Dietary fat interacts with QTLs controlling induction of Pgc-1α and Ucp1 during conversion of white to brown fat

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Coulter, Ann Allen, Christie M. Bearden, Xiaotuan Liu, Robert A. Koza, and Leslie P. Kozak. Dietary fat interacts with QTLs controlling induction of Pgc-1α and Ucp1 during conversion of white to brown fat. Physiol Genomics 14: 139–147, 2003. First published May 13, 2003; 10.1152/physiolgenomics.00057.2003.—To identify novel regulatory factors controlling induction of the brown adipocyte-specific mitochondrial uncoupling protein (Ucp1) mRNA in the retroperitoneal white fat depot, we previously mapped quantitative trait loci (QTLs) that control this trait to chromosomes 2, 3, 8, and 19. Since the peroxisome proliferator activator receptor-γ coactivator-1α (PGC-1α) regulates Ucp1 and other genes of energy metabolism, we have evaluated whether the QTLs controlling Ucp1 mRNA levels also modulate Pgc-1α mRNA levels by analysis of backcross progeny from the A/J and C57BL/6J strains of mice. The results indicate that a locus on chromosome 3 orchestrates expression of Pgc-1α and Ucp1 in retroperitoneal fat of mice fed a low-fat diet; however, the effect of this locus on Pgc-1α is lost, and a significant correlation between Ucp1 and Pgc-1α is severely reduced in mice fed a high-fat diet. An additional QTL located on chromosome 5 has also been identified for the selective regulation of Ucp1 mRNA levels. Similar to the effects of a high-fat diet on the chromosome 3 QTL, linkage of the chromosome 5 QTL is also lost in mice on a high-fat diet. Thus dietary fat has a profound influence on PGC-1α-regulated pathways controlling energy metabolism in white fat. The allelic variation observed in the regulation of Ucp1 and Pgc-1α expression in white adipocytes of white fat but not interscapular brown fat suggests that fundamentally different regulatory mechanisms exist to control the thermogenic capacities of these tissues.

adipocyte; differentiation; genetics; energy metabolism; fat depots by adrenergic stimulation appears to be a fairly common property among mammalian organisms that may also include fat tissues from adult humans (4, 11). The origin of the precursor cells that give rise to the brown adipocytes is still not resolved, but evidence showing the gradual acquisition of the brown adipocyte morphology by white adipocytes (12), the absence of significant de novo cell proliferation (16), and the very rapid induction of Ucp1 mRNA to maximal levels following adrenergic stimulation (12) all suggest that white adipocytes are converted to brown adipocytes. Whether this represents a form of transdifferentiation of a subpopulation of white adipocytes that have the capacity to respond to adrenergic signals to initiate the brown fat differentiation program without chromatin remodeling is unknown. A physiological role for an increase in the numbers of brown adipocytes in white fat depots was first suggested by Champigny et al. (5), who noted that dogs treated with a β3-adrenergic agonists had induced levels of Ucp1 mRNA in peritoneal fat depots and reduced girths; this correlation between increased brown adipocytes in white fat and reduced adiposity was subsequently corroborated in the rat (15) and in the mouse (7, 12). A significant reduction in triglyceride stores has also been demonstrated in a number of transgenic experiments that promote expression of Ucp1 in white fat (3, 10, 18, 36, 37).

The molecular mechanism by which brown adipocytes can be induced in white fat depots is poorly understood. Studies with transgenic mice indicate that transcription pathways associated the adrenergic signaling through the protein kinase A (PKA) pathway can initiate the brown adipocyte differentiation pathway (3, 36). However, mice with an inactivated gene for the translational inhibitor 4E-BP1 have an increase in brown adipocytes through an unresolved mechanism that causes twofold higher levels of PGC-1α protein in white adipose tissues (37). Alternatively, by analysis of the transcriptional machinery controlling Ucp1 expression, several transcription factors, including the thyroid hormone receptor, peroxisome proliferator activator receptors α and γ (PPARα and PPARγ), and the PPAR coactivator, PGC-1α, have been identified, which together can induce aspects of brown adipocyte differentiation following the introduction of expression vectors into preadipocyte cell cultures not previously shown to express Ucp1 (31). Taking advantage of a phenotypic difference in induction of brown adipocytes

THE IDENTIFICATION of brown adipocytes in the parametrial fat depots of female mice, a traditional white fat depot, was first described by Ashwell and colleagues (45). Subsequently, there have been many reports of an increase in the number of brown adipocytes in white fat of several mammalian species exposed to the cold or treated with β3-adrenergic agonists (5, 7, 9, 12, 15, 25). The ability to induce brown adipocytes in white fat
and Ucp1 mRNA levels between inbred strains of mice, we have mapped quantitative trait loci (QTLs) controlling Ucp1 expression to four different mouse chromosomes (19). Three of these QTLs are located in chromosomal regions that carry no candidate genes previously implicated in Ucp1 expression. Although the fourth QTL on chromosome 8 is located very near Ucp1, it is not known whether the variant gene in the QTL is Ucp1. To obtain more insight into the signaling and transcription pathways regulating induction of brown adipocytes in white fat, we have begun to ask how the QTLs controlling Ucp1 expression affect some of the upstream regulatory factors implicated in Ucp1 expression. The importance of PGC-1α as a modulator of Ucp1, as well as a tissue-specific transducer of physiological capacity of adipose tissues, including the developmental bifurcation that leads to white or brown adipocytes (27, 31). If this reasoning is correct, then mouse strains with a higher density of markers in chromosomal regions containing significant linkage to Ucp1 and Pgc-1α expression. Linkage F values were determined for each MIT marker by ANOVA single-factor analyses in Excel. LOD scores were generated for each composite group of 400 BC mice using MapManager QTb8 (28).

**RESULTS**

*Genetic variation in Pgc-1α induction in adipose tissue.* Male A/J and C57BL/6J (B6) mice at 2 mo of age were exposed to cold (5°C) for up to 7 days. Within 6 h of cold exposure Pgc-1α mRNA levels in the retroperitoneal fat depot (RP) of A/J mice increased fivefold, whereas a smaller, delayed increase in expression occurred in B6, resulting in a difference of two- to threefold between strains (Fig. 1A). As shown in several earlier studies, A/J has high levels of Ucp1 expression in RP fat after 7 days of cold exposure, whereas expression remains very low in the B6 strain (Fig. 1C). It is striking that although both Ucp1 and Pgc-1α were strongly induced by cold in interscapular brown fat, there was no difference in gene expression between the two strains (Figs. 1, B and D). The stimulation of Pgc-1α mRNA to peak levels is observed within 6 h in both retroperitoneal fat and interscapedar brown fat of A/J mice, even though retroperitoneal fat consists almost exclusively of white adipocytes and BAT mostly brown adipocytes. These data suggest that a form of transdifferentiation is occurring in retroperitoneal fat characterized first by early increases in Pgc-1α and Ucp1, the thermogenic effector of brown adipocytes, followed by a complex morphological conversion of white to brown adipocytes as shown in previous studies (12, 16). In addition to Ucp1 expression, mitochondrial biogenesis is another essential requirement of the acquisition of the brown adipocyte phenotype, since brown adipocytes have among the highest densities of mitochondria known in eukaryotic cells. The ectopic expression of Pgc-1α can stimulate mitochondrial biogenesis in preadipocytes and muscle cell lines by increasing the expression and transcrip-
tional activity of nuclear respiratory factor 1 (NRF1) (42). NRF1 is a transcriptional activator of nuclear genes encoding mitochondrial proteins and mitochondrial transcription factor A (mtTFA), an activator of transcription and replication of the mitochondrial genome (33, 39). Accordingly, we analyzed levels of Nrf1 mRNA in retroperitoneal fat and brown fat of cold-exposed animals and surprisingly did not find a cold-stimulated increase in Nrf1 mRNA in either fat depot (Fig. 1, E and F, P > 0.05 in RP), despite the observed increases in Pgc-1α. In addition, no statistically significant difference in Nrf1 levels between strains was observed in retroperitoneal fat (P > 0.05).

**Pgc-1α expression and induction of Ucp1.** If increases in Pgc-1α mRNA levels do not predict a priori increased expression of Nrf1, then what is the relationship between induction of Pgc-1α and Ucp1? Is the activity of Pgc-1α a necessary condition for induction of Ucp1 as previously suggested (27)? To address this question, we generated 400 male (B6×A/J)F1×A/J backcross progeny in which cold-induced Ucp1 levels in RP varied over 100-fold (19). If PGC-1α is regulating Ucp1 expression, then a high correlation should exist between the two gene products. Furthermore, genetic loci that control Pgc-1α should also control Ucp1. The correlation between Ucp1 and Pgc-1α mRNA levels in 400 backcross progeny exposed to cold for 7 days was high (Fig. 2A, r = 0.74), suggesting that ~55% of the variance in Ucp1 expression could be attributed to Pgc-1α mRNA levels under the conditions of this experiment in which mice were fed a low-fat diet and exposed to the cold at 2 mo of age. It is also evident from the data in Fig. 2A that variation in Pgc-1α mRNA is greater in this backcross population of recombinant genotypes than observed among the parents. In addition to (B6×A/J)F1×A/J backcross progeny, a similar effect of complex genetic control with respect to Ucp1 was previously observed in selected fixed recombinant inbred...
strains where the levels of RP Ucp1 mRNA varied 150-fold and the range greatly surpassed that of the A/J and B6 parental strains (12). We therefore analyzed Pgc-1α mRNA levels in the A×B and B×A recombinant inbred strains (19) were exposed to the cold 7 days and analyzed for mRNA levels. The number of animals for each recombinant inbred strain averaged about 5.

The relationship between QTLs controlling Ucp1 and Pgc-1α expression. To determine whether regulatory genes controlling Ucp1 expression also control Pgc-1α, QTLs were mapped in backcross mice as described previously (19). Consistent with our earlier study, the four QTLs controlling Ucp1 mRNA levels, located on chromosomes 2, 3, 8, and 19, also emerged as significant in this backcross (Table 1). However, unlike the previous study, a very strong QTL for Ucp1 mRNA levels \((P = 6.3 \times 10^{-7})\) was found on chromosome 5 following a genome wide scan. In addition, there were two weak associations \((P = 0.02)\) with chromosomes 4 and 10. Although a QTL with a \(P\) value of 0.02 could be considered of questionable genetic significance, the fact that these QTLs were also associated with \(Pgc-1α\) expression at a higher level of significance may be an indication that the effects of chromosomes 4 and 10 on \(Ucp1\) are significant, but secondary to \(Pgc-1α\) stimulation of \(Ucp1\) expression. Six chromosomes carried QTLs that affect \(Pgc-1α\) mRNA levels. Whereas the QTLs that affect expression of \(Pgc-1α\) also affect the expression of \(Ucp1\), the inverse is not observed. The QTL at the \(Ucp1\) locus on chromosome 8 was the strongest QTL detected, yet it has no significant association with \(Pgc-1α\) expression. Although several QTLs with high significance were associated with \(Ucp1\) expression, for example, those on chromosomes 3, 5 and 8, only the chromosome 3 QTL showed the same high level of significance for \(Pgc-1α\). The interval map shows a peak LOD score at \(D3Mit101\) for both \(Ucp1\) and \(Pgc-1α\) (Fig. 3), suggesting that a gene at this locus regulates cold-induced expression of both. The \(A/J\) allele on chromosome 3 confers high expression and is recessive to the \(B6\) allele. No QTL maps to chromosome 3 in a backcross of the \(B6\) parent to the \(F1\) (data not shown). It is also noteworthy that with only one highly significant QTL, the genetic complexity for \(Pgc-1α\) is much lower than that for \(Ucp1\), consistent with the idea that \(Pgc-1α\) is only one of several transcriptional and signaling mechanisms that are important for the control of \(Ucp1\) (32). Since the QTL on chromosome 5 at \(D5Mit182\) is located \(-14\) Mb proximal to the \(Pgc-1α\) structural gene, it was necessary to evaluate whether the \(Pgc-1α\) structural gene was the candidate gene for the QTL on chromosome 5. We sequenced \(A/J\) and \(B6\) \(Pgc-1α\) transcripts and failed to find any sequence polymorphisms and, in addition, did not find any polymorphisms within \(10\) kb of the genomic upstream flanking region between \(A/J\) and \(B6\) in the Celera database.

One interpretation of the data is that the amount of genetic variation controlling \(Pgc-1α\) is less than that controlling \(Ucp1\) so that only a subset of genes control-

Table 1. Chromosomal linkage to cold-induced expression of \(Pgc-1α\) and \(Ucp1\)

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Mit Marker</th>
<th>Position cM</th>
<th>(Pgc-1α)</th>
<th>(Ucp1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>(D2Mit66)</td>
<td>47.8</td>
<td>0.04</td>
<td>(4.3 \times 10^{-4})</td>
</tr>
<tr>
<td>3</td>
<td>(D3Mit101)</td>
<td>48.2</td>
<td>(9.8 \times 10^{-6})</td>
<td>(2.2 \times 10^{-6})</td>
</tr>
<tr>
<td>4</td>
<td>(D4Mit116)</td>
<td>40</td>
<td>(5.5 \times 10^{-4})</td>
<td>0.02</td>
</tr>
<tr>
<td>5</td>
<td>(D5Mit182)</td>
<td>24</td>
<td>0.02</td>
<td>(6.3 \times 10^{-7})</td>
</tr>
<tr>
<td>8</td>
<td>(Ucp1)</td>
<td>37</td>
<td>0.11</td>
<td>(5.1 \times 10^{-8})</td>
</tr>
<tr>
<td>10</td>
<td>(D10Mit214)</td>
<td>19</td>
<td>(2.0 \times 10^{-4})</td>
<td>0.02</td>
</tr>
<tr>
<td>19</td>
<td>(D19Mit86)</td>
<td>30</td>
<td>0.02</td>
<td>(3.4 \times 10^{-3})</td>
</tr>
</tbody>
</table>

Four hundred backcross mice were fed a diet containing 4.5 wt% fat from weaning to 2 mo of age and then exposed to the cold at 5°C for 7 days. Levels of mRNA, genotyping, and linkage were performed as described in METHODS.

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ling Ucp1 mRNA levels in A/J and B6 alleles is less for Pgc-1α than it is for Ucp1, our ability to reliably detect statistically significant QTLs in backcross progeny may be less robust for Pgc-1α than it is for Ucp1. In a parallel experiment, RP Nrf1 mRNA levels were measured in backcross mice, and a genome-wide scan was performed. No QTLs with significant linkage to cold-induced Nrf1 expression were found (P = 0.04).

The effects of an altered environment, i.e., diet, on the expression of Ucp1 and Pgc-1α. The emergence of a highly significant QTL on chromosome 5 for Ucp1 with 400 backcross progeny generated at the Pennington Biomedical Research Center between during 1999 and 2000 that was not detected in a similar backcross of A/J and B6 mice on a high-fat diet did not suggest that the high-fat diet was having a major effect on the QTLs controlling either Ucp1 or Pgc-1α (Table 2). However, the strong correlation between Ucp1 and Pgc-1α mRNA levels in mice fed a low-fat diet was lost when they were fed a high-fat diet (Fig. 4, r = 0.41). These data suggest that the pattern of QTLs associated with expression of Pgc-1α and Ucp1 would be altered in mice fed a high-fat diet.

A modest increase in the levels of Ucp1 and Pgc-1α mRNA in the A/J and B6 parental strains and in backcross progeny fed a high-fat diet did not suggest that the high-fat diet was having a major effect on the QTLs controlling either Ucp1 or Pgc-1α (Table 2). However, the strong correlation between Ucp1 and Pgc-1α mRNA levels in mice fed a low-fat diet was lost when they were fed a high-fat diet (Fig. 4, r = 0.41). These data suggest that the pattern of QTLs associated with expression of Pgc-1α and Ucp1 would be altered in mice fed a high-fat diet.

Accordingly, we mapped the QTLs controlling Ucp1 and Pgc-1α mRNA levels in the retroperitoneal fat of BC mice fed a high-fat diet (Table 3). Several striking differences occurred in the pattern of QTLs associated with gene expression. First, QTLs on chromosomes 4, 5, and 10 no longer had any linkage to Ucp1 or Pgc-1α mRNA levels. Second, the regulatory effect of chromosome 3 is completely lost for Pgc-1α and reduced by over two orders of magnitude for Ucp1. Chromosomes 2, 3, 8, and 19 continue to influence Ucp1 expression, whereas only chromosomes 2 and 19 affect Pgc-1α; however, the significance of both chromosomes are stronger in mice fed a high-fat diet. A genome-wide scan at 20-cM intervals indicated that no new QTLs could be detected in BC mice fed a high-fat diet.

Fig. 4. Correlation between Pgc-1α and Ucp1 mRNA levels in mice fed a 36 wt% fat diet. Pgc-1α and Ucp1 mRNA levels were determined in 400 backcross animals maintained on a high-fat diet (HFD) from weaning and exposed to the cold for 7 days.

Table 2. Effects of a high-fat diet on Pgc-1α and Ucp1 mRNA levels

<table>
<thead>
<tr>
<th>Strain and mRNA</th>
<th>LFD mRNA</th>
<th>HFD mRNA</th>
<th>P Value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/J Ucp1</td>
<td>222 ± 20</td>
<td>186 ± 42</td>
<td>0.43</td>
<td>12</td>
</tr>
<tr>
<td>B6 Ucp1</td>
<td>9.5 ± 2</td>
<td>33 ± 10</td>
<td>0.02</td>
<td>12</td>
</tr>
<tr>
<td>B × AF1 Ucp1</td>
<td>38 ± 7</td>
<td>55 ± 12</td>
<td>0.22</td>
<td>12</td>
</tr>
<tr>
<td>A/J Pgc-1α</td>
<td>13 ± 1</td>
<td>21 ± 2</td>
<td>3.3 × 10^{-4}</td>
<td>12</td>
</tr>
<tr>
<td>B6 Pgc-1α</td>
<td>5.7 ± 0.3</td>
<td>9.7 ± 0.9</td>
<td>1.1 × 10^{-4}</td>
<td>12</td>
</tr>
<tr>
<td>B × AF1 Pgc-1α</td>
<td>7.4 ± 0.4</td>
<td>9.3 ± 0.6</td>
<td>0.02</td>
<td>12</td>
</tr>
<tr>
<td>BC Ucp1</td>
<td>67.4 ± 2</td>
<td>77.8 ± 5</td>
<td>7.3 × 10^{-3}</td>
<td>400</td>
</tr>
<tr>
<td>BC Pgc-1α</td>
<td>8.2 ± 0.2</td>
<td>11.7 ± 0.3</td>
<td>1.6 × 10^{-24}</td>
<td>400</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE. A/J, C57BL/6J (B6), (B × A)F1, and backcross mice were exposed to cold at 5°C for 7 days while on chow (4.5 wt% fat, LFD) or high-fat diets (36 wt% fat, HFD) from weaning until the end of cold exposure. Pgc-1α mRNA levels increased 43%, and Ucp1 levels 15% in backcross mice on a high-fat diet.
Table 3. Linkage to cold-induced expression of Pgc-1α and Ucp1 in backcross mice fed a high-fat diet

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Mit Marker</th>
<th>Position, cM</th>
<th>Pgc-1α</th>
<th>Ucp1</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>D2Mit66</td>
<td>47.8</td>
<td>4.3×10⁻³</td>
<td>4.1×10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>D3Mit101</td>
<td>48.2</td>
<td>0.85</td>
<td>4.1×10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Ucp1</td>
<td>37</td>
<td>0.49</td>
<td>3.1×10⁻⁵</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>D19Mit86</td>
<td>30</td>
<td>7.1×10⁻³</td>
<td>1.7×10⁻⁵</td>
<td></td>
</tr>
</tbody>
</table>

Four hundred backcross mice were fed a diet containing 36 wt% fat from weaning age until 2 mo and then exposed to the cold for 7 days. Chromosomal linkage of Pgc-1α and Ucp1 expression in retroperitoneal fat was determined as described in Table 1.

DISCUSSION

In this study we demonstrate that cold exposure stimulates expression of Pgc-1α in white fat, and levels are significantly higher in the RP white fat depot of the A/J strain than in the B6 strain. The strong correlation between Pgc-1α and Ucp1 expression in RP fat in the A/J and B6 parental strains, backcross mice, and recombinant inbred strains is consistent with a role for PGC-1α in the regulation of Ucp1 and the differentiation of brown adipocytes in white fat depots. We also show that Nrf1 expression is not stimulated by cold exposure in either white adipose tissues or BAT despite the cold-stimulated increases in Pgc-1α. We cannot rule out the possibility that Nrf1 is regulated at the protein level in adipose tissues (13).

Similar to Ucp1 expression, induction of Pgc-1α expression in RP white fat is a multifactorial trait dependent on both genotype and environment. The latter includes both the ambient temperature and fat content of the diet. It is particularly curious that these variables are restricted to phenotypes of retroperitoneal fat. We have detected no significant variation between A/J and B6 mice for either Ucp1 or Pgc-1α in interscapular brown fat. One interpretation is that the difference is simply a consequence of the absence of any brown adipocytes in the retroperitoneal fat of B6 mice, whereas with equal numbers of brown adipocytes in interscapular fat in the two strains, there are no differences in Ucp1 or Pgc-1α expression. It would follow then that even in retroperitoneal fat all phenotypes associated with Ucp1 or Pgc-1α expression would simply reflect the number of brown adipocytes. However, we know that this simple interpretation is not valid from our initial studies of recombinant inbred lines of mice where we observed that although the A×B8 and A×B15 strains had similar levels of Ucp1 mRNA, they differed greatly in the number of brown adipocytes that could be detected in the tissue following cold induction and in the levels of Pgc-1α mRNA (12). We now show in this paper that the variation of Ucp1 and Pgc-1α is determined by some QTLs in common and others that independently act on either gene, signifying a complex interplay of allelic variation and environment to generate a phenotype. The molecular mechanisms for these effects of a high-fat diet are not known, but they reflect profound changes in the involvement of genes on several different chromosomes. As shown in the Venn diagram in Fig. 5, some highly significant QTLs that appeared to have a major role in the regulation of Ucp1 and Pgc-1α were lost completely, others had lower significance, and still others showed increased significance. One of the most prominent changes occurred in the QTL located on chromosome 3, since a highly significant linkage found in mice fed a low-fat diet was lost when the mice were fed a high-fat diet. Whatever the identity of the gene on chromosome 3, it appears to mediate strain-specific differences in the regulation of Ucp1 in the presence of a low-fat diet but not a high-fat diet. The 1-LOD confidence interval of this QTL is ~7 Mb extending from D3Mit76 to D3Mit75, and it includes 150 putative transcribed genes. None of the transcripts identified by the Celera and National Center for Biotechnology Information (NCBI) databases in this region have previously been implicated in expression of Pgc-1α, Ucp1, or adipogenesis, and experiments are underway to determine their expression patterns in RP fat. We need to understand why the loss of an allelic effect by chromosome 3 when mice are fed the high-fat diet has no apparent negative effect on Pgc-1α or Ucp1 expression. The effects of a high-fat diet on chromosome 3 will be more comprehensively evaluated when congenic strains for chromosome 3 become available (work in progress in our laboratory). Finally, the sensitivity of the gene(s) at the chromosome 3 QTL, as well as its effects on the expression of both Ucp1 and Pgc-1α, suggests that it could be a hot spot for regulation of obesity-related genes similar to the recently proposed hotspot on chromosome 2 (34).

Although the QTL on chromosome 5 regulates Ucp1 expression only, this linkage was also completely lost when mice were fed a high-fat diet. This result suggests that differences in diet may have been one of the environmental effects associated with the fact that this QTL was not detected in backcross mice generated at the Jackson Laboratory. The Pgc-1α gene on chromo-

Venn Diagrams for Chromosomes Involved in Ucp1 and Pgc1 Expression

- **7 days in cold/ Low fat diet**
  - Ucp1: 2, 5, 8, 19
  - Pgc1: 3, 4, 10

- **7 days in cold/ High fat diet**
  - Ucp1: 3, 8
  - Pgc1: 2, 19

Fig. 5. Venn diagram illustrating the chromosomal linkages to Pgc-1α and Ucp1 mRNA levels in backcross progeny depending on whether they were fed a low- or high-fat diet.
some 5 was a promising candidate; however, there were no nucleotide polymorphisms between strains in the open reading frame that could cause a difference in PGC-1α protein function. The other possible functional mutation, a Pgc-1α promoter/enhancer difference on chromosome 5 that regulates Ucp1 levels through the observed strain-specific differences in Pgc-1α expression, would show strong linkage to Pgc-1α levels. The absence of linkage of chromosome 5 to Pgc-1α mRNA levels does not support a role for Pgc-1α as the chromosome 5 QTL. This suggests that variation in Pgc-1α expression is determined by a transacting mechanism, and in fact there are QTLs on as many as five chromosomes, unlinked to Pgc-1α, that modulate its mRNA levels in a diet-dependent manner. In a recent QTL analysis of differences between C57BL/6J and DBA/2J by microarray analysis of 23,574 gene targets, Schadt et al. (34) have also found that multiple QTLs control mRNA expression levels. This data underscores the idea that genes determining phenotypes based upon variation in gene transacting mechanisms can be mapped. They also find that the strongest QTLs are associated with cis-acting elements; that is, the determinant of regulatory variation is tightly linked to the target gene. We have also found this effect in our study, since the strongest QTL is tightly linked to the Ucp1 gene itself on chromosome 8.

If Pgc-1α expression is elevated in mice fed a high-fat diet, then why is the association between Pgc-1α and Ucp1 reduced? One obvious mechanism to consider is that in mice fed a high-fat diet, PGC-1α is no longer necessary for Ucp1 expression. Simple inspection of the data in Fig. 4 shows that there are many mice with elevated levels of Pgc-1α mRNA but low levels of Ucp1 mRNA. Given the complexity of transcription factors associated with Ucp1 expression, some with apparently redundant functions, it is plausible that a requirement for PGC-1α in Ucp1 expression is reduced under some physiological conditions that activate other regulatory pathways. For example, dietary fat differentially increases circulating leptin levels in the A/J and B6 strains, and leptin regulates thermogenesis and fatty acid metabolism in adipose tissues (8, 22, 40). Fatty acids, endogenous ligands for PPARs, can increase PPARα binding to PGC-1α and activate transcription of Ucp1 as well as mitochondrial fatty acid oxidation enzymes (1, 38, 43). Another explanation to consider is that the Pgc-1α mRNA levels do not accurately reflect either the actual levels of PGC-1α protein in the cell nucleus or its functional state. With respect to the latter, it has been shown that cytokine-stimulated phosphorylation of PGC-1α by p38 MAP kinase enhances its transcriptional activity and that the activity of PGC-1α is also regulated by a repressor (17). Although p38 MAP kinase has been implicated in Ucp1 expression in cell cultures (2, 35), we know nothing about its potential role in the induction of Ucp1 in retroperitoneal fat.

A high-fat diet is also known to suppress adrenergic signaling through reduced β3-adrenergic receptor gene expression (7) and, correspondingly, could affect signaling mechanisms through PKA, CREB, and MAP kinase pathways to reduce the contribution of PGC-1α to Ucp1 transcription (2, 20, 32, 35, 46). Conversely, the higher carbohydrate content of the low-fat diet may promote the participation of Pgc-1α in Ucp1 regulation. A pivotal role for PGC-1α in carbohydrate and lipid metabolism is evident from recent studies showing that coactivation of PPARα, PPARγ, and glucocorticoid receptors by PGC-1α modulates expression of enzymes of lipid and glucose homeostasis (14, 26, 30, 31, 38, 44).

Although PGC-1α is associated with Ucp1 regulation in both the interscapular brown adipocyte and the diffuse brown adipocytes in white fat, genetic variation in cold-induced expression between strains is observed only in white fat. Fundamentally different mechanisms for regulating Ucp1 expression must exist for the brown adipocytes in the traditional white fat vs. brown fat tissues. We speculate that the function of discrete brown fat depots formed at birth and persisting through adulthood is primarily to protect the animal from the cold, whereas the diffuse brown adipocytes induced in white fat depots are associated with the regulation of body weight, thus the sensitivity of retroperitoneal fat to dietary fat. Consistent with this hypothesis are the findings that many of the transgenic models associated with UCP1 overexpression and reduced obesity are characterized by induction of brown adipocytes in white fat depots (3, 10, 18, 36, 37). In the human population, variation in nonexercise thermogenesis is responsible for 10-fold differences in fat storage (24). Identification of the genes underlying QTLs regulating thermogenesis in white adipose tissues will be important for the development of new drugs for treatment and prevention of obesity.

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DISCLOSURES

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REFERENCES


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