However, 2-keto[1-^{13}C]isocaproate oxidation tended to increase after dieting and was significantly higher than in controls (*P* = .015). These data suggest that the 2-keto[1-^{13}C]isocaproate breath test reflected the adaptive modifications in mitochondrial oxidation in response to caloric restriction in obese women.

THE 2-KETO[1-^{13}C]ISOCAPROATE breath test has been proposed as a noninvasive probe to study mitochondrial function in vivo.[1] The principle of this assay is that 2-ketoisocaproate, labeled with ^{13}C in the carboxylic acid group, is decarboxylated by a dehydrogenase complex located in the inner mitochondrial membrane,[2] generating $^{13}CO_2$ and isovaleryl-CoA. Thus, the in vivo assessment of the mitochondrial activity is possible by measuring the exhalation of $^{13}CO_2$ after an oral load of 2-keto[1-^{13}C]isocaproate.[1] Human research using this approach showed that the 2-keto[1-^{13}C]isocaproate breath test could be considered as a specific marker to detect mitochondrial impairment associated with alcoholism.[3-5]

The human branched chain 2-keto acid dehydrogenase complex is located not only in liver,[3-5] but also in the pancreas, kidney, brain, skeletal muscle, and adipose tissue.[6] The enzymatic activity is dependent on the mitochondrial integrity[2] and is regulated by several agents, such as redox status of cells, availability of CoA, or insulin.[7] Thus, the oxidation carried out by different tissues and the effect of endocrine factors could be involved in the outcome of the 2-keto[1-^{13}C]isocaproate breath test.[8,9] This raises the possibility for applying this breath test to study the mitochondrial oxidation of the tracer in obesity, because mitochondria are involved in the modulation of body weight by changes in human energy expenditure,[10] and adipose tissue is able to handle branched chain amino acids.[7]

Therefore, the aim of this study was to evaluate the 2-keto[1-^{13}C]isocaproate breath test on obesity and to study whether the response of the mitochondrial function to caloric restriction could be detected in vivo by the breath test in obese women under a dietary treatment to lose weight.

MATERIALS AND METHODS

Subjects

Patients. Fifteen obese women (age, 21 to 57 years and body mass index [BMI] between 31. 4 and 40.3 kg/m^2) were recruited to participate in this experimental trial. These volunteers were selected and monitored by a physician in the Department of Physiology and Nutrition of the University of Navarra and were in good health, as assessed by medical history, physical examination, and routine hematologic and biochemical analyses. Ten of these women were randomly assigned to a 10-week energy-restricted dietary program to lose weight. Five of these patients followed an energy-restricted hyperproteic diet (carbohydrates, 40%; lipids, 30%; proteins, 30%) and the other 5, a hypocaloric balanced diet (carbohydrates, 55%; lipids, 30%; proteins, 15%). During the first 2 weeks, the caloric restriction was adjusted to be 500 kcal less than the initial resting energy expenditure of each woman, based on current recommendations. The caloric restriction was gradually increased every 2 weeks by 10% of the measured resting energy expenditure, but intake was never under 1,000 kcal per day. The experimental group of obese women performed no vigorous exercise during the dietary intervention trial.

Controls. Fifteen lean women (age, 18 to 43 years and BMI between 20.0 and 22.2 kg/m^2) were included in the control group. These women were healthy volunteers with no history or laboratory evidence of disease. Laboratory investigation included fasting blood sampling for complete blood count, plasma electrolytes and glucose, renal and liver function tests, lipid profile, plasma insulin, thyroid hormones, and a urine analysis.

All women participating gave their written informed consent to be involved in this experimental trial, which was previously approved by the Ethical Committee of the University of Navarra.

Methods

The 2-keto[1-^{13}C]isocaproate breath test was performed according to previous studies.[3-5] After an overnight fast, the subjects, who rested for 15 minutes before and during the test, received 6.5 μmol/kg 2-keto[1-^{13}C]isocaproate sodium salt (Euriso-top, Saint-Aubin Cedex, France)

From the Departments of Clinical Biochemistry and Nutrition, Clínica Universitaria de Navarra, Pamplona. Spain.

Submitted May 29, 2002; accepted October 14, 2002.

Supported by a PIUNA grant received from the University of Navarra.

Address reprint requests to Álvaro González, PhD, Department of Clinical Biochemistry, Clínica Universitaria de Navarra, Av. Pío XII Navarra n° 36, 31008 Pamplona, Spain.

0026-0495/03/5204-0014$30.00/0

doi:10.1053/meta.2003.50075

Table 1. Biological Parameters Obtained in Control Group and in Obese Women

	Lean Women	Obese Women
Fat mass (%)	25.9 (19.4-30.0)	49.3 (45.1-53.9)*
Fat mass (kg)	14.9 (11.5-18.9)	39.8 (37.7-56.5)*
Fat-free mass (%)	74.0 (65.6-76.6)	50.7 (46.1-54.6)*
Fat-free mass (kg)	44.7 (38.9-47.4)	46.5 (43.8-53.8)
Resting energy expenditure (kcal/d)	1,422 (1,370-1,460)	1,520 (1,470-1,690)*
Resting energy expenditure adjusted for body composition (kcal/d)	1,509 (1,463-1,562)	1,493 (1,440-1,567)
Respiratory quotient	0.82 (0.76-0.88)	0.87 (0.84-0.92)

*Significant differences ($P < .050$) between lean and obese women.

together with 152.4 μmol/kg L-leucine USP (Sigma-Aldrich Chemical, Madrid, Spain) dissolved in 200 mL orange juice. Breath samples were recovered by exhaling through a straw into a tube (Labco, High Wycombe, England) before and at 10-minute intervals during the 2 hours after ingestion of the 2-keto[1-^{13}C]isocaproate. Enrichment of $^{13}CO_2$ in breath was measured by isotope ratio mass spectrometry on a BreathMAT plus spectrometer (Finnigan, Bremen, Germany). The results obtained by this technique, which are expressed as $\delta\ ^{13}C_{PDB}$ (‰) value,[11] were converted to a percentage of the ^{13}C-administered dose recovered in breath per hour (%^{13}C/h), according to Ghoos et al.[11] The curve, which represented the time curse of the transformed data, was analyzed to measure the maximal rate of tracer oxidation or peak exhalation percentage of the dose (%^{13}C/h). The percent of 2-keto[1-^{13}C]isocaproate oxidized at 2 hours after the test solution ingestion (%^{13}C) was calculated as the area under the kinetic curve.[12]

Body composition was measured by air displacement plethysmography[13] in the morning and in a fasted state. The analyzer used was a Bod Pod Body Composition System (Life Measurement Instruments, Concord, CA).

Oxidative metabolism was estimated by 30 to 45 minutes of indirect calorimetry using a Vmax 29 calorimeter (SensorMedics, Yorba Linda, CA). Calorimetry was performed in the morning after an overnight fast, with subjects resting before and during the calorimetry. The respiratory quotient and the resting energy expenditure were calculated.

All measurements were performed in all subjects at the beginning and at the end of the obese group dietary treatment. Fasting plasma insulin of obese patients was assayed before and after the dietary treatment by automated immunoassay on an IMMULITE analyzer (DPC, Los Angeles, CA). Similarly, fasting plasma leptin concentration was measured using the DSL-23100 inmunoradiometric assay kit (Diagnostic Systems Laboratories, Webster, TX).

Statistical Analysis

Nonparametric statistical assay was performed by Statistica software using Windows 97 (Microsoft, Redmond, WA). Differences between independent groups were determined by the Mann-Whitney *U* test and comparisons between dependent groups by the Wilcoxon matched pairs test. Correlation analysis was performed by the Spearman test, and the resting energy expenditure was adjusted for body composition by linear regression.

RESULTS

Effect of Obesity on the 2-Keto[1-^{13}C]Isocaproate Breath Test

Body composition analysis showed that the observed fat-free mass of obese women (n = 15) who participated in the study was similar to the control group values (n = 15) (Table 1). The respiratory quotient and the resting energy expenditure adjusted for body composition (fat and fat-free mass) were also similar between the groups (Table 1). Furthermore, the percentage of tracer oxidized during the 2-keto[1-^{13}C]isocaproate breath test did not differ from that obtained in the lean women group (obese: median, 27.9%; 25th to 75th percentiles: 22.0% to 29.3%; controls: median, 29.7%; 25th to 75th percentiles: 25.7% to 33.4%; $P = .205$). Also, no statistical differences between the groups in maximal oxidation rate were observed (obese: median, 22.5%/h; 25th to 75th percentiles: 17.4%/h to 27.0%/h; controls: median, 22.5%/h; 25th to 75th percentiles: 20.6%/h to 29.1%/h; $P = .534$).

The analysis of data revealed no correlation between the 2-keto[1-^{13}C]isocaproate breath test and the body composition (fat-free mass: $r = -.24$ $P = .228$ for the percentage of oxidized tracer and $r = -.28$, $P = .149$ for the maximal oxidation rate; fat mass: $r = -.19$, $P = .324$ for the percentage of oxidized tracer and $r = -.15$, $P = .149$ for the maximal oxidation rate). Additionally, no association between the respiratory quotient and the oxidation parameters obtained by 2-keto[1-^{13}C]isocaproate breath test was found ($r = -.24$, $P = .219$ for the percentage of oxidized tracer; $r = .09$, $P = .673$ for the maximal oxidation rate). The resting energy expenditure adjusted for the body composition showed no correlation with the result of the breath test ($r = .01$, $P = .981$ for the percentage of oxidized tracer and $r = -.13$, $P = .499$ for the maximal oxidation rate).

The lack of statistical association between the biological variables studied and the 2-keto[1-^{13}C]isocaproate breath test was maintained when obese patients and lean controls were analyzed in separate groups.

In the obese women group, the percentage of 2-keto[1-^{13}C]isocaproate oxidized did not correlate with plasma levels of insulin ($r = .04$, $P = .897$) or leptin ($r = .11$, $P = .749$), and no relationship between the maximal oxidation rate and the plasma levels of insulin ($r = -.06$, $P = .863$) or leptin ($r = .23$, $P = .500$) was found. Among the patients of this group, 6 had hyperinsulinemia (basal levels of insulin in plasma higher than 25 μU/mL) with the overload glucose test in normal range, although their body composition ($P = .200$ for fat-free mass and $P = .631$ for fat mass), respiratory quotient ($P = .169$), as well as the resting energy expenditure adjusted for body composition ($P = .054$), were similar to that of obese patients without hyperinsulinemia.

Effect of the Caloric Restriction Program on the 2-Keto[1-^{13}C]Isocaproate Breath Test

Ten patients included in the group of obese women agreed to participate in the dietary trial designed to lose weight by following 2 different types of hypocaloric diets. After the

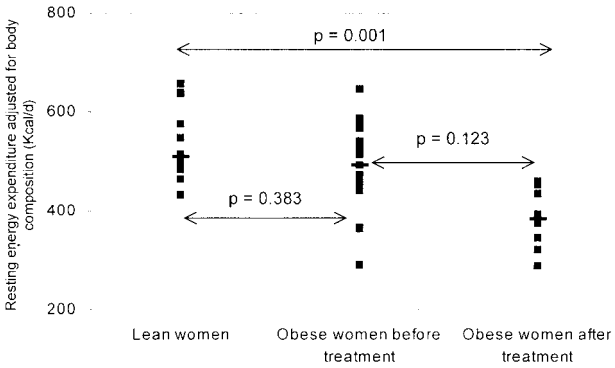

Fig 1. Resting energy expenditure in lean and obese women before and after the dietary trial to lose weight.

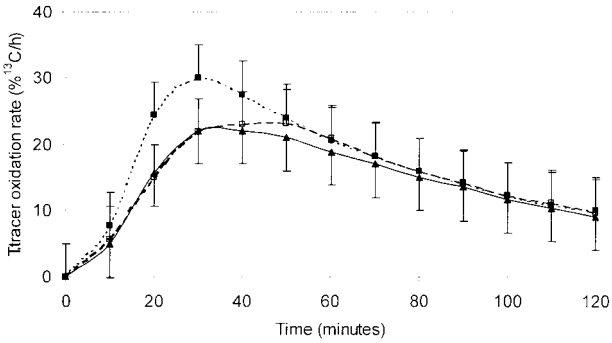

Fig 2. Kinetic curves describing the time course of the 2-keto[1-^{13}C]isocaproate oxidation measured by the breath test in lean and obese women before and after the dietary trial to lose weight (mean together with the 95% confidence interval). (--□--), Obese women before treatment; (--■--), obese women after treatment; and (—▲—), lean women.

dietary regimen, the weight reduction was between 3.8% and 8.3% (mean, 6%; SD, 1.7%) for 5 women who were fed the balanced diet according to current macronutrient recommendations for healthy adults and 2.5% and 12.4% (mean, 7%; SD, 3.4%) for 5 women receiving the energy-restricted hyperproteic diet. Because a similar response was observed between the 2 dietary groups concerning all the metabolic parameters studied, the findings were grouped together for further analysis.

The dietary program decreased fat mass ($P = .008$), but did not affect the fat-free mass ($P = .477$) of participants. Plasma levels of leptin (before diet: median, 53.7 μg/L; 25th to 75th percentiles: 49.2 μg/L to 76.4 μg/L; after diet: median, 31.0 μg/L; 25th to 75th percentiles: 21.4 μg/L to 38.5 μg/L; $P = .017$), as well as the basal levels of insulin (before diet: median, 22.9 μU/mL; 25th to 75th percentiles: 10.0 μU/mL to 32.3 μU/mL; after diet: median, 8.0 μU/mL; 25th to 75th percentiles: 7.2 μU/mL to 11.5 μU/mL; $P = .017$) decreased after treatment.

As expected, the respiratory quotient decreased (before diet: median, 0.88; 25th to 75th percentiles: 0.87 to 0.92; after diet: median, 0.87; 25th to 75th percentiles: 0.81 to 0.88; $P = .043$), as well as the resting energy expenditure adjusted for body composition (Fig 1). In fact, energy expenditure was lower in the obese women after the nutritional intervention than in lean women ($P = .001$) (Fig 1). In contrast, the 2-keto[1-^{13}C]isocaproate oxidation in the obese group who followed the dietary trial (n = 10) increased after the diet intervention (Fig 2), as reflected by the maximal oxidation rate (before diet: median, 22.5%/h; 25th to 75th percentiles: 17.4%/h to 27.0%/h; after diet: median, 29.9%/h; 25th to 75th percentiles: 22.5%/h to 30.5%/h; $P = .015$) and was higher than in the control group ($P = .034$). The amount of tracer oxidized also tended to increase after the caloric restriction trial (before diet: median, 27.9%; 25th to 75th percentiles: 22.0% to 33.0%; after diet: median, 35.4%; 25th to 75th percentiles: 27.6% to 36.1%; $P = .066$) and was statistically higher than in lean women ($P = .015$) (Fig 3). No correlation between the amount of weight loss and the percentage of tracer oxidized after dieting ($r = -.34$, $P = .328$) and the increment of oxidation ($r = -.25$, $P = .489$) was found.

DISCUSSION

Obesity is caused by a chronic imbalance between energy intake and energy expenditure and has been related to genetic predisposition, sedentary lifestyle, and overfeeding.[14,15] Although the involvement of mitochondrial dysfunction as a primary cause of obesity is unusual, these organelles could be involved in the disease due to their fat oxidation and energy production.[16] The oxidation of the 2-keto[1-^{13}C]isocaproate measured by a breath test has been proposed as a noninvasive method to study mitochondrial function in vivo.[1,3,17] The usefulness of this probe to detect mitochondrial impairment in alcoholic patients,[3,4] as well as in the differential diagnosis of alcoholic and nonalcoholic hepatic steatosis,[5] has been documented. Moreover, it is likely that this breath test may reflect not only the tracer oxidation in liver, but also the whole body mitochondrial metabolism of 2-keto[1-^{13}C]isocaproate.[8,9,18] Based on the oxidative information obtained through the 2-keto[1-^{13}C]isocaproate breath test, we studied whether the mitochondrial function reflected by this breath test suggested an impairment in obese women.

In agreement with previous studies,[19] the obese women

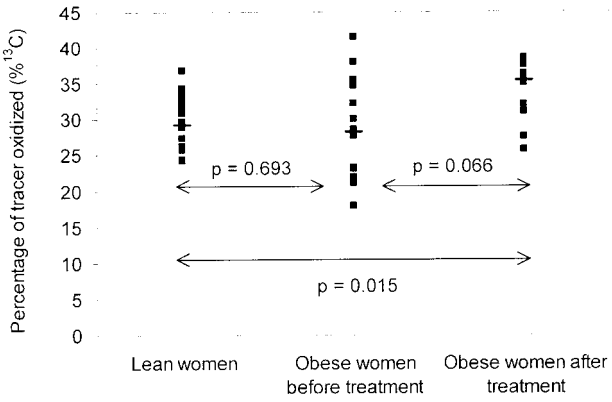

Fig 3. Percentage of tracer oxidized estimated by the 2-keto[1-^{13}C]isocaproate breath test in lean and obese women before and after the dietary program to lose weight.

participating in our trial had similar resting energy expenditure values than the control group when the parameter was adjusted for fat mass and fat-free mass. Moreover, the respiratory quotient was similar between both groups, indicating that the macronutrient utilization was not different between the obese and the lean women under the described experimental conditions. The 2-keto[1-^{13}C]isocaproate oxidation was also similar between groups, suggesting that the mitochondrial function estimated by this breath test was not impaired in the obese women. Similarly, other investigators have found no differences in the maximal mitochondrial function of obese and non-obese women, using ^{31}P magnetic resonance spectroscopy.[20]

Branched chain amino acid metabolism in adipose tissue is regulated by insulin.[7] For that reason, the insulin resistance associated with obesity could promote a decrease in 2-keto[1-^{13}C]isocaproate oxidation, but our results showed that obese patients with hyperinsulinemia oxidized a similar percentage of tracer to that measured in lean controls or in patients with plasma levels of basal insulin in the normal range. Previous studies have reported that obese-related insulin resistance did not affect leucine oxidation.[21] The oxidation of [1-^{13}C]leucine, as measured by the breath test (data not show), was similar in lean and in obese women and was not changed after the weight reduction in treated obese women. That this catabolic pathway is not affected by insulin resistance may account for why the 2-keto[1-^{13}C]isocaproate breath test was not altered in obese patients with hyperinsulinemia.

The 2-keto[1-^{13}C]isocaproate breath test performed in the obese women involved in this study did not reflect a mitochondrial dysfunction, however, observations in subjects with established obesity may reflect a state of compensated metabolism.[15] Based on this, we attempted to detect a dynamic mitochondrial response to perturbations in energy balance by using the breath test in obese women before and after a dietary treatment-induced weight loss. The decrease in respiratory quotient of obese women after the dietary intervention showed the impact that nutritional therapy had on peripheral metabolism by enhancing the utilization of lipids to reduce body weight. Previous studies with the 2-keto[1-^{13}C]isocaproate breath test were performed with subjects having similar body weights to reduce the potential variability of the breath test related to body composition.[3-5,8,22] Based on this, we studied the effect of changes in body composition on the breath test. We found no correlation between body composition and the 2-keto[1-^{13}C] isocaproate breath test performed in non-obese and obese women, before and after diet-induced weight reduction, suggesting that, at least in women, the breath test is independent of the size of fat and fat-free mass body compartments under the chosen experimental conditions.

While the contribution of low resting energy expenditure to the etiology of obesity is controversial,[15,19] it is well established that weight gain or loss is associated with compensatory changes in energy expenditure.[23] We observed an adaptive reduction in resting energy expenditure to increase the metabolic efficiency of caloric intake in obese women after the dietary intervention. In contrast, the oxidation of 2-keto[1-^{13}C]isocaproate measured by the breath test was increased after the caloric restriction.

Some investigators have used the 2-keto[1-^{13}C]isocaprote breath test and the resting energy expenditure to estimate changes in mitochondrial function associated with tacrolimus exposure on patients with liver transplantation.[24] They found relationships between the resting energy expenditure and the percentage of tracer oxidized,[5] while our results showed no correlation between the resting energy expenditure and the oxidation of 2-keto[1-^{13}C]isocaproate. These differences could be ascribed to individual characteristics of the subjects participating in both studies. On the other hand, resting energy expenditure mainly depends on the oxidative processes occurring in fat-free tissues,[24] while enzymes implicated in the oxidative pathway of 2-keto[1-^{13}C]isocaproate present a wider tissue distribution,[6] suggesting that the percentage of tracer oxidized and the resting energy expenditure may supply different and not directly related information about oxidative processes.

The metabolic response to caloric restriction reflected by resting energy expenditure could be partly determined by a feedback system linking the state of fat store depletion to a compensatory mechanism that suppresses thermogenesis.[10] We hypothesized that changes in mitochondrial oxidation of 2-keto[1-^{13}C]isocaproate could also be involved in this response. As expected, the reduction in fat mass was accompanied by a decrease in plasma levels of insulin, and the increase in 2-keto[1-^{13}C]isocaproate oxidation reflected by the breath test could be related to the improvement of insulin resistance in these treated obese patients. This hypothesis is supported by the improvement of liver mitochondrial respiratory function after the administration of insulin, as described in diabetic rats.[26]

In addition, the fat depletion in the dietary treated obese patients was accompanied by a decrease in plasma levels of leptin, which has been proposed to participate in the thermogenesis by uncoupling oxidative phosphorylation.[27] In isolated liver mitochondria from *ob/ob* mice, an increased proton leakage was described, as well as the reduction in this proton leakage after the acute administration of leptin.[28] In relation with this, mitochondrial proteins, named uncoupling proteins (UCPs), could be involved in thermogenesis.[29] Due to the dependence of 2-keto[1-^{13}C]isocaproate on the redox status in cells,[2] uncouplers of the oxidative phosphorylation could increase the percentage of tracer oxidized by increasing the NAD^+/NADH ratio.[22] This effect has been described after administration of mitochondrial uncouplers, such as salicylate,[22] or tacrolimus.[24] In the same way, mitochondrial UCPs could participate in the metabolic response to the weight reduction in treated obese women,[30] and the uncoupling of mitochondrial respiration mediated by them could increase the oxidation of 2-keto[1-^{13}C]isocaproate.

In summary, the 2-keto[1-^{13}C]isocaproate breath test suggests that obese patients have no mitochondrial oxidation impairment. However, obese women after a dietary intervention prescribed to lose weight, presented higher 2-keto[1-^{13}C]isocaproate oxidation than lean women, indicating the 2-keto[1-^{13}C]isocaproate breath test reflected the mitochondrial response to caloric restriction, independent of changes in body composition. Therefore, the 2-keto[1-^{13}C]isocaproate breath test could be considered as a noninvasive and safe probe useful for monitoring in vivo dynamic changes in mitochondrial metabolism during the treatment of obesity.

REFERENCES

1. Michaletz PA, Cap L, Alpert E, et al: Assesment of mitochondrial function in vivo with a breath test utilizing α-ketoisocaproic acid. Hepatology 10:829-832, 1989
2. Newby FD, Russ PS: Determinants of protein turnover in health and disease. Miner Electrolyte Metab 24:6-12, 1998
3. Lauterburg BH, Liang D, Schwarzenbach FA, et al: Mitochondrial dysfunction in alcoholic patients as assessed by breath analysis. Hepatology 17:418-422, 1993
4. Witschi A, Mossi S, Meyer B, et al: Mitochondrial function reflected by the decarboxylation of (^{13}C)ketoisocaproate is impaired in alcoholics. Alcohol Clin Exp Res 18:951-955, 1994
5. Mion F, Rousseau M, Brazier JL, et al: Human hepatic macrovesicular steatosis: A noninvasive study of mitochondrial ketoisocaproic acid decarboxylation. Metabolism 44:699-700, 1995
6. Suryawan A, Hawes JW, Harris RA, et al: A molecular model of human branched-chain amino acid metabolism. Am J Clin Nutr 68:72-81, 1998
7. Wagenmakers MAJ, Soeters BP: Metabolism of branched-chain amino acids, in Cynober LA (ed): Amino Acid Metabolism and Therapy in Health and Nutritional Disease. Boca Raton, FL, CRC, 1985, pp 67-87
8. Bendtsen P, Hannestad U, Pahlsson P: Evaluation of the carbon 13-labeled ketoisocaproate breath test to assess mitochondrial dysfunction in patients with high alcohol consumption. Alcohol Clin Exp Res 22:1792-1795, 1998
9. Grattagliano I, Vendemiale G, Lauterburg BH, et al: Reperfusion injury of the liver: Role of mitochondria and protection by glutathione ester. J Surg Res 86:2-8, 1999
10. Dulloo AG, Jacqet J: Adaptive reduction in basal metabolic rate in response to food deprivation in humans: A role for feedback signals from fat stores. Am J Clin Nutr 68:599-606, 1998
11. Ghoos Y, Maes BD, Geypens Bj, et al: Measurement of gastric emptying rate of solids by means of a carbon-labelled octanoic breath test. Gastroenterology 104:1640-1647, 1993
12. Gibaldi M (ed): Estimation of area under the curve, in Library of Congress Cataloging in Publications Data: Biopharmaceutics and Clinical Pharmacokinetics. Philadelphia, PA, Lea & Febiger, 1991, pp 377-378
13. McCrory MA, Wright NC, Kilmer DD: Nutritional aspects of neuromuscular diseases. Phys Med Rehabil Clin N Am 9:127-143, 1998
14. Martínez JA: Body-weight regulation: Causes of obesity. Proc Nutr Soc 59:337-345, 2000
15. Prentice AM, Black AE, Coward WA, et al: High levels of energy expenditure in obese women. BMJ 292:983-987, 1986
16. Ziegler O, Quilliot D, Guerci B, et al: Macronutrients, fat mass, fatty acid flux and insulin sensitivity. Diabetes Metab 27:261-270, 2001
17. Parra D, González A, Martínez JA, et al: Laboratory approach to mitochondrial disorders. J Physiol Biochem 57:269-288, 2001
18. Witschi A, Felix A, Schwarzenbarch A, et al: Mitochondrial function assessed by in vivo decarboxylation of α-ketoisocaproate is normal in patients with primary biliary cirrhosis. Hepatol Res 3:316-321, 1995
19. Astrup A, Gotzsche PC, van der Werken K, et al: Meta-analysis of resting metabolic rate in formerly obese subjects. Am J Clin Nutr 69:1117-1122, 1999
20. Larson-Meyer DE, Newcomer BR, Hunter GR, et al: Effect of weight reduction, obesity predisposition, and aerobic fitness on skeletal muscle mitochondrial function. Am J Physiol 278:E153-E161, 2000
21. Luzi L, Castellino P, DeFronzo RA: Insulin and hyperaminoacidemia regulate by different mechanisms leucine turnover and oxidation in obesity. Am J Physiol 270:E273-E281, 1996
22. Lauterburg BH, Grattagliano I, Gmür R, et al: Noninvasive assessment of the effect of xenobiotics on mitochondrial function in human beings: Studies with acetylsalycilic acid and ethanol with the use of the carbon 13-labeled ketoisocaproate breath test. J Lab Clin Med 125:378-383, 1995
23. Rosebaum M, Hirsch J, Murphy E, et al: Effects of changes in body weight on carbohydrate metabolism, catecholamine excretion, and thyroid function. Am J Clin Nutr 71:1421-1432, 2000
24. Gabe SM, Bjarnasson I, Tolou-Ghamari Z, et al: The effect of tacrolimus (FK506) on intestinal barrier function and cellular energy production in humans. Gastroenterology 115:67-74, 1998
25. Linder MC: Energy metabolism, intake and expenditure, in Linder MC (ed): Nutritional Biochemistry and Metabolism With Clinical Applications. Englewood Cliffs, NJ, Prentice-Hall, 1991, pp 277-304
26. Huang Q, Shao L, Jiang H, et al: Effect of insulin on oxygen free radicals and oxidative phosphorylation in liver mitochondria of diabetic rats. Chung Kuo Yao Li Hsueh Pao 22:455-458, 2001
27. Ricquier D, Bouillaud F: Mitochondrial uncoupling proteins: From mitochondria to the regulation of energy balance. J Physiol 529:3-10, 2000
28. Porter RK: Mitochondrial proton leak: A role for uncoupling proteins 2 and 3? Biochim Biophys Acta 1504:120-127, 2001
29. Nedergaad J, Golozoubova V, Matthias A, et al: UCP1: The only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. Biochim Biophys Acta 1504:82-106, 2001
30. Pedersen SB, Borglum JD, Kristensen K, et al: Regulation of uncoupling protein (UCP) 2 and 3 in adipose and muscle tissue by fasting and growth hormone treatment in obese humans. Int J Obes Relat Metab Disord 24:968-975, 2000