Caloric Restriction Results in Decreased Expression of Peroxisome Proliferator-Activated Receptor Superfamily in Muscle of Normal and Long-Lived Growth Hormone Receptor/Binding Protein Knockout Mice

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Resistance to growth hormone, reduced insulin-like growth factor 1 (IGF1) action, and enhanced insulin sensitivity are likely mediators of extended life span and delayed aging process in growth hormone receptor/binding protein knockout (GHR-KO) mice. Fat metabolism and genes involved in fatty acid oxidation are strongly involved in insulin action. Using real-time polymerase chain reaction and western blot we have examined expression of peroxisome proliferator-activated receptors (PPARs) and retinoid X receptor (RXR) genes in the skeletal muscle of normal and GHR-KO mice subjected to 30% caloric restriction. The results indicate that caloric restriction decreased the expression of PPARc, PPARa, and PPARb/d which would lead to down-regulation of fat metabolism. This suggested metabolic change clearly does not affect whole-body insulin action. These findings suggest that whole-animal insulin sensitivity is not regulated through skeletal muscle insulin action.

GROWTH hormone receptor/binding protein knockout (GHR-KO) mice have growth hormone resistance activity and reduced systemic levels of insulin-like growth factor 1 (IGF1) (1). Recent studies (2) have shown that GH deficiency, as well as GH or IGF1 resistance, lead to extended longevity and delayed aging in laboratory mice. As examples, Ames dwarf mice (Prop1df) and Snell dwarf mice (Pit1dw) are deficient in GH, prolactin, and thyroid-stimulating hormone, but live more than 40% longer than their normal siblings do (2–4). GHR-KO (in text KO) mice are also characterized by markedly extended longevity in comparison to normal controls (5). These GHR-KO mice have greatly reduced plasma IGF1 and insulin levels, and low glucose with significantly higher circulating GH level in plasma which is not active because of GHR disruption (1). The action of IGF1 is primarily mediated by IGF1 receptor, and the study of mice heterozygous for IGF1 receptor knockout revealed that this partial disruption extends the longevity of females by approximately 33% (6). Caloric restriction (CR) is the most effective intervention known to delay aging and increase life span. CR also is known to reduce body weight and the levels of plasma IGF1, insulin, glucose, and thyroid-stimulating hormone. We have shown that subjecting Ames dwarf mice to CR further extends the life span of these long-lived mutants, similar to the effects of CR in their normal siblings (7). These and other findings suggest that GH and insulin/IGF1 signaling have very important roles in the control of aging (8).

In this study we analyzed the expression of the peroxisome proliferator-activated receptor (PPAR) family and the retinoid X receptor (RXR) genes in skeletal muscle in GHR-KO and normal mice after long-term CR. PPARs are the members of the nuclear receptor superfamily which are ligand-dependent transcription factors that function to regulate expression of target genes by binding to specific peroxisome proliferator response elements (PPREs) in enhancer sites of regulated genes. PPARs form heterodimers with RXR and bind to their specific PPRE (9). In the PPAR family there are three isoforms encoded by separate genes, PPARγ, PPARα, and PPARδ/β (also known as PPARδ or PPARβ). These three nuclear receptors are subject of intense interest. PPARγ, the most explored gene of this superfamily, is known as a target receptor for thiazolidinediones (TZDs), which are used in type 2 diabetic patients as insulin sensitizers (10–13). Increasing the insulin sensitivity in type 2 diabetes improves glucose homeostasis, which is mainly regulated by improved insulin action in skeletal muscle (14). PPARγ is also known as a regulator of adipose tissue development as well as a regulator of gene expression in adipocytes, macrophages, and epithelial cells.

Another member of this superfamily, PPARα is highly expressed in the tissues with high levels of fatty acid
catabolism. It is highly expressed in the liver, where it modulates oxidation of fatty acids. PPARα also regulates genes which encode enzymes involved in the fatty acid metabolic pathway, such as β and ω oxidation. This regulation is made possible by the presence of PPREs, which are under transcriptional control of PPARα in the promoter regions of genes coding for the enzymes involved in this metabolic pathway (15). There are also suggestions that PPARα controls extracellular lipid metabolism. Fibrates, acting as PPARα activators, have an important role in high-density lipoprotein cholesterol levels control by controlling apolipoprotein A-I (apoA-I) and apolipoprotein A-II (apoA-II) gene expression. The study of PPARα-deficient mice by Peters and colleagues (16) has shown that a lack of PPARα increases the levels of total and high-density lipoprotein cholesterol. PPARα is also known to lower triglyceride in animals and humans.

The third gene from this nuclear receptor superfamily is PPARβ/δ. The function of PPARβ/δ is still not well explored, but it is known that it plays a critical role in epidermal maturation and skin wound healing (17,18). However, recent studies have shown that PPARβ/δ also plays an important role in lipid metabolism. Obese mice treated with a PPARβ/δ agonist demonstrated dramatic lipid depletion in tissues (19). Moreover, a study of PPARδ-deficient mice fed a high-fat diet showed reduced energy uncoupling, which is a predisposer to obesity. All in all, PPARβ/δ appears to coordinate fatty acid oxidation and energy uncoupling, thereby making it an important factor in fat burning (19).

As mentioned above, PPARs are active and bind to their PPREs as heterodimers with RXR. There are three RXR isoforms which are coded by separate genes: RXRα, RXRβ, and RXRγ (20).

In this study, expression of genes from PPARs and RXR families were analyzed in the skeletal muscle of normal and GHR-KO mice after long-term CR. The function of these genes in fat metabolism and insulin action suggests that they are likely to play a role in the control of aging and longevity.

METHODS

Animals

GHR-KO animals were produced at Southern Illinois University in a colony derived from animals provided by Dr. J. J. Kopchick. This was accomplished by mating knockout (−/−) males and heterozygous (+/−) females. The animals were maintained under temperature- and light-controlled conditions (20–23°C, 12-hour light/dark cycle). All animal protocols for this study were approved by the Southern Illinois University Laboratory Animal Care and Use Committee. Animals were group housed according to sex and phenotype. Mice matched for average body weight within phenotype at 8 weeks of age were divided into two treatment groups: those on CR and those fed ad libitum (AL). AL animals were allowed unlimited access to food; CR animals were subjected to 30% CR starting at 2 months of age using a previously described protocol (21). There were four experimental groups: normal-AL (N-AL), N-CR, KO-AL, and KO-CR, consisting of eight males per group. At the age of 21 months, the animals were anesthetized using isoflurane, blood was taken by cardiac puncture, and mice were killed by decapitation. Hind-limb muscles were rapidly collected, washed with 0.9% saline, and snap frozen on dry ice for analysis.

Insulin Sensitivity: Homeostasis Model Analysis

Insulin and glucose were analyzed using previously described procedures (22). Insulin resistance (or sensitivity) was estimated by homeostasis model analysis (HOMA) according to the method described by Matthews and colleagues (23).

Free Fatty Acids Assay

Free fatty acid (FFA) concentration was determined with a Half Micro test (Roche Applied Science, Philadelphia, PA) using a protocol provided by the manufacturer. The concentration of FFA in plasma was determined by using 25 μl of plasma, and muscle FFAs were determined by analysis of a solution containing 200 μg of muscle protein (prepared as described below, see “Western Blots”).

Triglyceride Assay

Triglyceride concentration was determined with a Triglyceride-GPO Reagent Set (Pointe Scientific, Lincoln Park, MI) using a protocol provided by the manufacturer. The concentration of plasma triglycerides was determined by using 10 μl of plasma, and muscle triglycerides were determined by analysis of a solution containing 2 μg of muscle protein (prepared as described below, see “Western Blots”).

Cholesterol Assay

Plasma cholesterol concentration was determined with a Cholesterol Reagent Set (Pointe Scientific) using a protocol provided by the manufacturer.

 Extraction of Messenger RNA and Complementary DNA Synthesis

After grinding in liquid nitrogen, total muscle RNA was extracted using the phenol/chloroform procedure of Chomczynski and Sacchi (24). The quantity and quality of total RNA was analyzed on agarose gel using electrophoresis. Afterwards, complementary DNA (cDNA) was synthesized from 2 μg of total RNA using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacture’s protocol.

Real-Time Polymerase Chain Reaction

The real-time polymerase chain reaction (PCR) was performed using the Smart Cycler instrument (Cepheid, Sunnyvale, CA) with iQ SYBR Green Supermix (Bio-Rad Laboratories). Each reaction contained 12.5 μl of iQ SYBR Green Supermix, 0.4 μM each of backward and forward primer (Table 1), and 2 μl of cDNA (after dilution 3 H2O:1 cDNA mix). The three steps of the PCR included denaturation at 94°C for 2 minutes, annealing at 62°C for 30 seconds with fluorescence reading, and extension at 72°C for 30 seconds. In addition, a melting curve was done for each reaction to evaluate the potential of nonspecific
products. All samples were analyzed in a single reaction for each gene to avoid false differences caused by different efficiency in separate PCR reactions. β-2-microglobulin (B2M) was used as a housekeeping gene. Relative expression from real-time PCR was calculated from the equation $2^{\Delta \text{A} - \Delta \text{C} - \text{D}}$ (where $\text{A} = \text{Cycle Threshold (Ct)}$ number for the gene of interest in the first control sample; $\text{B} = \text{Ct number for the gene of interest in the analyzed sample; C = Ct number for the housekeeping gene in the first control sample; and D = Ct number for housekeeping gene in the analyzed sample}). The first control was expressed as 1.00 by this equation, and all other samples were calculated in relation to this value. Afterward, the results in the control group (N-AL) were averaged, and all other outputs were divided by the mean value of the relative expression in the control group to yield the fold change of the genes of interest expression compared to the control group.

**Western Blots**

After crushing the muscle in liquid nitrogen, 1× PBS was added, 250 µl was taken for RNA extraction, and the remaining homogenate was used for protein extraction. Western blot procedure was performed using PPARγ (Cell Signalling, Beverly, MA), PPARα, and PPARδ (both obtained from Santa Cruz Biotechnologies, Santa Cruz, CA) using protocol described earlier (22). Monoclonal anti-β-actin antibody (Sigma, St. Louis, MO) was used after stripping the membrane as a loading control.

**Statistical Analysis**

Data are expressed as mean ± standard error (SE). To evaluate the effects of the phenotype and diet, two-way analysis of variance (ANOVA) was used followed by Fisher’s protected least significant difference (PLSD) as a post hoc test. A $t$ test was used to evaluate the effect of diet within the phenotypes and phenotypes within diet. Values of $p < .05$ were considered significant.

**RESULTS**

The insulin-sensitivity HOMA test shows a decreased value (increased sensitivity) in KO animals in comparison to their normal siblings ($p < .0003$). There was also a significant decrease in normal and KO animals after CR (mean values: N-AL 36.65, SE 7.01; N-CR 9.09, SE 2.35; KO-AL 6.00, SE 0.83; and KO-CR 1.27, SE 0.33) ($p < .0018$ and $p < .0001$, respectively). The determination of FFA by colorimetric assay did not reveal any differences between N-AL and KO-AL mice in either plasma or muscle homogenate, but there was a significant increase of FFA in both normal and KO mice after CR in plasma ($p < .0028$ and $p < .0083$, respectively) and in muscle homogenate ($p < .0281$ and $p < .0331$, respectively) (Figure 1).

The analysis of triglycerides in plasma and muscle homogenate similarly failed to reveal significant differences between normal and KO mice. However, there was a significant decrease of triglyceride content in both normal and KO mice subjected to CR in plasma ($p < .0394$ and $p < .0406$, respectively) and in the muscle ($p < .0422$ and $p < .0279$, respectively) (Figure 1).

Plasma cholesterol level was reduced in KO mice in comparison to their normal siblings ($p < .0479$). There was also a significant diet effect in normal animals ($p < .0424$) with no CR effect in KO mice (Figure 1).

Using two-way ANOVA to analyze the PPARγ messenger RNA (mRNA) and protein expression, we found that there were significant diet effects ($p < .0001$ and $p < .0012$, respectively), and there was also an effect of the phenotype, but only at the protein level ($p < .0190$). The expression of PPARγ mRNA did not differ between N-AL and KO-AL mice, whereas the PPARγ protein level was decreased in KO-AL mice in comparison to N-AL mice ($p < .0027$ using $t$ test). CR significantly decreased PPARγ mRNA expression in both normal and KO animals (by $t$ test; $p < .0093$ and $p < .0012$, respectively). Moreover, PPARγ mRNA levels in KO-CR were reduced compared to those in N-CR mice ($p < .0077$) (Figure 2A). Analysis of protein levels by $t$ test did not reveal statistically significant effects of CR in either normal or KO mice; however, there was an apparent trend consistent with the findings at the mRNA level ($p < .0706$ and $p < .0881$, respectively) (Figure 2B).

PPARα mRNA and protein levels were significantly affected by the diet ($p < .0001$ and $p < .0015$, respectively), with no significant effect of the phenotype. However, results of the $t$ test indicate that the levels of the PPARα protein were reduced in KO-AL in comparison to N-AL mice ($p < .0347$). CR led to a significant decrease in PPARα expression in both normal and KO mice at the mRNA and protein level ($t$ test; $p < .0229$ and $p < .0001; p < .0148$ and $p < .0349$, respectively) (Figure 2, C and D). There was also a significant decrease in PPARα mRNA expression in KO-CR in comparison with N-CR mice ($p < .0112$).

The analysis of expression of the third gene from PPARs family, PPARδ, revealed significant effects of diet and phenotype with reductions at both mRNA ($p < .0001$ and $p < .0085$, respectively) and protein levels ($p < .018$ and $p < .0009$, respectively). However, analysis by $t$ test detected no difference between N-AL and KO-AL at the mRNA level, whereas the PPARδ protein levels were significantly reduced.

### Table 1. Sequence of Primers Used for Real-Time Polymerase Chain Reaction

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPARγ-forward</td>
<td>5′-CAAAGTAGAAGCCTGACATCCTC</td>
<td>3′-CAATCTGGCCAAACAGCTT</td>
</tr>
<tr>
<td>PPARγ-backward</td>
<td>5′-CTCGAGATGAGAAGTGGCA</td>
<td>3′-AGAGTACCTGCTGCTCTAG</td>
</tr>
<tr>
<td>PPARα-forward</td>
<td>5′-CGAAGCTCTACGTAAGAGATTT</td>
<td>3′-CCTCCTCTACAGACTATTAG</td>
</tr>
<tr>
<td>PPARα-backward</td>
<td>5′-TGACATACCTGAGGAGCCAAACA</td>
<td>3′-GGACAGGAGGACACGAAGA</td>
</tr>
<tr>
<td>PPARδ-forward</td>
<td>5′-GACATACCTGCATGATCTGA</td>
<td>3′-GGACAGGAGGACACGAAGA</td>
</tr>
<tr>
<td>PPARδ-backward</td>
<td>5′-GACATACCTGAGGAGCCAAACA</td>
<td>3′-GGACAGGAGGACACGAAGA</td>
</tr>
</tbody>
</table>

Note: Gene bank sequence number appears beside each forward primer.

**Statistical Analysis**

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The analysis of expression of the third gene from PPARs family, PPARδ, revealed significant effects of diet and phenotype with reductions at both mRNA ($p < .0001$ and $p < .0085$, respectively) and protein levels ($p < .018$ and $p < .0009$, respectively). However, analysis by $t$ test detected no difference between N-AL and KO-AL at the mRNA level, whereas the PPARδ protein levels were significantly reduced.
in KO-AL in comparison to N-AL mice \( (p < .0117) \). PPAR\(\delta\) RNA message appeared to be lower in N-CR in comparison to N-AL \( (p < .0859) \), and PPAR\(\delta\) protein was significantly decreased in N-CR as compared to N-AL \( (p < .0403) \). In KO animals, there was a significant decrease in PPAR\(\delta\) after CR at both the mRNA and protein levels \( (p < .0001\) and \( p < .0001 \), respectively). There was also a significant decrease in PPAR\(\delta\) in KO-CR mice in comparison to N-CR at both mRNA and protein levels \( (p < .0001\) and \( p < .0418 \), respectively) (Figure 2, E and F).

Two-way ANOVA indicated that CR significantly altered mRNA expression for RXR\(\gamma\), RXR\(\alpha\), and RXR\(\delta\) \( (p < .0001\),
Figure 2. Expression of peroxisome proliferator-activated receptor (PPAR) superfamily genes in muscle tissue of normal (N) and growth hormone receptor/binding protein knockout (KO) mice fed ad libitum (AL) or subjected to 30% caloric restriction (CR). The data from real-time polymerase chain reaction were normalized by the housekeeping gene, β-2-microglobulin. Equal loading of protein for western blots was verified using β-actin. Means ± standard error of the mean. a, b, and c: values that do not share the same letter in the superscript are significantly different (p < .05). A, PPARγ messenger RNA (mRNA) expression; B, PPARγ protein level; C, PPARα mRNA expression; D, PPARα protein level; E, PPARβ/δ mRNA expression; F, PPARβ/δ protein level.
p < .0001, and p < .0008, respectively), whereas phenotype significantly affected only the expression of RXRa (p < .0305). RXRγ mRNA expression did not differ between phenotypes, and CR treatment significantly decreased levels of this message in KO-CR mice in comparison to KO-AL mice (p < .0001). There was no decrease in RXRγ in normal animals subjected to CR, but there was a trend in the same direction as in KO mice (p < .0965) (Figure 3A). The expression of RXRa mRNA did not differ between phenotypes, but there was a significant decrease in N and KO animals after CR treatment (p < .029 and p < .0009, respectively) with a striking decrease in KO-CR mice in comparison with the N-CR mice (p < .0001) (Figure 3B). RXRδ was expressed at the same level in normal and KO mice, but CR down-regulated mRNA level in N and KO as compared to the corresponding AL controls (p < .0357 and p < .0052, respectively). This effect was more pronounced in KO than in N mice (p < .0007) (Figure 3C).

**DISCUSSION**

In this study we analyzed expression of PPARs and RXR genes in the skeletal muscle of normal and long-lived GHR-KO mice in combination with long-term CR. Consistent with GH resistance, as a consequence of disruption of GH receptor, KO animals exhibit significant body mass reduction. We have recently reported that CR produced additional decreases in body weight of KO mice, and also reduced body weight of N-CR animals in comparison to N-AL animals, indicating that these animals were under effective CR (22). Analysis of blood components revealed that circulating IGF1 levels were reduced in normal animals after CR, and were not detectable in KO mice regardless of the diet (22). Blood glucose was significantly decreased in both phenotypes subjected to 30% CR. Insulin levels were reduced in N-CR, KO-AL, and KO-CR mice as compared to N-AL mice (22).

According to HOMA analysis, KO animals are characterized by having higher insulin sensitivity than their normal siblings, which agrees with the results of an insulin...
tolerance test performed on GHR-KO and normal mice by Liu and colleagues (25). There was also a significant increase in insulin sensitivity after CR in both normal animals and in insulin-hypersensitive KO mice.

Elevated plasma level of FFAs can impair insulin action, constituting a risk factor for the development of type 2 diabetes (26). Our findings show increased levels of FFA in plasma and muscle homogenate in both phenotypes after CR, which could lead to insulin resistance. However, CR is known to increase insulin sensitivity in normal and KO mice as shown by the HOMA test. Perhaps the reduced dietary intake of fat inherent to the CR condition is associated with limited storage of fat. Additionally, it is possible that most of the fat consumed may be used as an energy source after the daily allotment of food has been consumed. Due to the lower levels of blood glucose in CR animals, higher levels of fat usage in these animals may represent a compensatory mechanism. Lower fat intake and higher FFA levels in plasma and muscle homogenate of CR animals are likely related to decreased triglyceride levels in plasma and muscle because, under these conditions, the storage of FFA as triglycerides in the plasma and muscle of these animals is expected to be reduced. This finding may suggest that, in contrast to AL mice, the animals subjected to CR do not produce energy from stored fat, but preferentially use fatty acids freshly obtained from food and present in peripheral circulation. Under this condition the fatty acid oxidation has to be accommodated to the needs of the CR animals to keep the balance between FFA oxidation and energy usage when food is not available.

Cholesterol level was significantly affected by genotype, and a decrease in total cholesterol plasma levels in KO in comparison to normal animals confirms our previous findings (27). CR reduced cholesterol level in normal mice to the levels measured in KO animals. However there was no effect of CR on cholesterol in KO animals, which may suggest that these long-lived animals have optimal “healthy” levels of cholesterol and defend it from a further decrease.

PPARγ is involved in the regulation of energy, lipid, and glucose homeostasis. Because PPARγ is known to increase insulin sensitivity, and is the target for TZD treatment in type 2 diabetics (10–13), we were particularly interested in the possible changes of its expression in the long-lived GHR-KO mice which are hypersensitive to insulin. Our data on PPARγ mRNA expression showed no effects of the phenotype, but unexpectedly the PPARγ protein levels were down-regulated in these highly insulin-sensitive KO animals as compared to their normal siblings. It was also surprising that PPARγ expression was down-regulated in both phenotypes after CR at the mRNA level with the protein levels showing a similar trend. However, in muscle-specific PPARγ KO mice, disruption of PPARγ does not cause insulin resistance in muscle, but causes whole-body insulin resistance (14). Relating this finding to our results concerning PPARγ expression is difficult because long-lived GHR-KO mice are highly sensitive to insulin and CR treatment also increases insulin sensitivity, which was confirmed using HOMA analysis. Moreover, muscle-specific PPARγ KO mice were reported to respond normally to the insulin-sensitizing action of rosiglitazone. These data imply that PPARγ in muscle plays an important role in the regulation of whole-body responsiveness to insulin, but may not be necessary for the antidiabetic actions of TZDs (14). On the basis of this information, we suggest that whole-body insulin sensitivity in the GHR-KO mice may be regulated independently from muscle PPARγ, as in the case of TZD treatment of PPARγ(--)/c mice. Similarly to PPARγ-independent regulation of responsiveness to insulin in the muscle by rosiglitazone treatment, we suspect that CR may stimulate additional pathways to increase insulin sensitivity. This suspicion may suggest existence of yet to be discovered mechanisms of CR action.

PPARα is known to modulate the oxidation of fatty acids (15). The expression of PPARα mRNA did not differ between N-AL and KO-AL animals, whereas the PPARα protein content was lower in KO mice. These data resemble our findings concerning PPARγ. Down-regulation of the PPARα protein in KO mice suggests that these long-lived KO mice may have a naturally lower level of fatty acid oxidation in the muscle. Down-regulation of PPARα mRNA and protein expression by CR in normal and KO mice suggests that fatty acid oxidation may be silenced in the skeletal muscle of these animals. As proposed above, lower levels of blood glucose in CR animals may compensate for a higher level of FFA as a source of energy. However, CR animals have a lower intake of fat in their diet which leads to reduced storage of fat. Perhaps a decrease of PPARα in CR animals slows down fatty acid oxidation, thus increasing the reliance on carbohydrates as the energy source. However, this would lead to a competition for this scarce resource in CR animals. We propose that PPARα may be responsible for preventing the muscle from using all of the available FFA immediately after food intake and thus maintaining a balance between energy availability and energy usage during a fasting period. PPARα is also known to regulate the total cholesterol level. However, reduced levels of plasma cholesterol in KO animals and in normal animals after CR suggest that, in these animals, the action of PPARα as a factor controlling this component may not be required. In contrast, in N-AL animals which have a lower level of cholesterol the relatively greater expression of PPARα may be required for regulation of cholesterol in plasma.

PPARδ is the least explored gene from this family; however, there are some studies that link this receptor to lipid metabolism. In our study, the PPARδ mRNA expression did not differ between KO-AL and N-AL mice, however, similar to other PPARs, the PPARδ protein level was decreased in KO-AL as compared to N-AL mice. This lack of correspondence of the data from western blot and real-time PCR is difficult to explain. It may reflect regulation at the posttranscriptional level, or differential stability of RNA and/or protein in normal and KO mice. Feeding high-fat diets to PPARδ-deficient mice was shown to reduce energy uncoupling and promote obesity (19). This finding suggests that the reduced level of PPARδ protein expression in GHR-KO animals may contribute to increased adiposity of these animals, which is supported by our previous data showing that fat content is significantly higher in KO than in normal animals (2). There was a trend for expression of PPARδ mRNA to be lower in N-CR than in
N-AL mice, whereas PPARδ protein levels were significantly decreased by CR. This finding conflicts with the data presented by Lee and colleagues (28), which indicated that the expression of PPARδ was increased by CR in C57BL/6 mice. This difference could be due to using much older animals, a different method of analysis, a smaller number of animals per group, a different protocol for CR, and/or analyzing gastrocnemius muscle instead of all hind-limb muscles (which were used in the present study because of the small size of GHR-KO animals). CR reduced PPARδ expression in KO mice at both the mRNA and protein levels. However, it is well known that CR promotes fat depletion and prevents obesity. Results of studies in PPARδ-deficient mice on a high-fat diet would predict that reduced levels of PPARδ in the muscles of N-CR and KO-CR mice should increase lipid accumulation and promote obesity. However, reduced dietary fat intake in CR animals may alter these relationships. We suspect that CR may act to silence the pathway of lipid metabolism and accommodate it to the circumstances of restricted food intake. Reduced lipid metabolism in CR animals prevents disruption of fatty acid homeostasis.

We also analyzed mRNA expression of all three RXR isoforms: RXRγ, RXRα, and RXRβ. These receptors play a very important role in PPAR-dependent gene activation by forming PPAR/RXR heterodimers (20). Real-time PCR analysis of the expression of RXRs revealed apparent correlations with the changes in the expression of PPAR genes. This argues against the possibility that decreased expression of PPARs was compensated for by easier access to RXR and supports our conclusions concerning the expression of PPARs.

Summary

The present study of the PPAR family genes indicates that CR affects the molecular pathway of fatty acid metabolism in muscle. Involvement of PPARs in mediating the effects of CR raises the intriguing possibility of devising CR mimetics based on enhancing or silencing their action.

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