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Leptin and the control of respiratory gene expression in muscle

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Abstract

Leptin plays a central role in the regulation of fatty acid homeostasis, promoting lipid storage in adipose tissue and fatty acid oxidation in peripheral tissues. Loss of leptin signaling leads to accumulation of lipids in muscle and loss of insulin sensitivity secondary to obesity. In this study, we examined the direct and indirect effects of leptin signaling on mitochondrial enzymes including those essential for peripheral fatty acid oxidation. We assessed the impact of leptin using the JCR:LA-cp rat, which lacks functional leptin receptors. The activities of marker mitochondrial enzymes citrate synthase (CS) and cytochrome oxidase (COX) were similar between wild-type (+/?) and corpulent (cp/cp) rats. In contrast, several tissues showed variations in the fatty acid oxidizing enzymes carnitine palmitoyltransferase II (CPT II), long-chain acyl-CoA dehydrogenase (LCAD) and 3-hydroxyacyl-CoA dehydrogenase (HOAD). It was not clear if these changes were due to loss of leptin signaling or to insulin insensitivity. Consequently, we examined the effects of leptin on cultured C_2C_{12} and Sol8 cells. Leptin (3 days at 0, 0.2, or 2.0 nM) had no direct effect on the activities of CS, COX, or fatty acid oxidizing enzymes. Leptin treatment did not affect luciferase-based reporter genes under the control of transcription factors involved in mitochondrial biogenesis (nuclear respiratory factor-1 (NRF-1), nuclear respiratory factor-2 (NRF-2)) or fatty acid enzyme expression (peroxisome proliferator-activated receptors (PPARs)). These studies suggest that leptin exerts only indirect effects on mitochondrial gene expression in muscle, possibly arising from insulin resistance.

Keywords: Leptin; Fatty acid; Muscle; Mitochondrion; Enzyme; JCR:LA rat

1. Introduction

Leptin is a peptide hormone that controls whole-body lipid metabolism with regulatory influences on fatty acid homeostasis in multiple tissues. In storage tissues, such as adipose, leptin promotes triacylglyceride (TAG) storage, whereas in peripheral tissues, such as skeletal muscle, leptin enhances lipid oxidation [1–3]. Models with defects in leptin signaling show pronounced changes in whole-body metabolism, including obesity, insulin resistance and cardiovascular pathologies [4–8]. The JCR:LA rat model, used in the present study, possesses an autosomal recessive mutation in the leptin receptor gene (cp) that disrupts the extracellular domain of the leptin receptor. Homozygous cp/cp rats become obese and insulin-resistant, whereas homozygous wild-type (+/+) or heterozygotes (+/cp) (collectively +/?) are lean and metabolically normal. By 4 weeks of age, cp/cp rats show skeletal TAG levels that are elevated by more than 4-fold but insulin levels remain normal. After 6 weeks of age, the obese phenotype emerges. While plasma glucose remains constant (10 mM), cp/cp rats show increases in plasma fatty acids (0.37 vs. 0.27 mM), plasma TAG (6 vs. 2 mM) [6], and soleus muscle TAG (160 vs. 40 mmol/g) [4]. The cp/cp rats have 10-fold higher insulin levels (2746 vs. 272 pM) and insulin resistance in muscle [4,6]. The complex metabolic phenotype arises from the primary lesion (i.e. loss of leptin signaling) in addition to secondary effects from hyperphagia, hyperlipidemia and insulin resistance [4–8].

Despite the importance of muscle in whole-body lipid metabolism, the direct effects of leptin on the muscle metabolic phenotype remain poorly understood. In particular, little is known about how leptin influences the expression of the mitochondrial genes that encode the enzymes responsible for lipid oxidation and energy metabolism.

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Skeletal muscle of leptin-deficient mice respond rapidly to leptin infusion, activating JAK and PI3K pathways [9]. Muscle also activates AMP-activated kinase (AMPK) in response to leptin [10,11], enhancing fatty acid oxidation through effects on acetyl-CoA carboxylase (ACC) [12]. However, chronic leptin treatment of rats did not alter the activities of either citrate synthase (CS) or β-hydroxyacyl-CoA dehydrogenase (HOAD) in soleus muscle [13]. Microarray analyses identify few genes in muscle that are influenced by leptin treatment [14]. Leptin treatments of whole animals lead to an increase in muscle UCP3 levels and increases in proton leak, although it is likely that the effects are indirect [15].

Despite recent advances in understanding leptin signal transduction (see Ref. [16]) and respiratory gene expression (see Ref. [17]) relatively few studies have considered interaction between the two pathways. Signaling via the leptin receptor can involve the JAK/STAT [18,19] as well as the p38- and ERK−MAPK pathways [19–21]. Mitochondrial gene expression is regulated by a number of transcriptional regulators. Nuclear respiratory factor (NRF)-1 and NRF-2 appear to regulate the enzymes of oxidative phosphorylation [22], whereas the fatty acid oxidizing enzymes are regulated by peroxisome proliferator-activated receptors (PPARs) [23]. PPAR appears to mediate changes in fatty acid enzymes in response to diet and food deprivation [24,25]. In addition, the co-activator PPAR-gamma coactivator-1 (PGC-1) interacts with PPARs and NRF-1 [26]. While there is potential for cross-talk between leptin signaling and mitochondrial gene expression, the connections have not been explored.

In this study, we assessed the effects of leptin on the expression and activity of the enzymes of fatty acid oxidation and mitochondrial metabolism using a combination of in vitro and in vivo models. Cultured rodent myoblasts were used to explore potential direct effects of leptin on mitochondrial gene expression and transcriptional control. We also assessed the changes in bioenergetic enzymes seen in JCR:LA rats tissues, including multiple striated muscle fiber types. While the JCR:LA corpulent rat showed changes in the levels of several bioenergetic enzymes, we found no evidence for direct effects of leptin on the expression of mitochondrial enzymes in muscle.

2. Methods

2.1. Animals

Tissues were collected from JCR:LA-cp rats in accordance with CCAC guidelines and with the approval of animal care of the University of Alberta. Male rats, cp/cp (obese, 497 ± 13 g) or +/? (lean, 317 ± 9 g) were bred in the established colony at the University of Alberta. At 12 weeks of age, six animals from each group were euthanized and individual skeletal muscles, liver, and heart were extracted and immediately placed in liquid N_2 and stored at − 80 °C until analysis.

2.2. Cell culture

All cell culture media and sera were supplied by GIBCO. Cells were grown at 37 °C in 10% CO_2. Penicillin, streptomycin, and neomycin (PSN) were included in all media, which was refreshed every 3 days. Sol8 cells were grown in Dulbecco's modified Eagle's medium (DMEM; 25 mM glucose, 4 mM glutamine, and 1 mM pyruvate) supplemented with 20% fetal bovine serum (FBS). C_2C_{12} cells were grown in DMEM supplemented with 10% FBS. Once cells had reached 90% confluency, the medium was changed to DMEM supplemented with 2% horse serum (HS) with 0, 0.2, or 2 nM recombinant rat leptin (R&D Systems Inc., Minneapolis, MN) refreshed daily for 3 days.

2.3. Enzyme activities

Tissue homogenates of soleus, tibialis anterior (TA), gastrocnemius (gastroc), liver, and heart were prepared by powdering in liquid N_2 and weighing out approximately 50–100 mg of tissue. To this, 20 volumes of extraction buffer consisting of 20 mM Hepes (pH 7.4), 1 mM EDTA and 0.1% Triton X-100 was added. C_2C_{12} and Sol8 cells were harvested in extraction buffer to yield approximately 1 mg protein/ml. Citrate synthase (EC 4.1.3.7), HOAD (EC 1.1.1.35) and cytochrome oxidase (COX, EC 1.9.3.1) were assayed as previously described [27], except HOAD was assayed in 50 mM imidazole buffer (pH 7.2). Long-chain acyl-CoA dehydrogenase (LCAD, EC 1.3.99.13) was assayed according to Davidson and Schulz [28] in 0.1 M potassium phosphate buffer containing 28 μM 2,6-dichlorophenolindophenol (DCPIP), 0.65 mM phenazine methosulfate (PMS), 0.2 mM *N*-ethylmaleimide and 0.45 mM KCN. The reaction was started with the addition of 0.1 mM palmitoyl-CoA. Carnitine palmitoyltransferase II (CPT II, EC 2.3.1.21) was assayed in 20 mM Tris buffer (pH 8.0) containing 0.1 mM DTNB and 5 mM L-carnitine. The reaction was started with the addition of 0.1 mM palmitoyl-CoA. This activity represents CPT II activity and was completely insensitive to malonyl-CoA inhibition since CPT I activity is inactivated by freezing [29] and detergent treatment [30]. Protein concentrations were determined by the Bradford method (Biorad). All assays were performed at 37 °C on a SpectraMAX Plus spectrophotometer (Molecular Devices).

2.4. Northern analysis

Total RNA was purified from rat tissues and cell culture from guanidinium thiocyanate (GTC) extracts [27]. RNA was electrophoresed on 1% agarose/2% formaldehyde gels. cDNA probes for CS and HOAD were obtained by PCR as previously described [27]. Probes for CPT Iα, CPT II,

Table 1
Primer sequences and response elements used for cDNA probe and luciferase reporter construct production via PCR from rat ventricle cDNA produced from total RNA

Gene	Forward primer (5′–3′)	Reverse primer (5′–3)′	Annealing temperature (°C)	Size (bp)
PGC-1	gtggatgaagacggattgccc	cctgcagttccagagagttcc	55	601
CPT Iβ	gcaaactggaccgagaagag	aagaaagcagcacgttcgat	54	397
CPT II	agacgctcagcttcaacctc	ccatmgctgcytctttggt	54	409
LCAD	tggcattagcctcttttgg	tggatgtgtgcgactgtttt	51	300
hCS promoter	aaaacgcgtgctttccaattgcccttatagc	ttttctcgaggcgggcgatctccgggatc	64	2113
Response element				
NRF-1	gtacctagtgcgcacgcgcaggatagtgcgcacgcgcaggatagtgcgcacgcgcaggataggtgcgcacgcgcagg			
NRF-2	caccggaagagaccggaagagaccggaagagaccggaagagggagagct			

Binding site sequences are underlined.

LCAD and PGC-1 were obtained by PCR using rat ventricle cDNA with primers outlined in Table 1. PCR fragments were cloned into PCR 2.1-TOPO (Invitrogen) and transformed into XL-1 Blue *E. coli* cells. Probe identities were confirmed by sequencing (Cortec, Inc., Kingston, Canada). The cDNA probes (50 ng) were labeled with 50 μCi of [α-^{32}P] dCTP using Ready-to-Go DNA labeling beads (Amersham Pharmacia Biotech Inc.) and signals were measured using a phosphoimager and quantified using Imagequant software (Molecular Dynamics).

2.5. Plasmids and constructs

To generate citrate synthase promotor (pGL2-hCS), 2113 bp upstream of the human citrate synthase (hCS) translation initiation start site was amplified from human genomic DNA by PCR with restriction sites introduced using primers in Table 1. The resulting PCR product was cloned into the firefly luciferase reporter vector (pGL2-basic, Promega) using the *Mlu*I and *Xho*I restriction sites.

The NRF1-TK$_{Luc}$ plasmid is a reporter construct that contains four tandem copies of the mouse mitochondrial RNA processing RNA promoter's NRF-1 binding site [31] (underlined in Table 1) cloned into *Kpn*I and *Sac*I sites in TK$_{Luc}$. TK$_{Luc}$ contains the 757 bp HSV (thymadine kinase) TK promoter fragment digested from pRL-TK (Promega) at the *Bgl*II and *Hin*dIII sites, and subcloned into pGL2-basic (Promega). The NRF2-TK$_{Luc}$ reporter consists of four tandem copies of the NRF-2 response element [32], cloned into *Kpn*I and *Sac*I sites in TK$_{Luc}$. Reporter constructs for PPARα response element PPRE-TK$_{Luc}$ derived from peroxisomal acyl-CoA oxidase gene promotor were a gift from D. Kelly (Washington University) [33]. The Renilla luciferase reporter construct, pRL-TK (Promega), was used as a cotransfection control for transfection efficiency.

2.6. Transfections

Plasmids were prepared for transfection using Maxi Prep kit (Qiagen). Cells were transfected using FuGENE 6 (Roche) as per the manufacturer's protocol. Cells were grown to ~ 50% confluence in 24-well cell culture plates then transfected with fresh growth medium containing 250 ng of firefly luciferase plasmid (test plasmid) in combination with 50 ng Renilla luciferase plasmid (control plasmid, pRL-TK, Promega).

2.7. Luciferase measurements

Cells were harvested and frozen 3 days post-differentiation in 100 μl 1× passive lysis buffer (PLB, Promega). Thawed lysates were assayed on a Lmax Luminometer (Molecular Devices) using the automatic injector. To measure firefly luciferase, 100 μl of luciferase assay reagent

Table 2
Enzyme activities of liver, heart, gastrocnemius (gastroc), tibialis anterior (TA) and soleus muscle from obese (cp/cp) and lean (+/?) JCR:LA-cp rats

		COX	CS	LDH	CPT II	HOAD	LCAD
Liver	+/?	27 ± 2	17 ± 1	796 ± 45	1.1 ± 0.03	75 ± 3	1.1 ± 0.06
	cp/cp	27 ± 1	19 ± 1	1272 ± 71*	1.6 ± 0.2*	84 ± 6	1.2 ± 0.1
Heart	+/?	73 ± 4	133 ± 8	509 ± 22	2.0 ± 0.2	42 ± 6	1.8 ± 0.1
	cp/cp	73 ± 5	140 ± 1	646 ± 18*	2.7 ± 0.3	55 ± 6	2.0 ± 0.02
Gastroc	+/?	14 ± 3	24 ± 1	1110 ± 95	0.43 ± 0.02	4.4 ± 0.4	0.46 ± 0.05
	cp/cp	16 ± 1	27 ± 1*	1181 ± 126	0.56 ± 0.05*	4.5 ± 0.2	0.62 ± 0.05*
TA	+/?	9 ± 1	38 ± 3	1353 ± 121	0.60 ± 0.06	3.7 ± 0.2	0.44 ± 0.04
	cp/cp	14 ± 2	40 ± 2	1530 ± 56	0.63 ± 0.04	5.3 ± 0.4*	0.52 ± 0.06
Soleus	+/?	6.3 ± 0.4	24 ± 1	178 ± 7	1.3 ± 0.2	7.0 ± 0.5	0.98 ± 0.06
	cp/cp	7.1 ± 0.8	29 ± 1	207 ± 12	1.6 ± 0.2	9.5 ± 0.7*	1.02 ± 0.05

Values are means ± S.E. of $n = 6$ expressed as μmol substrate converted min^{-1} g tissue wet weight^{-1}.
* Significantly different from +/?, $P < 0.05$.

(LARII, Promega) was added to 20 μl of room-temperature cell lysate in a 96-well plate. Relative light units (RLUs) were measured for 10 s, with a 2-s pre-measurement delay. Then the LARII luminescence was quenched and Renilla luciferase activity measured by adding 100 μl of Stop & Glow solution (Promega) to the same well. RLUs were again measured for 10 s, with a 2-s delay. Values of reporters are standardized relative to Renilla luciferase values.

2.8. Statistical analysis

All data presented are means ± S.E. Results were analyzed using a *t*-test, one-way and two-way ANOVA (JMP Statistical Software, SAS Institute Inc.).

3. Results

3.1. Enzyme activities

The apparent V_{max} values for COX, CS, HOAD, LCAD, CPTII and LDH from several skeletal muscles, heart and liver appear in Table 2. There was no significant change in COX or CS activities, with the exception of the gastrocnemius muscle (CS +13%), in cp/cp rats. The cp/cp rats did show significantly ($P<0.05$) greater activity of CPT II (+45% in liver and +30% in gastroc), HOAD (+43% in TA and +36% in sol) and LCAD (+35% in gastrocnemius) in peripheral tissues. The activity of LDH was elevated in both the heart (+27%) and the liver (+60%) of cp/cp rats.

C_2C_{12} and Sol8 cell lines were induced to differentiate by serum starvation and treated with leptin at 0, 0.2, or 2.0 nM. After 3 days of differentiation, there was no effect of leptin on the activities of COX, CS, HOAD or LCAD in either cell line (Table 3).

3.2. Changes in mRNA

Northern analysis was conducted on heart and TA muscles to assess the pattern of mRNA for selected enzymes. There were no differences between genotypes in CS mRNA in either TA or heart (Fig. 1A and B). In TA muscle, the cp/cp

Table 3
Enzyme activities of C_2C_{12} and Sol8 cells measured after 3 days of differentiation with and without leptin

	Leptin (nM)	COX	CS	HOAD	LCAD
C_2C_{12}	0	43 ± 2	51 ± 1	29 ± 1	1.7 ± 0.3
	0.2	42 ± 2	53 ± 3	28 ± 2	1.9 ± 0.4
	2.0	50 ± 2	53 ± 2	29 ± 2	2.0 ± 0.5
Sol8	0	66 ± 5	56 ± 6	30 ± 3	1.4 ± 0.3
	0.2	66 ± 3	60 ± 5	30 ± 5	1.3 ± 0.4
	2.0	67 ± 4	53 ± 3	32 ± 4	1.4 ± 0.1

Values are means ± S.E. of $n=3$ expressed as μmol substrate converted min^{-1} mg cellular $protein^{-1}$.

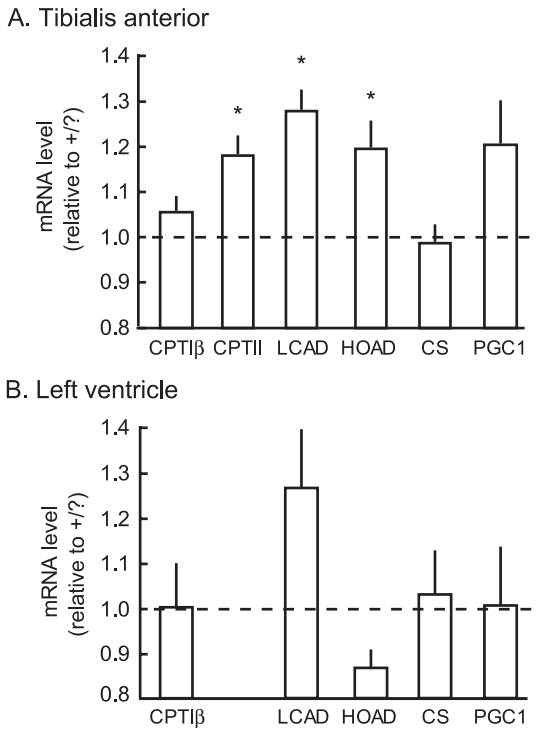

Fig. 1. Northern analysis of the JCR:LA-cp rat (A) TA and (B) heart muscle in cp/cp relative to wild-type (+/?) controls. Hybridization was with cDNA probes for CPT Iβ, CPT II, LCAD, HOAD, CS and PGC-1. $N=6$.

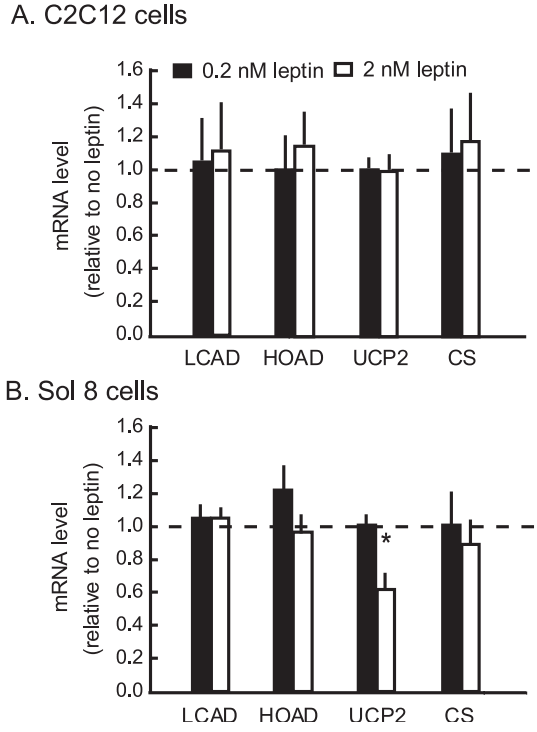

Fig. 2. Northern analysis for (A) C_2C_{12} and (B) Sol8 cell lines treated with 0, 0.2 and 2 nM recombinant rat leptin. Hybridization was with cDNA probes for LCAD, HOAD, UCP-2, and CS ($N=3$).

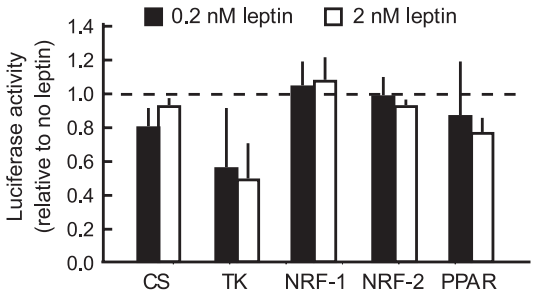

Fig. 3. Transient transfections of C_2C_{12} cells with luciferase reporter constructs for PPAR, NRF-1, NRF-2, and CS. TK was used as a basal promoter. Cells were allowed to differentiate for 3 days in media containing either 0.2 or 2 nM recombinant rat leptin. $N=3$.

rats had higher mRNA levels for CPT II (+19%), LCAD (+29%) and HOAD (+20%) ($P<0.05$). In TA muscle PGC-1 ($P=0.09$) and CPT Iβ ($P=0.12$) mRNA levels appeared to increase but not to a level that was significantly different than controls (Fig. 1A). There were no significant differences in mRNA levels for the genes of interest in heart compared to controls (Fig. 1B).

In the cell culture models (C_2C_{12} and Sol8), there was no significant effect of the leptin treatments on mRNA levels of LCAD, HOAD or CS (Fig. 2A and B). There was a significant decrease in UCP2 (−26%) mRNA in Sol8 cells at leptin concentration of 2.0 nM.

3.3. Reporter gene analysis

C_2C_{12} cells were transiently transfected with luciferase reporters for PPAR, NRF-1, NRF-2 and human CS promoter. Treatment of differentiated cells with 0.2 (Fig. 3A) and 2 nM (Fig. 3B) leptin had no significant effect on the transcription of these reporters.

4. Discussion

4.1. Leptin signaling and gene expression

Our analysis of leptin-treated cultured myocytes was used to assess the potential for direct effects of leptin. Murine myocytes possess the leptin receptor [20]. Treatment of cultured muscle cells (C_2C_{12} and Sol8) with leptin failed to induce any changes in gene expression. There were no changes in the activities of mitochondrial enzymes, including those involved in fatty acid oxidation (Table 3). Similarly, there were no differences in mRNA for selected enzymes (LCAD, HOAD, CS). There was an apparent decrease in mRNA for UCP2 in Sol8 cells.

Recent studies have shown that the JAK/STAT pathway in muscles can respond within minutes to changes in leptin levels [9]. In contrast, we found that 3 days of treatment with leptin failed to induce any change in transcription factor reporter genes. Leptin did not alter the activity of reporter genes for NRF-1 and NRF-2, which control the expression of many mitochondrial genes that are induced with adaptive remodeling of mitochondria [17,22,31]. Similarly, the activity of a reporter gene driven by a complex promoter from the CS gene was unresponsive to leptin (Fig. 3). Similarly, the activity of the PPAR reporter was unchanged by leptin (Fig. 3). In other experimental contexts, these reporters increase several fold in response to hormone treatment. Vega et al. (e.g. Ref. [33]) have used the PPAR construct to show the responses to PPAR agonists. Similarly, we have found that the NRF-1, NRF-2 and CS constructs increase in activity 5–10-fold with cellular differentiation (unpublished). Thus, the results argue that leptin does not affect gene expression in these cells.

4.2. Metabolic phenotype of the JCR:LA corpulent rat

The results from in vitro studies failed to find support for any effects of leptin on the expression of mitochondrial genes. However, when mitochondrial enzyme activities and mRNA levels were assessing the JCR:LA rats, which are deficient in leptin signaling, we saw a complex pattern of changes. It is important to stress that the enzyme changes (activity and mRNA) in this model could, in principle, be due to (i) the primary mutation in the gene for the leptin receptor, (ii) the compensatory changes in insulin signaling, or (iii) changes in metabolite/nutrient regulation. When we compared the enzyme activities between cp/cp and +/? littermates, there were no detectable changes in the activities of select citric acid cycle (CS) and electron transport chain (COX) enzymes. This is consistent with what we observed in vitro. Similarly, no changes in CS were seen when rats were treated directly with leptin [13]. However, when other enzymes were analyzed, we saw complex changes in enzyme/mRNA levels and stoichiometries.

Under most conditions, the genes of fatty acid oxidation are regulated in parallel allowing tissues to retain intrinsic stoichiometries [34,35]. However, the cp/cp genotype caused complex changes in fatty acid oxidation enzyme patterns in different tissues. The activities of HOAD were elevated in TA and soleus but not liver, heart or gastrocnemius. The activities of CPT II were elevated in liver and gastrocnemius but not the other tissues. LCAD activities were increased only in gastrocnemius. In TA muscle, the pattern of change in mRNA reflected the changes in enzyme activity, suggested that the changes were controlled by transcription. Several studies have implicated PGC-1 in the coordination of changes in mitochondrial gene expression in relation to diabetes and obesity [33]. However, we found no significant change in the mRNA levels of PGC-1. The non-stoichiometric changes in the different muscles and other tissues are likely due to tissue specific peculiarities in the control of gene expression.

Another enzyme that appears to respond differently in each tissue was LDH. It was elevated in heart and liver of cp/cp rats but not the skeletal muscles. Consistent with our

observations, well-fed rats possess higher cardiac LDH activity than caloric-restricted rats [36]. The functional significance of elevated LDH depends on which intracellular pool has increased [37,38], and to a lesser extent, any change in isozyme composition [39]. The increases in heart LDH likely arose through effects on expression of the LDH-B gene. Conversely, the increases in liver LDH likely arose through effects on the LDH-A gene. Surprisingly little is known about the control of the LDH gene expression. LDH activities, which can be diagnostic indicators of liver and heart disease [40], are generally thought to contribute to nutrient sensing pathways that balance glycolytic and oxidative metabolism [41].

The importance of leptin itself in the metabolic phenotype of cp/cp rats is difficult to assess because of confounding effects on insulin levels and insulin sensitivity. Insulin resistance is a key component of the metabolic syndrome [7] that results in a decrease in fatty acid metabolism in muscle [42]. Obesity in humans shows a decrease in muscle fatty acid oxidative capacity leading to increased vastus lateralis TAG accompanied by decreases in COX and CPT activity [42]. This differs from cp/cp rats (Table 2) and may reflect taxa-, muscle- or model-specific differences in the effects of insulin resistance on mitochondrial gene expression. Differences seen in cp/cp rats may reflect the combination of loss of leptin signaling and insulin resistance on muscle respiratory gene expression. Loss of insulin sensitivity in streptozotocin-diabetic mice leads to an increase in all of the β-oxidation enzymes in muscle [35]. The changes in enzyme stoichiometries seen in this study may reflect interactions between leptin and insulin signaling pathways. In cp/cp rats, the obese phenotype does not appear until insulin levels increase [4], suggesting that insulin signaling is more important than leptin signaling.

4.3. Hyperlipidemia

Fatty acids are thought to act as signaling molecules and can activate PPARα [43]. This transcriptional pathway appears to be disrupted in cp/cp rats leading to variable increases in synthesis of fatty acid enzymes. In fact, hyperlipidemia in this model does not result in a coordinated increase in mitochondria biogenesis and fatty acid enzymes that help regulate body composition under normal circumstances [44]. Free fatty acids also stimulate insulin secretion from β-cells [45], which may provide a link between hyperlipidemia, increased circulatory insulin and insulin resistance. The hyperlipidemia may also trigger insulin resistance through activation of the IκB kinase-β pathway and inhibition of the insulin signal cascade [46]. Insulin deficiencies in rats cause an increase in fatty acid uptake by muscles [42,47] and this increased uptake may trigger gene expression for enzymes involved in fatty acid oxidation [44]. This does not hold true in hearts of cp/cp rats which show increased accumulation of TAG in response to an increase in fatty acid supply but does not alter fatty acid oxidation or the activities of key enzymes of lipid metabolism do not change (Ref. [8]; Table 2).

4.4. Conclusions and implications

We found that cp/cp rats showed changes in gene expression culminating in alterations in select enzymes of fatty acid oxidation in peripheral tissues. It is not clear if these enzymatic changes contribute to the obese phenotype or if they are a response to the metabolic changes in cp/cp rats. It is noteworthy that the obese JCR:LA rats showed normal or elevated levels of fatty acid oxidation enzymes, whereas obesity is normally associated with a decrease in fatty acid oxidation [48,49]. Superimposed upon the enzymatic changes are the effects of allosteric regulators (e.g. malonyl-CoA) that change in response to hormonal and metabolic conditions. Furthermore, changes in fatty acid metabolism have the potential to alter the expression of bioenergetic genes (e.g. UCP-2 and UCP-3 [50]). Our studies focused on enzymes of intermediary metabolism, primarily those in mitochondria oxidative metabolism. We conclude that changes in leptin signaling act indirectly on the metabolic phenotype, acting through metabolic changes or insulin resistance. However, it is important to recognize the potential effects of leptin, both direct and indirect, on other enzymes related to the uptake, storage and hydrolysis of lipid in muscle.

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