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Expression of Genes Regulating Malonyl-CoA in Human Skeletal Muscle

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Abstract In humans and animal models, increased intramuscular lipid (IML) stores have been implicated in insulin resistance. Malonyl-CoA plays a critical role in cellular lipid metabolism both by serving as a precursor in the synthesis of lipids and by inhibiting lipid oxidation. In muscle, Malonyl-CoA acts primarily as a negative allosteric regulator of carnitine palmitoyl transferase-1 (CPT1) activity, thereby blocking the transport of long chain fatty acyl CoAs into the mitochondria for oxidation. In muscle, increased malonyl-CoA, decreased muscle CPT1 activity, and increased IML have all been reported in obesity. In order to determine whether malonyl-CoA synthesis might be under transcriptional as well as biochemical regulation, we measured mRNA content of several key genes that contribute to the cellular metabolism of malonyl-CoA in muscle biopsies from lean to morbidly obese subjects. Employing quantitative real-time PCR, we determined that expression of mitochondrial acetyl-CoA carboxylase 2 (ACC2) was increased by 50% with obesity ($P < 0.05$). In both lean and obese subjects, expression of mitochondrial ACC2 was 20-fold greater than that of cytoplasmic ACC1, consistent with their hypothesized roles in synthesizing malonyl-CoA from acetyl-CoA for CPT1 regulation and lipogenesis, respectively. In addition, in both lean and obese subjects, expression of malonyl-CoA decarboxylase was approximately 40-fold greater than fatty acid synthase, consistent with degradation, rather than lipogenesis, being the primary fate of malonyl-CoA in human muscle. No other genes showed signs of increased mRNA content with obesity, suggesting that there may be selective transcriptional regulation of malonyl-CoA metabolism in human obesity. J. Cell. Biochem. 99: 860–867, 2006.

Key words: obesity; acetyl-CoA carboxylase; lipogenesis; fatty acid synthase; pyruvate carboxylase; sterol regulatory element binding protein 1

It has long been recognized that obesity is associated with whole body insulin resistance, although the causal mechanisms are not fully understood. Accumulation of lipids in adipose tissues can negatively influence insulin action in other tissues via endocrine secretion from enlarged adipocytes [Lafontan, 2005; Lazar, 2005]. Also, an accumulation of excess lipid in non-adipose cells can inhibit insulin action in insulin target tissues [Virkamaki et al., 2001; Boden, 2002; Hegarty et al., 2003; Lam et al., 2003]. Understanding cellular lipid metabolism in insulin target tissues is, therefore, vital to studying the etiology of insulin resistance.

Skeletal muscle is the tissue responsible for the majority of glucose uptake in the body [DeFronzo, 1988]. Evidence suggests that lipid accumulation within muscle cells can interfere with the response to insulin. Levels of intramyocellular lipid (IML) in human skeletal muscle correlate with whole body insulin resistance [Virkamaki et al., 2001]. In addition, the accumulation of lipids in cultured muscle cells results in impaired insulin signaling

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[Schmitz-Peiffer et al., 1999]. Impaired oxidation of lipids likely contributes to the accumulation of IML in obese subjects despite increased availability of plasma lipid [Kelley et al., 1999; Kelley and Mandarino, 2000]. Work by McGarry [2002] and Ruderman et al. [1999] has shown that the metabolite malonyl-CoA regulates cellular lipid oxidation rates by inhibiting the activity of carnitine palmitoyl transferase 1 (CPT1), an enzyme complex that determines the rate of lipid entry into the mitochondria. Correspondingly, acute manipulation of dietary energy intake and energy expenditure result in changes of malonyl-CoA levels in skeletal muscle, which correlate inversely with changes in whole body lipid oxidation [Ruderman et al., 2003]. Although studies in humans are lacking, malonyl-CoA levels are elevated in a mouse model of obesity and insulin resistance [Saha et al., 1994]. In humans, CPT1 activity decreases with increasing adiposity [Simoneau et al., 1999].

Despite its key role in muscle lipid metabolism, the regulation of malonyl-CoA metabolism in human muscle has not been completely characterized. Synthesis of malonyl-CoA occurs under conditions of surplus carbohydrate supply and high glycolytic rates [Ruderman et al., 1999; Schmitz-Peiffer et al., 1999]. Studies from other tissues suggest that there are three principal sites of regulation in the synthesis and degradation of malonyl-CoA. One is the conversion of acetyl-CoA to malonyl-CoA by one of the isoforms of acetyl-CoA carboxylase (ACC), which is the prime site of metabolic regulation in liver and muscle cells [Brownsey et al., 1997]. Another potential site of regulation is the conversion of pyruvate to oxaloacetate (OAA) by the enzyme pyruvate carboxylase (PC), which allows entry into the TCA cycle in support of biosynthetic reactions involving TCA intermediates. This step is rate limiting for malonyl-CoA production in pancreatic beta cells and other lipogenic tissues [Farfari et al., 2000], but its contribution to muscle metabolism is largely unknown. Finally, the turnover of malonyl-CoA can be accomplished by incorporation into long chain fatty acyl CoA (LCFACoA) through either the activity of fatty acid synthase (FAS) or by conversion back to acetyl-CoA by the enzyme malonyl-CoA decarboxylase (MCD). Although FAS expression and de novo lipogenesis is observed in cultured human muscle cells [Aas et al., 2004], the presence of this enzyme and metabolic pathway in human skeletal muscle tissue is not established.

While the minute to minute regulation of malonyl-CoA synthesis occurs largely by modification of ACC activity [Ruderman et al., 2003], there is evidence in several tissues for transcriptional upregulation of many of these key enzymes under conditions of chronic nutrient oversupply such as obesity [Foretz et al., 1999a,b; Kaput and Rodriguez, 2004; Gosmain et al., 2005]. This suggests that synthesis of malonyl-CoA is under transcriptional as well as substrate control.

In order to determine whether human obesity is associated with a coordinated transcriptional regulation of genes involved in malonyl-CoA metabolism, we have examined the concurrent expression of ACC1, ACC2, PC, MCD, FAS, and the nutrient-regulated transcription factors SREBP1a and SREBP1c in skeletal muscle biopsies from subjects of varying degrees of adiposity.

MATERIALS AND METHODS

Subjects

The subjects for this study were individuals undergoing elective abdominal surgery (hysterectomy or gastric bypass). Body mass, height, ethnicity, gender, age, and diabetes status were recorded as part of pre-operative procedures. A fasting blood sample was obtained for later analysis of insulin and glucose. During surgery, a biopsy specimen of the rectus abdominus was obtained and immediately frozen under liquid nitrogen. All procedures were approved by the University Review Board and informed consent was obtained prior to any procedures.

Plasma Analysis

Plasma was analyzed for glucose (YSI 2300 STAT Plus Glucose and Lactate Analyzer, YSI, Inc., Yellow Springs, OH) and for insulin with a microparticle enzyme immunoassay (IMx, Abbott Laboratories, Abbott Park, IL). Glucose and insulin concentrations were used to determine homeostasis model assessment HOMA values [fasting glucose (mg/dl) 0.05551) $\times$ fasting insulin (μU/ml)/22.1] as an index of in vivo insulin action [Matthews et al., 1985].

Quantitative Real-Time PCR (QRT PCR)

RNA was extracted from frozen skeletal muscle biopsies using Qiagen RNeasy Fibrous

Tissue Mini kits (Qiagen, Valencia, CA). Total RNA was quantified by a Nanodrop ND-1000 UV-Vis Spectrophotometer. First strand cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). QRT PCR was performed in triplicate in 50 μl reaction volumes consisting of 1× PCR buffer A (Applied Biosystems, Foster City, CA), 5.5 mM $MgCl_2$, 0.025 U/μl Taq Gold (Applied Biosystems), and 5 μl of the appropriate RT reaction. The final primer and probe concentrations were optimized for each primer/probe combination. Two-step PCR cycling was carried out as follows: 95°C 12 min × 1 cycle, 95°C 15 s, 60°C 1 min × 40 cycles. At the end of the PCR, baseline and threshold values were set in the software and the Ct values were exported to Microsoft Excel for analysis. Gene expression was expressed relative to β-glucuronidase (GUS) to correct for RNA input variability and gene copy number was calculated with the equation copy number $= 2^{-\Delta Ct}$.

Probes and primers were either ready-designed and optimized by Applied Biosystems, were designed using the Primer Express Software (Applied Biosystems), and supplied by Integrated DNA Technologies (Coralville, IA), or were custom made and optimized by Applied Biosystems (see Table I).

Statistical Analyses

All data analysis was performed employing MedCalc statistical software (Mariakerke, Belgium). Values are presented as mean ± SEM. Differences between obese and non-obese groups were analyzed by Student's *t*-test. Simple correlations were determined by Pearson correlation coefficient. Identification of independent determinants of gene expression was performed by multiple regression analysis. Significance was accepted as $P < 0.05$.

RESULTS

Subject Data

The subject characteristics are shown in Table II. Body mass index (BMI) ranged from 21 to 77 kg/m^2. 12 subjects out of 46 were non-obese (BMI of less than 30) and the rest were classified as obese or morbidly obese (BMI greater than 40). Non-obese subjects were significantly more insulin sensitive as determined by HOMA values (1.7 ± 0.9 vs. 4.0 ± 0.9, non-obese vs. obese respectively, $P < 0.05$). Two obese subjects were diabetic with fasting plasma glucose (FG) over 120 mg/dl. Removing these subjects from the study did not influence the data. All of the patients, with the exception of two of the obese group, were female.

Relative Expression Levels in Skeletal Muscle

Expression levels of genes believed to play a role in the metabolism of malonyl-CoA have not been systematically studied in human skeletal muscle. In order to determine the potential for known regulatory genes to contribute to malonyl-CoA synthesis, degradation, or conversion in muscle, we employed quantitative PCR to measure the expression of levels of ACC1, ACC2, MCD, FAS, and PC in biopsies of rectus abdominus muscle.

TABLE I. Real-Time PCR Probe and Primer Sequences

GUS	Probe	5′-6FAM-TGAACAGTCACCGACGAGAGTGCTGG-BHQ1-3′
	Forward	CTCATTTGGAATTTTGCCGATT
	Reverse	CCGAGTGAAGATCCCCTTTTTA
ACC2	Probe	5′-6FAM-CATCGAGAAGGTGCTTATTGCCAACAAC-BHQ1-3′
	Forward	TGAGTTTGTCACACGCTTTGG
	Reverse	CGTTGCGGAACATCTCATAGG
ACC1	Probe	5′-6FAM- CAGCTCTGGAGGTGTACGTTCGAAGGG-BHQ1-3′
	Forward	CATCTATTTTTGATGTCCTACCAAACTTC
	Reverse	ACGCTGTTAAGTTCATAGGCAATATAAG
MCD		ABI 20× Taqman assay Hs00201955_m1
FAS		ABI 20× Taqman assay Hs00188012_m1
PC	Probe	5′-6FAM-TTGCCTCCGCAGATAGTGTCTGCCTT-BHQ1-3′
	Forward	CCACCTGTTGGGCTCTTTCTT
	Reverse	GTGGAGGTTCGGCGGATT
SREBP1c	Probe	5′-6FAM-CATGGATTGCACTTTCG-NFQ-3′
	Forward	GGGCGGGCGCAGAT
	Reverse	GTTGTTGATAAGCTGAAGCATGTCT
SREBP1a		ABI 20× Taqman assay Hs00231674_m1

Forward and reverse primer sequences are designated as forward or reverse and are presented 5′-3′. When the probe/primer sets were bought already-designed and tested from Applied Biosystems, the catalog number is shown below.

TABLE II. Subject Characteristics

	Age (y)	BMI (kg/m^2)	FG (mg/dl)	FI (μU/ml)	HOMA
Non-obese (BMI <30) N = 12	42 ± 2	25 ± 1*	85 ± 5	9.0 ± 5.5	1.7 ± 0.9†
Obese (BMI ≥30) N = 34	40 ± 2	51 ± 2	104 ± 8	15.4 ± 1.8	4.0 ± 0.6

Significant difference between non-obese and obese subjects.
*$P < 5 \times 10^{-9}$.
†$P < 0.05$.

Relative gene copy number for each gene of interest was determined by normalizing against the control gene GUS. In initial studies, we compared the expression of a panel of putative housekeeping genes consisting of GUS, cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and histone H3. Comparison of expression levels of these genes in biopsies of a diverse group of subjects indicated that GUS produced a lower coefficient of variance between samples, and absolute expression levels of GUS did not vary as a function of obesity (data not shown). The analysis of the data presented below is not qualitatively different when copy number is normalized to either cyclophilin, GAPDH, or histone H3.

Profiling the expression of ACC isoforms in human muscle indicated that ACC2 was more than 20-fold more abundant than ACC1 (average mRNA relative to GUS; ACC2 13 and ACC1 0.5). Although PC activity is required in the malonyl-CoA synthesis pathway in many tissues, expression of this enzyme in human skeletal muscle is at low levels, similar to ACC1 (Table III).

The metabolic fate of malonyl-CoA in lipogenic tissues can either be decarboxylation by MCD or incorporation into long chain acyl-CoA by FAS. Although dramatically lower than expression of MCD, we find evidence of FAS expression in human skeletal muscle. In order to determine whether the FAS mRNA measured in these biopsies was likely to be of muscle origin, rather than the result of contamination by adipose or other cell types, we examined FAS mRNA content in myotubes cultured from biopsies from several of the subjects included in the present study. We observed appreciable expression of FAS mRNA in cultures of pure differentiated muscle cells (data not shown) as has been reported by others [Aas et al., 2004; Guillet-Deniau et al., 2004].

We next looked at expression of transcription factors SREBP1a and SREBP1c. We found that SREBP1c was highly expressed in human rectus abdominus muscle and was approximately eightfold more abundant than SREBP1a mRNA (Table III).

TABLE III. mRNA Levels Relative to GUS

	Non-obese	Obese
PC	1.0 ± 0.2	1.2 ± 0.3
ACC1	0.47 ± 0.05	0.48 ± 0.03
MCD	19.5 ± 2.7	21.3 ± 2.1
FAS	0.47 ± 0.15	0.50 ± 0.09
SREBP1c	21.7 ± 5.4	14.0 ± 3.6
SREBP1a	2.4 ± 0.5	2.0 ± 0.4

Gene copy number determined by quantitative RT-PCR from muscle biopsies of non-obese (BMI <30) and obese (BMI >30) subjects.
Significantly different from non-obese, *$P < 0.05$.

Gene Expression in Non-Obese Versus Obese

When the subject pool was divided into two groups, non-obese (BMI ≤30) and obese (BMI >30), we found a significantly elevated expression of ACC2 in muscle from obese subjects (9.6 ± 1.6 non-obese, 14.2 ± 1.1 obese, $P < 0.05$), (Fig. 1). No significant difference was observed in the expression of other genes as a result of obesity (Table III).

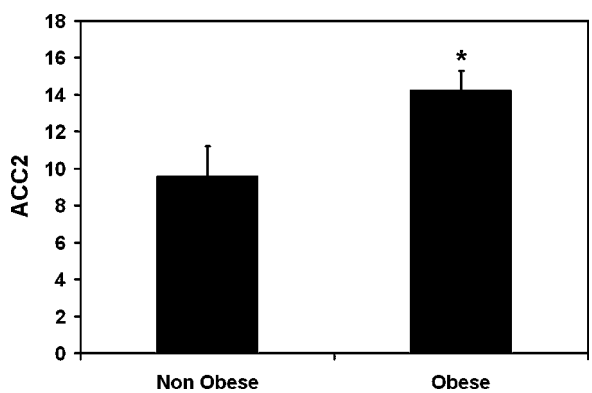

Fig. 1. ACC2 mRNA levels are increased in obese subjects. Gene copy number determined by quantitative RT-PCR from muscle biopsies of non-obese (BMI <30) and obese (BMI >30) subjects. *$P < 0.05$.

Expression Correlations

To find evidence of coordinated expression of genes in the malonyl-CoA pathway, we next performed correlational analysis of the gene copy number and select clinical data. We observed a significant correlation of ACC1 mRNA with MCD mRNA ($r = 0.57$, $P < 0.001$) (Fig. 2). There was no correlation between mRNA content for other genes involved in malonyl-CoA metabolism.

Although the absolute expression levels of SREBP1a and SREBP1c were very different, the mRNA levels of the two isoforms were highly correlated ($r = 0.82$, $P < 0.0001$). SREBP1a mRNA levels were significantly associated with fasting insulin across all subjects ($r = 0.50$, $P < 0.05$). However, despite a similar trend for SREBP1c, the association between this isoform and fasting insulin did not reach statistical significance ($r = 0.32$, $P = 0.18$).

Analysis of the data from the whole subject group suggested that BMI was not significantly correlated with the copy number of any of the genes examined. However, the ACC2 and BMI data did fit a linear regression for subjects with BMI <50 ($r = 0.4$, $P < 0.05$, $N = 29$) with ACC2 increasing with BMI. At BMI >50 ($N = 17$), ACC2 mRNA plateaued and was independent of BMI. HOMA values correlated with BMI over the whole range presented by these patients ($r = 0.5$, $P < 0.005$) but no correlation was observed between HOMA and gene expression for any of the genes examined here.

DISCUSSION

We investigated the expression of genes regulating malonyl-CoA metabolism in human skeletal muscle biopsies. These studies were undertaken to identify relative expression levels of enzyme isoforms that could potentially contribute to alternate pathways of malonyl-CoA synthesis and degradation in skeletal muscle. In addition, we explored whether, in human skeletal muscle, obesity might be associated with a coordinated change in expression of genes from the malonyl-CoA, lipogenesis pathway, or the transcription factors that regulate their expression.

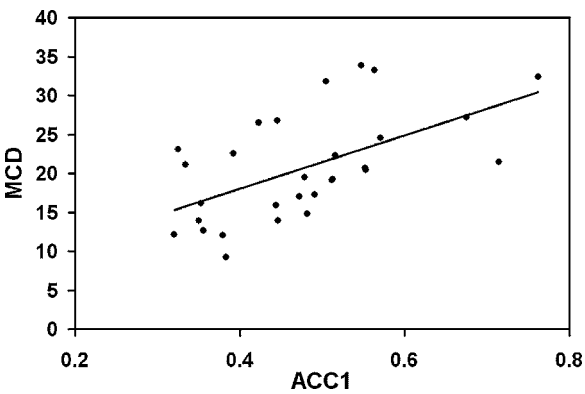

Fig. 2. ACC1 mRNA in skeletal muscle correlates to MCD mRNA. $r = 0.57$, $P = 0.001$.

As malonyl-CoA is a committed substrate in the synthesis of long chain fatty acyl CoA (LCFACoA), the enzymes responsible for its synthesis are categorized as part of the lipogenic pathway. While regulation of malonyl-CoA plays a key role in muscle lipid metabolism and insulin action (Ruderman et al., 1999), the components of the malonyl-CoA synthetic pathway have largely been studied in classic lipogenic tissues, rather than in skeletal muscle.

Malonyl-CoA is synthesized by the carboxylation of acetyl-CoA by one of the two isoforms of ACC. ACC2 is prevalent in skeletal muscle and heart in which lipid oxidation is a major source of energy production [Bianchi et al., 1990; Brownsey et al., 1997; Munday, 2002]. ACC2 is localized adjacent to the outer mitochondrial membrane thereby producing malonyl-CoA in close proximity to CPT1 [Abu-Elheiga et al., 2000]. This isoform can, therefore, facilitate the regulation of lipid oxidation by malonyl-CoA. ACC1 is predominant in lipogenic and anabolic tissues such as liver, adipose, and lactating mammary gland. ACC1 lacks the mitochondrial membrane-binding motif possessed by ACC2 and is believed to be cytosolic. ACC1 is thus speculated to be more important in de novo lipogenesis by producing substrate for the cytosolic enzyme FAS [Bianchi et al., 1990; Brownsey et al., 1997; Ruderman et al., 1999]. The different tissue expression patterns of ACC1 and ACC2, as well as this distinct subcellular localization of the two isoforms suggest that they synthesize separate pools of malonyl-CoA that would have separate metabolic fates, consistent with the largely separate roles of malonyl-CoA in lipogenic tissues versus tissues with a large capacity for, and tight regulation of lipid oxidation.

Given that skeletal muscle is not a classical lipogenic tissue, the predominant fate of malonyl-CoA in this tissue is undoubtedly decarboxylation by MCD, rather than de novo

lipogenesis via FAS. However, there is evidence that muscle lipogenesis does occur, and may even play an important role in skeletal muscle metabolism. While FAS expression in muscle biopsies, as observed in the present study, could be due to contamination with fat or other tissues, FAS expression and de novo lipogenesis has been demonstrated in cultured muscle cells from humans and rodents [Aas et al., 2004; Guillet-Deniau et al., 2004]. In addition, it has been hypothesized that muscle lipogenesis might serve a functional role as a metabolic sink in thermogenesis [Dulloo et al., 2004].

We found expression of both ACC1 and FAS in human muscle biopsies. However, given the relatively low expression level for these genes compared to mRNA levels for both ACC2 and MCD, it is obvious that substrate flux through ACC1 and FAS in lipogenesis likely represents a comparatively minor pathway. ACC2 mRNA abundance was more than 20 times greater than that of ACC1. While this ratio had not been previously documented in human muscle, the results are in agreement with previous reports which concluded that ACC2 is prevalent in tissues in which lipid oxidation is important, while ACC1 is higher in lipogenic tissues such as liver and lactating mammary gland [Bianchi et al., 1990; Witters et al., 1994; Brownsey et al., 1997; Munday, 2002].

We found that ACC2 transcription was upregulated in muscle from obese humans. This observation is in agreement with studies of human skeletal muscle in which ACC2 expression decreased following weight loss through life style intervention [Mensink et al., 2003] and biliopancreatic diversion [Fabris et al., 2004]. However, it had not been previously reported that ACC2 expression in humans was altered as a function of body weight in the absence of acute nutrient intervention. Insulin is known to regulate ACC2 expression in muscle and other tissues via the SREBP1 transcription factors [Guillet-Deniau et al., 2004]. Although previous reports have attributed decreased ACC2 expression with weight loss to reductions in insulin, and while the fasting insulin levels were significantly elevated in our obese subjects, we did not observe any significant correlation between fasting insulin levels and ACC2 expression. Nor did we observe any correlation between ACC2 and other surrogate markers of insulin sensitivity.

It is not clear to what extent transcriptional regulation of ACC2 contributes to malonyl-CoA flux in the cell. Malonyl-CoA synthesis in muscle is primarily a factor of both ACC activity and substrate availability. Modulation of ACC activity occurs via phosphorylation and inactivation by AMP-activated protein kinase (AMPK). ACC is also activated allosterically by citrate, which is a precursor to acetyl-CoA, and thus stimulates production of malonyl-CoA in a feed-forward manner [Munday, 2002].

Synthesis of malonyl-CoA occurs when glycolytic rates are high resulting from high levels of glucose and insulin [Ruderman et al., 1999] and requires export from the mitochondria of TCA cycle intermediates (termed cataplerosis). This process must be balanced by the replenishment of TCA carbons in the process of anaplerosis. In several cell types, this export of precursors of malonyl-CoA is dependent on PC activity. In these cells, when the pyruvate levels are high, the export of TCA cycle intermediates is balanced by conversion of pyruvate to OAA by PC [Jitrapakdee and Wallace, 1999].

In lipogenic tissues such as liver and adipose tissue, as well as other cells in which the anaplerotic pathway contributes significantly to cellular function, such as pancreatic beta cells, PC activity is rate limiting for biosynthetic reactions including malonyl-CoA synthesis, gluconeogenesis, glycerogenesis, and urea synthesis [Martin-Requero et al., 1992; Jitrapakdee and Wallace, 1999; Farfari et al., 2000; Liu et al., 2002]. PC expression is dramatically increased in liver and fat of genetically obese, hypherphagic rodents [Jitrapakdee et al., 1998; Jitrapakdee et al., 2005] and in beta cells, hyperglycemia results in an increased expression of PC [MacDonald, 1995]. These results suggest that increased substrate flux through the TCA cycle results in a transcriptional upregulation of PC expression in some tissues. While PC mRNA levels were approximately 25% higher in muscle biopsies from obese subjects in the present study, this trend did not reach statistical significance. Whether this result is due to differences between muscle and other tissues with a higher anaplerotic capacity, or to the substantial differences between human and rodent obesity, is not clear. While human obesity is logically associated by a net positive energy balance over the long term, this condition is less likely to reflect a continual oversupply of carbohydrates present in a

genetically hypherphagic rodent model of obesity. Further studies will be needed to determine whether transcriptional regulation of muscle PC content is influenced by nutrient availability or obesity.

We did observe a very strong association between MCD and ACC1 expression. The reasons for this association are not clear. While both enzymes are components of the pathway for malonyl-CoA turnover, MCD expression is regulated as part of a package of genes controlling lipid oxidation by the nuclear receptor PPARα [Lee et al., 2004], while ACC expression is coordinated by the transcription factor SREBP1c along with a family of genes involved in glucose and lipid metabolism [Guillet-Deniau et al., 2004].

We found that obesity was not accompanied by altered expression levels of either SREBP1a or SREBP1c mRNA. In agreement with this work, no differences in SREBP1 mRNA were found in human vastus lateralis biopsies before and after weight loss resulting from biliopancreatic diversion [Fabris et al., 2004]. The functional significance of the two isoforms of the SREBP1 transcription factors is not completely understood, and the relative abundance of the two isoforms varies in different tissues [Shimomura et al., 1997; Guillet-Deniau et al., 2002]. Both the mRNA levels and the transcriptional activity of SREBP1s are regulated by insulin, glucose, and fasting/feeding nutritional status [Guillet-Deniau et al., 2002, 2004; Gosmain et al., 2005]. SREBP1c regulates transcription of lipogenic genes [Gosmain et al., 2004] and the glycolytic enzyme hexokinase [Gosmain et al., 2005]. SREBP1a has higher transcriptional activity than SREBP1c [Shimano et al., 1997], and is independent of acute changes in insulin, glucose, and nutritional status in rat muscle [Gosmain et al., 2005]. We observed that expression of SREBP1c was eightfold higher than SREBP1a in human skeletal muscle. Further, we found that across lean and obese subjects, expression of SREBP1a and SREBP1c mRNA were highly correlated. However, only SREBP1a expression was significantly correlated with fasting insulin levels, while the association between insulin and SREBP1c mRNA did not reach significance ($P = 0.15$). We did not observe significant associations between mRNA levels for either SREBP1 isoform and any of the genes believed to be regulated by these transcription factors, although this may be due to the important roles that modification and localization of the SREBP1 proteins have on their transcriptional activity.

In conclusion, due to the role these genes play in controlling malonyl-CoA levels, we measured mRNA levels of ACC1, ACC2, MCD, FAS, and PC, in rectus abdominus muscle biopsies from humans of varying degrees of obesity. We found that ACC2 mRNA was higher in muscle from obese patients. This result is consistent with the role of malonyl-CoA as fuel sensor, and suggests that in humans, there may be some degree of obesity-linked transcriptional regulation of genes involved in malonyl-CoA synthesis, and therefore lipid oxidation.

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