[Frontiers in Bioscience 7, a109-116, May 1, 2002]

DECREASED MITOCHONDRIAL CARNITINE TRANSLOCASE IN SKELETAL MUSCLES IMPAIRS UTILIZATION OF FATTY ACIDS IN INSULIN-RESISTANT PATIENTS

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TABLE OF CONTENTS

1. ABSTRACT

Insulin resistance (IR) and its health consequences (diabetes, hypertension, cardiovascular disease, obesity etc.) affect between 25 and 35% of Westernized populations. Decreased fatty acid (FA) oxidation in skeletal muscle is implicated in obesity-related IR. Carnitine-acylcarnitine translocase (CACT) transports long-chain FAs both into mitochondria (as carnitine esters for energy-generating processes) and out of mitochondria. To determine whether CACT activity correlates with decreased FA oxidation we measured CACT concentrations in cellular and mitochondrial extracts from the skeletalmuscle of 19 obese IR individuals and of 19 lean controls. We also evaluated carnitine transport in skeletal muscle mitochondria in both groups. Mitochondrial CACT was decreased at translational and transductional level, and carnitine-carnitine and acylcarnitine-carnitine exchange rates were significantly lower in IR subjects. Aberrant acylcarnitine flux into mitochondria was not correlated with decreased activity of other components of the mitochondrial carnitine system (i.e., carnitine palmitoyl transferase-I and II). Our data suggest that by restraining entry of FA-coenzyme A into mitochondria, low CACT levels increase cytosolic FA levels and their incorporation into glycerolipids. The low level of CACT in IR muscle may contribute to the elevated muscle concentrations of triglycerides, diacylglycerol, and FA-coenzyme A characteristic of IR muscle.

2. INTRODUCTION

From 25 to 35 percent of Western populations has some degree of insulin resistance (IR) and health consequences associated with this metabolic derangement (1). Insulin resistance is a major feature of type 2 diabetes and obesity (2). The condition is now recognized as a state of reduced responsiveness to normal circulating

concentrations of insulin in specific target tissues such as fat and skeletal muscle. Quantitatively, skeletal muscle has the greatest impact on whole-body glucose disposal, and hence on IR (3). Skeletal muscle is important not only for glucose utilization mediated by insulin signaling, but also for fatty acid (FA) metabolism. Fatty acids are the main substrate for energy production in muscle during fasting or exercise (4, 5). Skeletal muscle capacity for FA utilization is important for systemic fat balance (6). Increased muscle fat in IR obesity has been related to increased FA availability and uptake (7, 8). Instead, during fasting or aerobic exercise training, when the capacity for FA oxidation significantly increases, FA oxidation is reduced in skeletal muscle in IR and in type 2 diabetes (7-9). Again, impaired muscle oxidation of lipids has been invoked in individuals at risk for obesity (9). A perspective clinical study demonstrated that decreased capability to utilize FAs is a risk factor for weight gain (10). Furthermore impaired FA oxidation in skeletal muscle can persist after weight loss in post-obese subjects (11). Despite numerous studies (12-14), the reduced FA oxidation in IR remains an enigma.

Since the carnitine system mediates the entry of FAs into mitochondria for energy-generating processes (12), we measured the concentrations of carnitine-acylcarnitine translocase (CACT), which is a crucial component of this system, in lean subjects and in IR obese individuals. Long-chain FAs are translocated into mitochondria as carnitine esters (15). Consequently, cytosolic acyl coenzyme A esters must necessarily be transesterified to L-carnitine in a reaction catalyzed by carnitine palmitoyl transferase I (E.C. 2.3.1.21) on the outer membrane of mitochondria. Carnitine-acylcarnitine translocase shuttles long-chain acylcarnitine esters into mitochondria, and carnitine may exit the mitochondrion as such or as a short-chain acylcarnitine ester, mainly via this specific carrier (16). In mitochondria, short- and medium-chain FAs such as acyl-CoA esters arising from beta-oxidation and other mitochondrial processes are transesterified to carnitine by carnitine acetyl transferase (E.C. 2.3.1.7). Again, CACT transports acylcarnitine out of mitochondria. This ping-pong mechanism by which short-, medium-, and long-chain acylcarnitines are transported across the mitochondrial membrane in exchange for carnitine (17) is unique among mitochondrial transporters. The unidirectional transport of carnitine is slow (generally concentration- and gradient-driven), since there is no second substrate (carnitine/acylcarnitine) to bind.

Thus, CACT equilibrates the concentrations of its reactants in both the cytosolic and mitochondrial compartments. In case of an increase of acylcarnitines in the mitochondrial matrix, CACT may export acylcarnitine-esters to the cytosol and import carnitine into the mitochondria. This pathway provides a means to regenerate intramitochondrial free coenzyme A (CoA) under conditions in which short-chain acyl-CoA esters are produced at a rate faster than they can be utilized. By this mechanism CACT buffers the excess acyl groups in the cytosol or in the mitochondrial matrix, so preventing overacylation of CoA and therefore ensuring that free CoA is available for other essential CoA-requiring reactions such as pyruvate oxidation. Moreover, rapid removal of long-chain acylcarnitines from the cytosol gives the 'two-way' transesterification reaction a high degree of unidirectionality towards the formation of acyl-carnitine and CoA from long-chain acyl-CoA and free carnitine.

We hypothesized that in obesity-related IR, FA oxidation would be decreased in skeletal muscle because of decreased CACT activity. To test this hypothesis we evaluated CACT at transcriptional and transductional levels in patients with phenotypic IR and in control subjects. We also evaluated the transport of carnitine in isolated mitochondria obtained from skeletal muscle tissue of the same subjects to determine whether carrier activity was decreased.

3. MATERIALS AND METHODS

3.1. Clinical Characteristics of Patients

Nineteen IR obese (body mass index [BMI] > 40 kg/m^2) patients (11 men and 8 women) were enrolled in the study (Table 1). None had diabetes mellitus or any other endocrine or non-endocrine diseases. Patients and controls were on a similar diet, i.e., 60% carbohydrate, 30% fat, 10% protein (at least 1 g per kg of body weight). This dietary regimen was maintained for one week before the study. Diet composition was similar in controls and obese subjects. The 19 lean subjects underwent abdominal surgery for cholecystectomy. The IR obese patients underwent bilio-pancreatic diversion, consisting of a partial gastrectomy with a distal *Roux-en-Y* reconstruction. During surgery, the investing fascia of the rectus abdominis muscle was opened and multiple biopsies were obtained. Each sample was free of visible contamination from subcutaneous adipose tissue and was immediately placed in liquid nitrogen and stored at –80°C until analyzed. The study protocol was approved by the Institutional Review Board of the Catholic University (Rome, Italy); the nature and purpose of the study were carefully explained to all subjects before written consent was given.

3.2. Body composition

The day before surgery, body weight was measured to the nearest 0.1 kg by a beam scale, and height to the nearest 0.5 cm with a stadiometer (Holatin, Crosswell, Wales, UK). Total body water was determined using 0.19 Bq of tritiated water in 5 ml of intravenous-bolus-injected saline solution (18, 19).

3.3. Euglycemic hyperinsulinemic clamp

After a 12–14-h overnight fast at the Metabolic Diseases Department, School of Medicine, Catholic University (Rome), the obese IR subjects and controls underwent the euglycemic hyperinsulinemic glucose clamp (EHC) procedure described by De Fronzo et al. (20). Obese patients underwent the procedure two days before surgery.

3.4. Skeletal muscle lipid analysis

The concentration of skeletal muscle lipids was measured as previously described (21). Briefly, lipids extracted from biopsies were dissolved in

Table 1. Whole body glucose uptake, steady-state plasma insulin, plasma lipid (triglycerides and NEFA) concentrations, and HPLC fatty acid profile of skeletal muscle (SM) triglycerides in control and obese subjects

		Controls n. 19	Obese subjects n. 19	P
Whole body glucose uptake	micromol/kg/min	50.12 ± 9.78	21.76 ± 4.15	<0.01
Steady-state plasma insulin	pM	497.15 ± 32.23	567.31 ± 50.28	NS
Plasma triglycerides	mM	0.92 ± 0.18	1.63 ± 0.24	<0.01
Plasma NEFA	mM	422.55 ± 77.31	570.84 ± 82.19	<0.01
Total SM triglycerides	mg	6.112 ± 2.390	19.33 ± 7.010	<0.0001
SM myristic acid	mg	0.008 ± 0.002	0.010 ± 0.002	<0.01
SM palmitoleic acid	mg	0.084 ± 0.032	0.106 ± 0.045	NS
SM oleic acid	mg	1.328 ± 0.350	1.678 ± 0.639	NS
SM palmitic acid	mg	0.713 ± 0.188	0.901 ± 0.128	<0.01
SM linoleic acid	mg	0.179 ± 0.472	0.226 ± 0.322	NS
SM stearic acid	mg	0.147 ± 0.039	0.186 ± 0.026	<0.01
Intramyocytic triglycerides	mg	2.450 ± 0.641	3.107 ± 0.443	<0.01
Unsaturated/Saturated ratio	mg	1.846 ± 0.077	1.318 ± 0.036	<0.01

The values are expressed as mean ± SD; SM mean values are referred to 100 mg of wet tissue. Total SM triglycerides are the global amount of muscle triglycerides including both those between myocytes and those inside myocytes.

chloroform:methanol (2:1 vol/vol) and fractionated into their various components by thin-layer chromatography. The area of silica gel corresponding to the ratio of fractionation time (R_f) of a triolein and tripalmitin standard mixture was scraped off and extracted with peroxide-free diethyl ether. FAs were obtained, separated and measured as described elsewhere (19).

3.5. Northern blot anakysis

Frozen skeletal muscle biopsies were pulverized in liquid nitrogen (Omni 5000, Bioquip, Inc.), and total RNA was isolated by the TRIzol (Life Technologies, Paisley, UK) procedure. The integrity and the amount of RNA were checked by ethidium bromide staining of ribosomal RNA. Poly(A)$^+$ RNA was isolated, blotted onto a positive nylon membrane, and immobilized by UV cross-linking using a UV Stratalinker 1800 (Stratagene, La Jolla, CA, USA) following a standard procedure (22). The RNA recovered was quantified by spectrometry. After denaturation, equal amounts of RNA from control and test samples were loaded on the agarose-formaldehyde gel. CACT cDNA was labeled with [^{32}P]dCTP and [^{32}P]dATP) (50 microCi each at a specific activity of 3,000 Ci/mmol; Amersham Pharmacia Biotec, Milan, Italy) using the random hexanucleotide-primed second-strand synthesis method (22). Samples were hybridized for 24 h at 42°C and the filters were washed three times for 30 min at 58°C. The blots were stripped and rehybridized, under the same conditions, with a beta-actin probe obtained by digestion of a pUC19 vector containing a 1.9-kb human beta-actin insert between BamHI sites (American Type Culture Collection, Rockville, MD, USA). Autoradiographic exposure intensities were compared using an Ultrascan XL enhanced laser densitometer (two-dimensional gel scan program, Amersham Pharmacia Biotech, Milan, Italy). The relative carnitine translocase RNA levels were expressed as a percentage of their corresponding beta-actin bands. The northern analyses shown are representative of three or more experiments.

3.6. Isolation of mitochondria

Mitochondria were isolated by differential centrifugation of fresh unfrozen muscle samples (23). Manipulations were conducted at 4°C or on ice to minimize mitochondrial-membrane and protein degradation. Skeletal muscle specimens were finely minced with a Thomas tissue slicer, immersed in isotonic homogenization buffer (225 mM mannitol; 75 mM sucrose; 10 mM Mops; 1 mM EGTA; 0.5% BSA, pH 7.2) and homogenized (homogenizer, IKA Works, Wilmington, NC, USA). Mitochondrial pellets were resuspended in the same buffer (150-600 microl). An aliquot of mitochondrial preparation suspended in the buffer without BSA was analyzed for protein concentration (BCA protein assay, Pierce, Milan, Italy).

3.7. Western blot analysis

Western blots were prepared using affinity purified rabbit polyclonal antibodies against mitochondrial CACT. Antisera were collected and purified by affinity chromatography on respective peptide-Sepharose columns. Total cellular lysate (20 microg) or isolated mitochondrial lysate (15 microg) was electrophoresed, and blotted onto nitrocellulose membranes. Blots were preincubated with 5% non-fat dry milk in Tris buffered saline (TBS) before immunostaining. For specific immunodetection of CACT, rabbit anti-human polyclonal CACT antibody was used at the appropriate concentration in a solution containing 0.5% non-fat dry milk and 1% BSA in TBS containing 0.1% Tween 20. We also checked the amount of protein loading with a rabbit anti-actin antibody (Sigma, Milan, Italy). Immunoreactive proteins were visualized with an enhanced chemiluminescence detection system (Biorad, Milan, Italy). The Western blots were subject to densitometry (three

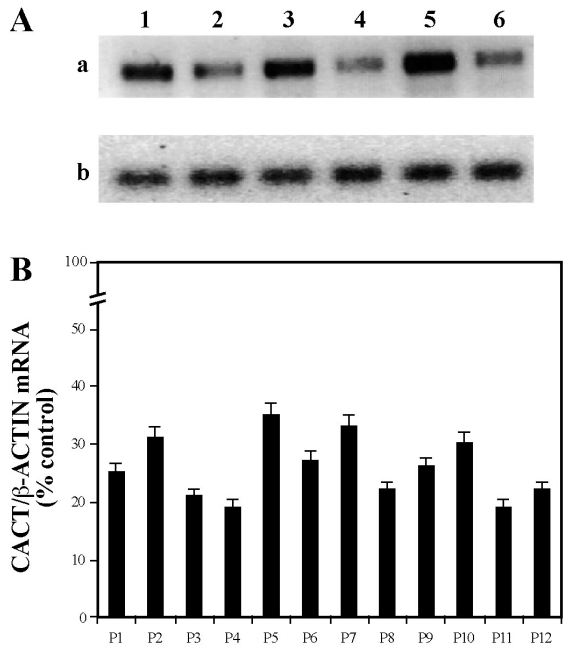

Figure 1. Decreased CACT mRNA levels in skeletal muscle specimens from IR patients. A. Northern blot analysis showing the expression of CACT mRNA in control (lane A: 1, 3 and 5) and IR subjects (lane A: 2, 4 and 6). mRNA was isolated as described in Materials and Methods, run in a 1% (w/v) agarose-formaldehyde gel and transferred to nylon filters. The filters were hybridized with the ^{32}P-labeled CACT cDNA; the blot was rehybridized to beta-actin probe (lane B) as control. Quantitatively similar results were obtained in three independent experiments. B. Quantitative analysis of CACT mRNA expression normalized with beta-actin mRNA for 12 representative patients. Autoradiographic signal intensities were determined with densitometry. The CACT: beta-actin intensity ratio was expressed as percentage of control values. Values are the mean ± S.D. of three independent experiments. *Significantly different from control values ($p<0.005$). Data from single patients (P1 through P12).

replicates) and the data were processed with an imaging densitometer (GS-6470 model Bio-Rad, Milan, Italy). Data were analyzed with Molecular Analyst software. The densitometric values of the bands representing CACT immunoreactivity were normalized with respect to values obtained for the corresponding anti-actin bands.

3.8. Carnitine-acylcarnitine translocase and carnitine palmytoyltransferase II assays

CACT activity was measured as described elsewhere using two assays (24, 25). In assay 1, the reaction was initiated by adding isolated mitochondria (~0.5 mg protein) loaded with 2 mM L-[methyl-^{14}C]-carnitine (specific activity ~7,200 dpm/mmol) to 0.5 ml of a solution containing 200 mM mannitol, 50 mM Tris (pH 7.4), and unlabelled carnitine or palmitoylcarnitine. Exchange rates were calculated as described by Paradies et al. (24). In assay 2, the initial reactants were 125 microM [^{14}C] L-carnitine (~280,000 cpm), 125 microM palmytoyl-L-carnitine, and ~0.75 mg of mitochondrial protein (25), and appearance of label in palmitoyl-L-carnitine was monitored for 4 min. To measure carnitine palmytoyltransferase (CPT) II activity, the mitochondrial suspension was brought to 1% (w/v) with octyl glucoside and kept on ice for 30 min before assay. This treatment destroys the CPT I isoform. Reactions were initiated by adding ~0.25 mg of octyl glucoside-treated mitochondrion protein to the reaction mixture containing 25 microM palmitoyl -[^{14}C] L-carnitine (~30,000 cpm) and 100 microM CoASH, and the rate of loss of label from palmitoyl-L-carnitine was monitored for over 4 min. Reactions were terminated with 0.5 ml of 1.2 N HCL and the palmitoyl -[^{14}C] L-carnitine was extracted with butanol and quantified by scintillation counting.

3.9. Statistical analysis

Results are reported as the mean ± SD, unless otherwise specified, of at least three separate experiments. Statistical significance of differences was examined with the independent *t*-test or one-way ANOVA when required. *Post-hoc* multiple comparisons were performed using Student-Newman-Keuls tests. Intergroup comparisons were made by a Mann-Whitney U test. Correlations between anthropometric and metabolic data were assessed by the Spearman rank nonparametric correlation coefficient. Linear regression was used to evaluate relationships between continuous variables. The Fisher exact test was used to assess differences between groups of categorical variables like sex.

4. RESULTS

4.1. Skeletal muscle lipid levels in Patients and Normal Individuals

Control and obese subjects did not differ in height, age or sex. During the EHC procedure, steady-state plasma glucose was maintained close to initial values, the average coefficient of variation was below 10%. Fasting insulin was up to 2 times higher in obese versus control subjects (Table 1). Muscle triglyceride (mTG) content was significantly lower in controls than in obese subjects (6.11±2.39 vs 19.33±7.01 mg%; $p<0.0001$) respectively. The concentrations of individual FFAs, separated and quantified by HPLC, were significantly higher and the unsaturated/saturated ratio was significantly lower in obese subjects (Table 1). The BMI was directly related to triglyceride levels in muscle ($r^2=0.75$; $p<0.0001$).

4.2. CACT mRNA and protein levels

Northern blot analysis of poly(A)$^+$ RNA from muscle specimens was carried out with a cDNA probe for human CACT to determine if the changes in carnitine mitochondrial translocase level correlated with decreasing sensitivity to insulin (Figure 1 A, B). Poly(A)$^+$ mRNA from control and IR subjects hybridized as a single band that corresponded to the CACT transcript. However, the CACT mRNA was 3.5-fold higher in controls (densitometric analysis: ratio of CACT mRNA/beta-actin mRNA). Western blot analysis was performed with cellular and mitochondrial extracts from control and IR subjects

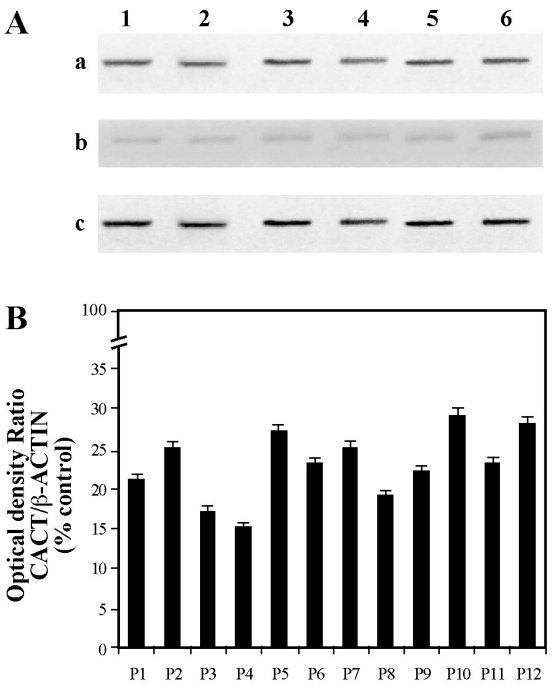

Figure 2. Decreased CACT protein levels in skeletal muscle specimens from IR patients. A. Western blot showing the levels of the CACT protein in control (row A) and IR (row B) patients. Row C shows an actin control for protein loading. Protein extracts were electrophoresed, and blotted onto nitrocellulose membranes. Membranes were incubated with rabbit polyclonal anti-CACT antibody; chemiluminescent detection showed a single band of approximately 33 kDa. B. Quantitative analysis of CACT protein normalized to actin levels for 12 representative IR patients. Autoradiographic signal intensities were determined with densitometry. The CACT:actin intensity ratio was expressed as percentage of control values. Values are the mean ± SD of three independent experiments. *Significantly different from control values ($p<0.005$).

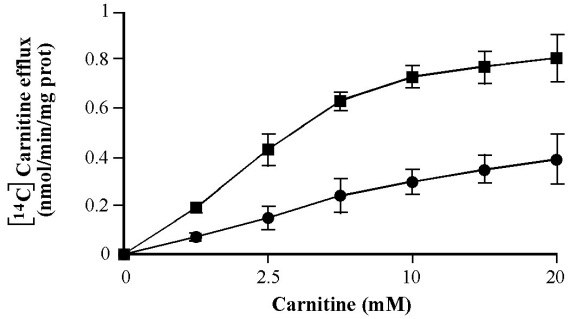

Figure 3. Rates of carnitine-carnitine exchange in skeletal muscle mitochondria isolated from control and IR subjects. Rates of carnitine-carnitine exchange were measured by adding [^{14}C]-carnitine-loaded mitochondria to 0.5 ml of a mixture containing different concentrations of unlabeled carnitine (for details see 'Materials and Methods'). Values are expressed as means ± S.D.

and probed for CACT. The decrease in CACT transcripts in IR subjects correlated with a significant decrease in CACT protein (Figure 2). Examination and scanning of muscle proteins by silver-stained SDS-PAGE did not show other differences between the control and IR state. The decrease in CACT in IR muscle appeared to be specific. The densitometric values depicted in Figure 2 A, B were normalized for actin.

4.3. Carnitine-acylcarnitine translocase activity

To assess whether the partial CACT deficiency in mitochondria could affect the exchange of extramitochondrial palmitoylcarnitine for matrix carnitine and thus be responsible for the diminished stimulation of palmitoylcarnitine oxidation, we measured the rate of carnitine-carnitine exchange and carnitine-palmitoylcarnitine exchange in mitochondria from controls and IR subjects. To prevent palmitoylcarnitine micelles from affecting the membrane, palmitoylcarnitine concentration was kept below 150 microM. The rate of the carnitine-carnitine exchange reaction was significantly decreased in mitochondria from IR individuals compared with controls (Figure 3). To evaluate if the reduced CACT activity reflected an altered affinity of the carrier for carnitine or a reduction in the maximal exchange velocity, we analyzed the effect of substrate concentration on the rate of carnitine efflux in carnitine-preloaded mitochondria. The maximal velocity of carnitine-carnitine exchange was significantly diminished in mitochondria from IR patients, whereas the Km for carnitine was unaffected.

As in the case of carnitine-carnitine exchange, palmitoylcarnitine-carnitine exchange rates were dramatically decreased at all palmitoylcarnitine concentrations tested in mitochondria from IR patients compared with controls. Since carnitine exchange could be related to matrix carnitine concentrations, the decreased rates of carnitine exchange in mitochondria from IR subjects could reflect reduced loading of labeled carnitine by mitochondria during preincubation. To test this possibility, we performed an assay in which the initial external reactants were [14C] L-carnitine and unlabeled palmitoyl-L-carnitine. This assay monitors the combined activity of CACT and internal CPT II in intact mitochondria without the need for preloading. Indeed, CPTI does not operate without external CoASH, but palmitoyl-[14C] L-carnitine is still formed because of the combined activity of CACT and internal CPT II. Both the external [14C] L-carnitine and unlabeled palmitoyl-L-carnitine can enter the matrix via CACT, and label is exchanged between them through the action of CPT II using the matrix pool of CoASH (palmitoyl-L-carnitine + CoASH → L-carnitine + palmitoyl- CoA). In the presence of labeled L-carnitine, CPT II activity in the reverse direction may produce the formation of labeled palmitoyl-L-carnitine. Since under these conditions palmitoyl-[14C] L-carnitine is formed only by CPT II activity, one must ensure CPT II activity is not decreased in the mitochondria used in the assay. There were no differences in CPT II activity between control and IR specimens, whereas CACT activity was again significantly decreased in mitochondria from IR subjects (Figure 4).

5. DISCUSSION

Muscle lipid content as a determinant for IR in humans is attracting keen interest (26). In obesity, TG levels are increased in skeletal muscle (27), the principal site of insulin-mediated glucose disposal. Enhanced

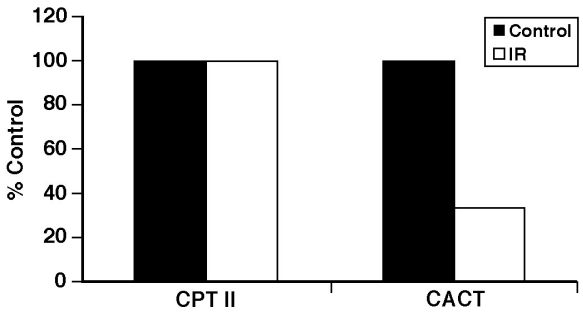

Figure 4. A 'typical' measurement of CACT and CPT II activity in skeletal muscle mitochondria isolated from control and IR subjects. CPT II activity was evaluated in octyl glucoside-treated mitochondria; CACT activity was measured in intact mitochondria (for details see 'Materials and Methods'). Values represent the mean of three experiments that yielded similar results.

skeletal TG has been implicated in insulin-resistant glucose metabolism, independently of central and overall adiposity (28-30). The degree of TG storage in skeletal muscle biopsies is inversely correlated with whole body glucose uptake (19). Moreover, by reducing the amount of adipose tissue infiltrating muscle, weight loss improves insulin sensitivity (30, 31).

The concept that perturbed muscle FA beta-oxidation and as a consequence skeletal muscle lipid storage can contribute to IR is not new, but the mechanism(s) underlying the lipid metabolism defect in IR obese skeletal muscle is unknown. Lipid oxidation is decreased in skeletal muscle mitochondria of obese subjects (8, 13) – one of the findings linking increased skeletal muscle fat deposits in IR obesity with CPT I alterations. But whereas decreased long-chain FA oxidation could be explained by decreased CPT I activity, it is difficult to explain the significant decrease in long-chain acylcarnitine oxidation, which is independent of CPT I. Consequently, other facets of the muscle carnitine system must be deranged in the mitochondria of obese IR subjects.

We studied the transport of FAs into skeletal muscle mitochondria to try to discern whether the defect in muscle lipid oxidation of IR patients is related to decreased CACT activity. Before analyzing CACT expression and function, we verified that the obese subjects were indeed resistant to insulin by examining muscle TGs and their respective FA patterns in skeletal muscle biopsies from control and IR individuals. Muscle TG content and whole-body glucose uptake were inversely correlated and differed significantly between the two groups as did muscle FA profiles. Both total saturated fat and the absolute amount of palmitic acid were significantly higher in IR obese individuals. These results show that beta-oxidation is altered and that lipid esterification is increased in skeletal muscle of IR individuals.

Acylcarnitine transport into mitochondria is an important route of FA beta-oxidation in skeletal muscle. The inner membranes of mitochondria contain a specific carrier protein, CACT, that transports acylcarnitines into the matrix space where acylgroups used for FA oxidation are released (12). The importance of CACT is demonstrated by the observation that CACT deficiency is generally lethal at an early stage of life. CACT-deficient patients have survived severe neonatal conditions (32-36), possibly because of the rate of long-chain FA oxidation in fibroblasts (32). Conceivably, in these patients either a secondary pathway bypasses the CACT block or the CACT deficiency may be present only in such tissues as skeletal muscle. However, little is known about primary and secondary partial CACT deficiencies and how these may affect the metabolic phenotype.

Using specific cDNA probes and antibodies against CACT, we now show that CACT content is dramatically reduced at both transcriptional and transductional levels in skeletal muscle of obese IR patients. To verify whether this decrease affects carrier activity, we measured the decline in carnitine transport in isolated mitochondria. Palmitoylcarnitine-carnitine exchange represents the physiological expression of CACT activity as it affects the metabolic oxidation of FAs. Because palmitoylcarnitine-mediated energy production is decreased in IR and because, as shown here, carnitine-carnitine exchange is also decreased, it seemed plausible that decreased extramitochondrial palmitoylcarnitine/ matrix carnitine exchange could cause the decline in lipid beta-oxidation. Consistent with this hypothesis, palmitoylcarnitine-carnitine exchange was significantly reduced in mitochondria from IR patients compared with control subjects.

This novel finding of a correlation between alterations of acylcarnitine transport into mitochondria and IR has implications for IR in obesity. Our results suggest that by restraining entry of LCFA-CoA into mitochondria, a low level of CACT would increase both the FA concentration in the cytosol and its incorporation into glycerolipids. Thus, a low level of CACT may contribute to the elevated concentrations of TGs, diacylglycerol, and LCFA-CoA observed in IR muscles. Studies on the carnitine carrier system might shed light on the pathogenesis of obesity and obesity-related comorbid conditions such as type 2 diabetes mellitus.

A scheme that links CACT to IR in skeletal muscle is shown in Figure 5. The common denominator in the proposed model is increased concentrations of cytosolic LCFA-CoA, which may result from decreased CACT. Increased LCFA-CoA could secondarily lead to increased concentrations of diacylglycerol (DAG), phosphatidic acid and TGs.

Reduced glucose transport in IR could be due to the effects of FAs on insulin action rather than to competition between FA and glucose for substrate oxidation (35, 36). The Randle model (35) predicts that FAs inhibit phosphofructokinase activity (due to increased intracellular citrate concentrations), and thereby lead to increased intramuscular glucose-6-phosphate concentrations. In contrast, in agreement with others (36), we propose that the

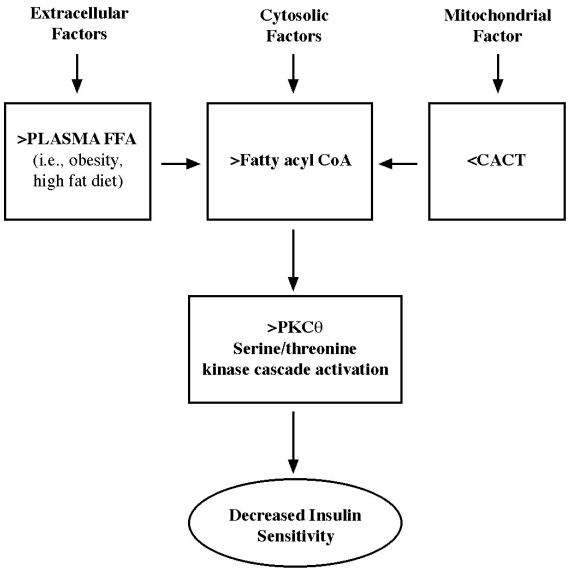

Figure 5. Proposed interrelations between carnitine-acylcarnitine translocase (CACT) activity and development of insulin resistance. Increased delivery of fatty acids to muscle, consequent to obesity or to a high fat diet, and the decreased mitochondrial lipid beta-oxidation due to perturbation of the acylcarnitine transport system together lead to an increase in cytosolic fatty acid metabolites such as fatty acyl-coenzyme A (FA-CoA). Alteration of the CACT-dependent mechanism prevents carnitine buffering of the excess acyl groups in the cytosol and in the mitochondrial matrix, thereby inducing overacylation of CoA, which is then less available for other essential CoA-requiring reactions. The increased FA-CoA activates a serine/threonine kinase cascade via protein kinase Cθ (PKCθ), which affects insulin receptor signaling and glucose transport activity. The proposed mechanism links the carnitine system to other cell events related to insulin resistance, e.g., protein acylation and altered membrane fluidity.

increase in intracellular FA metabolites such as diacylglycerol, fatty acyl CoAs, and ceramides activates a serine/threonine kinase cascade with subsequent phosphorylation of serine/threonine sites on insulin receptor substrates. The phosphorylated forms of these target molecules fail to activate PI 3 kinase thereby decreasing activation of glucose transport and other downstream events. As a consequence, any perturbation that results in accumulation of intracellular fatty acyl CoAs or other FA metabolites in skeletal muscle might be expected to induce IR. For example, an alteration in the ability of skeletal muscle cells to metabolize FAs, e.g., inherited or acquired defects of mitochondrial function, would lead to intracellular accumulation of FA metabolites and defects of insulin signaling and action.

Our model suggests that the cytosolic concentration of LCFA-CoA in muscle may be increased by a number of the mechanisms implicated in IR. Increased LCFA-CoA could occur: (i) if muscle receives excess lipids from the circulation because of high plasma FFA or TG levels; (ii) if the concentration of CACT is decreased and as a consequence less LCFA-CoA enters the mitochondria, and (iii) if muscle TG levels are increased, increased generation of LCFA and secondarily LCFA-CoA may result by mass action. The latter process could account for the well-documented relationship between muscle TG content and IR in humans and experimental animals. The proposed model agrees with clinical trials that carnitine treatment, which preserves CACT function in mitochondria (37, 38), increases the sensitivity and responsiveness of glucose transport and glycogen synthesis to insulin stimulation. Further work is needed to determine whether the decrease in CACT is a primary or a secondary deficiency and, in the latter case, the molecular basis of the diminished biosynthesis.

6. ACKNOWLEDGMENTS

The authors are indebted to Dr. John P. Blass for his suggestions and advice, and to Jean Ann Gilder for revising the manuscript.

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Key Words: Carnitine, Insulin Resistance, Obesity, Fatty Acids, Muscle

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