Developmental and tissue-specific involvement of PPARα in the control of mouse UCP3 gene expression

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ABSTRACT

The impact of PPARα gene targeted deletion on UCP3 gene expression depends on the tissue and developmental stage. In adults, UCP3 mRNA expression is unaltered in skeletal muscle from PPARα–knockout (PPARα-KO) mice both in basal conditions and under the stimulus of starvation. In fact, this occurs despite increased levels of free fatty acids due to fasting in PPARα-KO mice. In contrast, UCP3 mRNA is down regulated in heart both in the fed and fasted conditions. In neonates, PPARα-KO mice show an impairment of UCP3 mRNA expression in skeletal muscle in response to the initiation of milk intake, and this is not due to reduced free fatty acid levels. The murine UCP3 promoter is activated by fatty acids through either PPARα or PPARδ, but not by PPARγ or RXR. However, changes in PPARδ gene expression in skeletal muscle respect to heart, during development or in response to the lack of PPARα are not consistent with the extent of compensation in UCP3 gene expression. Although PPARδ-dependent activation could be a potential compensatory mechanism to ensure appropriate expression of UCP3 gene in skeletal muscle in the absence of PPARα, other mechanisms of transcriptional regulation of the UCP3 gene by fatty acids cannot be excluded.

INTRODUCTION

UCP3 is a mitochondrial protein similar to the thermogenic UCP1 protein present in brown adipose tissue. In rodents, UCP3 is expressed preferentially in skeletal muscle, brown fat and to a minor extent in heart and white adipose tissue (1). Although this protein appears to be capable of lowering mitochondrial membrane potential, its precise biological function is a matter of debate. Various reports suggest that it can play a role in mitochondrial fatty acid metabolism (2) as well as in the control of mitochondrial reactive oxygen species production (3). In any case, UCP3 appears to be involved in the regulation of biological processes associated to mitochondrial energy metabolism and, accordingly, gene expression of UCP3 is tightly regulated in physiological situations associated with major changes in fuel metabolism, such as the early neonatal period (4).

Birth leads to a major change in nutrient utilization for energy metabolism. In rodents, there is a sudden change after delivery from a glucose-based fetal nutrition to a lipid-based diet coming from milk intake. Thus, the metabolic adaptation to birth requires the induction of hepatic gluconeogenesis to maintain glycemia and the activation of lipid oxidation in most tissues in order to use this fuel for energy metabolism. In extra-hepatic tissues such as skeletal muscle or heart, fatty acids, together with ketone bodies, increase dramatically their use as fuel supply instead of glucose (5). These metabolic events, which resemble those elicited by fasting in adults, are induced in neonates by the fed state, as they result from the sudden imbalance between glucose and lipid availability in the transition from the fetal to the neonatal period.

The UCP3 gene is almost no expressed in the fetal period and it is dramatically induced after birth under the stimulus of the rise of circulating free fatty acids due to the initiation of milk intake (6). In fact, free fatty acid levels determine UCP3 gene expression in skeletal muscle regardless of their origin and the feeding status of mice. This is exemplified in adult rodents, in which it is fasting what up-regulates UCP3 mRNA expression in response to the rise in circulating free fatty acids, in this case coming from lipolysis of stored triacylglycerols in white adipose tissue (7).

Acute treatment of adults or neonates with fibrates, drugs capable of activating either PPARα or PPARα and PPARδ, were shown to mimic the effects of fatty acids on UCP3 gene expression(6;8). Thus, PPARα and/or PPARδ could mediate the effects of fatty acids on human UCP3 gene transcription. Studies on the transcriptional control of the human UCP3 gene established that both PPARα and PPARδ were able to activate UCP3 gene transcription and to mediate fatty acid effects through a PPRE in the proximal region of the promoter (9). However, PPARα was always more powerful than PPARδ in trans-activating the human UCP3 gene promoter and, in rodents, Wy14,643, a PPARα-specific activator, was also capable of maximal induction of UCP3 mRNA expression (6;8). This led to the proposal that PPARα could be the preferential mediator of UCP3 gene regulation in response to fatty acids.

Mice with targeted disruption of PPARα provide a unique tool to establish the involvement of this nuclear receptor in the control of UCP3 gene expression "in vivo" in response to fatty acids. Previous reports have shown that the responsiveness of the UCP3 gene to be induced by fasting in adults is unaltered in skeletal muscle of PPARα-KO mice but it is down-regulated in heart (10). In the present study we report that PPARα is required for the induction of UCP3 gene in response to milk intake both in skeletal muscle and

heart from neonatal mice. This indicates that compensatory mechanisms to mediate fatty acid-dependent responsiveness of the UCP3 are activated during skeletal muscle development whereas they are never capable to compensate for the absence of PPARα in heart. The capacity of PPARδ to mediate fatty acid effects on the mouse UCP3 gene promoter can provide such a compensatory mechanism.

MATERIALS AND METHODS

Materials. Oleic acid, Wy14,643 (pirinixic acid), phytanic acid, GW501516 and bezafibrate were obtained from Sigma. Rosiglitazone and AGN 194204 were a kind gift from Dr.L.Casteilla (University of Toulouse, France) and Dr. R. Chandraratna (Allergan, Irvine, CA, USA), respectively.

Animals. The care and use of mice were in accordance with the European Community Council Directive 86/609/EEC and approved by the *Comitè Ètic d'Experimentació Animal of the University of Barcelona*. For studies in PPARα-null mice, heterozygous females carrying the corresponding targeted deletion (11) were mated with heterozygous males, and the day of gestation was determined by the presence of vaginal plugs. For studies in neonates, pups were studied at birth (considered to be the time at which pups had been born but had not yet started suckling) and 8h and 16h after birth. In adult mice, the effects of fasting were determined by food withdrawal for 30 h. Mice were killed by decapitation and blood was collected into heparinized tubes and centrifuged to obtain plasma. Hearts and whole muscle from the leg in neonates and gastrocnemius muscle from adult mice were extracted and frozen in liquid nitrogen. Fed and fasted animals, as well as wild-type compared with homozygous gene-disrupted mice, were taken from the same litter in each experiment and at least three different litters per experiment were analyzed.

Quantitative real-time RT-PCR. Total RNA was extracted from heart and skeletal muscle using Tripure (Roche). The quality and concentration of the RNA was determined by measuring the absorbance at 260 and 280 nm,

and the RNA integrity was confirmed by electrophoresis in agarose/MOPS gel. The RNA was treated with DNase I to remove genomic DNA (DNA-free, Ambion). Reverse transcription was performed in 20 μl, using random hexamer primers (Applied Biosystems) and 0.5 μg RNA. PCR reactions were conducted in duplicate for increased accuracy. 25 μl of reaction mixture contained 1 μl of cDNA, 12.5 μl of TaqMan Universal PCR Master Mix (Applied Biosystems), 250 nM probes and 900 nM primers from Assays-on-Demand Gene Expression Assay Mix (UCP3 and 18S rRNA) or Assays-by-design Gene Expression Assay Mix (PPARδ) (Applied Biosystems). The amplification was performed as follows: 2 min at 50°C, 10 min at 95°C, then 40 cycles each at 95°C for 15 sec and 60°C for 60 sec in the ABI/Prism 7700 Sequence Detector System. After PCR was completed, baseline and threshold values were set to optimize the amplification plot, and the data were exported to an Excel spreadsheet.

Plasma metabolites. Serum free-fatty acid levels were quantified using a colorimetric acylCoA synthase and acylCoA oxydase-based method (NEFA C, Wako Chemicals, Neuss, Germany). Serum glucose levels were determined with a glucose oxidase-based test (Accutrend, Roche). Serum β-hydroxybutyrate levels were quantified using a spectrophotometric β-hydroxybutyrate dehydrogenase-based assay (Sigma).

Construction of transfection plasmids. A fragment of the mouse UCP3 gene was amplified using 200ng of mouse genomic DNA. The complementary 3' primer corresponded to bases from +60 to +35 downstream to the transcription initiation site, according to GenBank/EMBL data (Accession AB011070). The complementary 3' primer corresponded to –1946 to

–1924. The 3' and 5' complementary primers generated included 6 base pair non-complementary extensions capable of generating *Kpn*I and *Hind*III restriction sites. Reaction was performed using Expand Long Term PCR System (Roche) in 50 μl final volume containing 15 pm of each primer, 350 μM each dNTP, 1,75 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase. Ten cycles were performed at 94°C for 10 sec, 60°C for 30 sec, and 68°C for 2 min and twenty cycles were performed at 94°C for 10 sec, 60°C for 30 sec, and 68°C for 2 min 30 sec. The resulting DNA product of ~2 kb was digested with *Kpn*I and *Hind* III, purified and ligated into pGL3-basic (Promega) (opened by *Kpn*I and *Hind*III), which contains the cDNA for firefly (*Photinus pyralis*) luciferase as a reporter gene. The whole fragment was sequenced by the dideoxy method using flanking and internal oligonucleotides. The insertion of the fragment from –1946 to +60 of the mouse UCP3 gene into pGL3-basic generates –1946mUCP3-Luc.

Cell culture and transient transfection assays. Myoblastic L6 cells were obtained from American Type Culture Collection (Rockville,MD) and were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS). Transfection experiments were carried out in L6 cells at 50% confluence by using FuGene6 Transfection Reagent (Roche) and were performed according to manufacturer's instructions. For L6 transfection each point was assayed in triplicate in a 6-well plate and contained 1.5 μg of luciferase reporter vector, 0.3 μg of the mammalian expression vectors pCMV-MyoD (12), pRSV-RXRα, pSG5-PPARα, pSG5-PPARδ, pSG5-PPARγ (13-15), and 3 ng of pRL-CMV (Promega), an expression vector for the sea pansy (*Renilla reniformis*) luciferase used as an internal transfection control. Cells were incubated for 48 h after transfection and when indicated cells were treated for

24 h prior to harvest with or without 10 μM Wy14,643, 100 μM bezafibrate, 1 μM GW501516, 1 μM AGN194204, 30 μM phytanic acid, 10 μM rosiglitazone and 500 μM oleic acid.

Firefly luciferase and Renilla luciferase activities were measured in a Turner Designs Luminometer (model TD20/20) using the Dual Luciferase Reporter assay system kit (Promega). Homogenates from cells were prepared with 500 μl of PLB (passive lysis buffer, Promega). Cells were lysed in agitation for 15 min. 20 μl of homogenate was used for measurement. Luciferase activity elicited by UCP3 promoter constructs was normalized for variation in transfection efficiency using Renilla luciferase as an internal standard.

Statistical analysis. Where appropriate, statistical analysis was performed by Student's t test; significance is indicated in the text.

RESULTS AND DISCUSSION

Differential regulation of UCP3 mRNA in heart and skeletal muscle in adult wild-type and PPARα-KO mice. To analyze the effects of the lack of expression of PPARα in skeletal muscle on the regulation of UCP3 gene, UCP3 mRNA levels were measured in gastrocnemius skeletal muscle in adult PPARα-KO mice in comparison with their control littermates (Fig 1A). The results showed that under fed conditions, UCP3 mRNA levels were similar between both genotypes. UCP3 mRNA response to 30h of starvation was also similar in control and PPARα-KO mice, showing an increase in the mRNA levels of about 5-fold. Also in tibialis anterior or soleus muscle tissues the increase due to starvation was not impaired (Amat, Rosell and Iglesias, data not shown). In the same experimental conditions UCP3 mRNA levels were analyzed in heart and the results showed a significant reduction in PPARα-KO respect to wild-type mice (Fig 1B). In the fed condition, UCP3 mRNA was 40% reduced in KO mice compared to controls. Also when animals were starved for 30h, the levels of UCP3 in heart increased more than 7-fold in controls whereas in PPARα-KO mice the induction was significantly lower respect to fed PPARα-KO mice (around 5-fold) and the total amount of UCP3 mRNA was just 30% of the levels reached in the heart of the starved control littermates. These findings are similar other reports (10) and had been interpreted either as indicating a minor role for PPARα in the control of skeletal muscle UCP3 gene expression "in vivo" or as evidence of the existence of compensatory mechanisms acting preferentially in muscle to maintain normal regulation of UCP3 gene expression.

To analyze the metabolites related to energy metabolism in these mice, glucose, free fatty acids (FFA) and ketone bodies (β-hydroxybutyrate) were checked in plasma of fed and fasted conditions (Table I). Although glucose levels were similar in wild-type and PPARα-KO mice in fed conditions and the levels of glucose decrease in both genotypes due to starvation, in PPARα-KO mice the levels of glucose were statistically lower than in wild-type. The levels of FFA were similar under fed conditions but in response to starvation, FFA from PPARα-KO were significantly higher than in wild-type. The analysis of β-hydroxybutyrate levels showed no differences in fed conditions between wild-type and PPARα-KO mice and, as expected, an increase in the levels of this metabolite due to starvation was observed. Nevertheless, this response was highly impaired in PPARα-KO mice being 60% less than in their wild-type littermates. The pattern of alterations in the metabolic response to starvation in wild-type and PPARα-KO mice is in agreement with the first characterization of the phenotype of these mice (16;17). However, as the increase in FFA concentration is considered the signal inducer for UCP3 mRNA expression in response to fasting in muscle, it is noteworthy that the higher FFA induction observed in fasted PPARα-KO mice results just in an equal induction of UCP3 mRNA respect to wild-type. Previous data in rodents at different physiological and experimental situations indicated a very close correlation between the levels of circulating FFAs and skeletal muscle UCP3 mRNA levels (4). The differential relationship between FFAs and UCP3 mRNA levels in PPARα-KO mice may indicate that there is some extent of impairment or "resistance" in the responsiveness of UCP3 gene expression to fatty acids due to the lack of PPARα.

UCP3 mRNA expression depends on PPARα during perinatal period in skeletal muscle and heart. To study the PPARα-dependent UCP3 regulation in the perinatal period, hindlimb muscle and heart were obtained from wild-type and PPARα-KO mice just after birth (0h, no suckling), and 8 and 16 hours after birth, with pups being suckling (Fig 2A). As expected the levels of UCP3 mRNA in skeletal muscle of wild-type mice increased rapidly after birth, 8-fold after 8 hours, and remain high 16 hours later. In PPARα-KO mice, UCP3 mRNA levels increased at a lower extent: they were just two-fold and 3-fold 8h and 16h after birth, respectively. The regulation of UCP3 by PPARα in heart was similar to adults (fig 2B). In wild-type mice, 16 hours after birth, UCP3 mRNA levels increased 5-fold respect to pups at birth. In the PPARα-KO the increase observed on UCP3 mRNA at 16 hours was just 2-folds, significantly lower than in wild-type. These results indicate a major impact of the lack of PPARα in skeletal muscle of neonatal mice respect to adults whereas the behavior of UCP3 gene expression in heart was similarly impaired as in adult PPARα-KO mice.

The levels of glucose, FFA and ketone bodies were also measured in plasma of wild-type and PPARα-KO mice 16 hours after birth (Table II). Glucose and FFA levels did not show any statistically difference between both groups of mice whereas the levels of the β-hydroxybutyrate showed a remarkable 75% reduction in PPARα-KO respect to wild-type pups. Unaltered levels of FFAs in PPARα-KO mice indicate that there is no impairment in their suckling behavior, as FFAs in neonatal blood depend on milk intake. Thus, lowered UCP3 mRNA in PPARα-KO pups could not caused by the exposure of skeletal muscle and heart to lower levels of circulating FFAs and should be due to a lack of responsiveness of the tissues. Similarly to adults, lowered

ketone bodies are indicative of reduced hepatic oxidation of FAs, in this case coming from milk intake. This is in agreement with the reported impairment in gene expression for ketogenic enzymes in perinatal liver of PPARα-KO mice (18).

PPARα and PPARδ can mediate fatty acid-dependent activation of mouse UCP3 gene promoter. We undertake the analysis of the responsiveness of the UCP3 gene transcription to fatty acids and the involvement of PPARα respect to other nuclear receptors. For this purpose, 2 kb of the mouse UCP3 promoter was fused to luciferase reporter gene (2mUCP3-Luc) and transient transfection assays were performed in L6E9 rat muscle cell line. Results obtained from these experiments showed that, in the absence of co-transfected MyoD, mouse UCP3 promoter showed a very low activation and was not sensitive to induction neither by fatty acids nor PPARα or PPARδ agonists. Nevertheless, in the presence of MyoD, the basal activity of the promoter increased by 15-fold and the response of the promoter to induction by fatty acids was observed. In the presence of MyoD, induction of UCP3 mouse promoter by oleic acid was observed when PPARα or PPARδ but not PPARγ was co-transfected. In addition, in the presence of PPARα, treatment of the cells with PPARα agonists (Wy 14,643 and bezafibrate) increased the activity of the promoter similarly of what is observed for oleic acid. Also, the addition of PPARδ agonists (GW 501516 or bezafibrate), when co-transfected with PPARδ, increased the activity of the promoter significantly. Since the induction by fatty acids could be mediated by RXR (19), parallel experiments were performed co-transfecting this nuclear receptor and treating the cells with oleic acid and also with RXR agonists (phytanic acid and AGN 194204). The specific RXR ligand AGN 194204 caused a small but significant induction of

the UCP3 promoter. No statistically significant effects due to oleic or phytanic acid were observed, indicating that the RXR pathway is not mediating mouse UCP3 regulation in response to fatty acids, at least “in vitro”.

The present findings indicate that the mouse UCP3 gene promoter showed a similar behavior respect to the human promoter (9): it is dependent on MyoD and responsiveness to fatty acids can be mediated by PPARα or PPARδ. In fact, despite the large divergence in overall promoter sequence (20), the one base-spaced direct repeat identified in the human UCP3 promoter to mediate fatty acid effects through PPARs is completely conserved in the mouse proximal promoter region at –51/-38.

Gene expression of PPARδ in PPARα-KO and wild-type mice. Results above suggest that the sensitivity of the UCP3 gene transcription to PPARδ can constitute a major mechanism for compensation of the lack of PPARα and that this mechanism is never operational in heart but it is enough for compensating UCP3 mRNA expression at least in adult skeletal muscle. PPARδ gene expression studies were undertaken in wild-type and PPARα-KO mice in the situations studied before (Fig 4). PPARδ mRNA levels in skeletal muscle were not modified in PPARα-KO mice respect to wild-type neither in newborns nor in adults in the fed condition. Starvation caused an increase in skeletal muscle PPARδ mRNA levels in wild-type mice, in agreement with previous reports (21). However, this induction was lower in PPARα-KO mice. In newborns there was a significant reduction in skeletal muscle PPARδ mRNA levels 16h after birth, as already reported (9), and this reduction was also observed in PPARα-KO pups. In heart, PPARδ mRNA levels were not significantly different in wild-type and PPARα-KO adult mice either fed or

fasted. Heart PPARδ mRNA levels in 16h-old pups were lower than in neonates just after birth, but no significant differences were observed between wild-type and PPARα-KO neonates neither at birth nor 16h later.

Present results indicate that the absence of PPARα does not elicit any preferential compensatory up-regulation of PPARδ mRNA expression in skeletal muscle respect to heart or in adult respect to neonatal skeletal muscle.

Overall, these findings indicate that the requirement of PPARα for the regulation of UCP3 gene expression is tissue- and development-specific. In skeletal muscle it appears that compensatory mechanisms of fatty acid-dependent regulation of UCP3 gene transcription, not active in the neonatal period, are present in adults to ensure normal UCP3 mRNA expression when muscle is exposed to high levels of circulating free fatty acids. Although other mechanisms of fatty-acid dependent regulation of transcription cannot be excluded, PPARδ is the most likely candidate to mediate this compensation considering its activity on UCP3 gene promoter regulation in response to fatty acids. However, the performance of these compensatory effects does not require up-regulation of PPARδ mRNA expression in different tissues and developmental stages.

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FIGURE LEGENDS

Figure 1. UCP3 mRNA levels in gastrocnemius skeletal muscle and heart from wild-type and PPARα-KO mice. 8 week old mice were used in basal conditions (feeding) or under 30h of starvation (starvation). Gastrocnemius skeletal muscle or heart were isolated and UCP3 gene expression was analyzed by real time PCR as described in materials and methods. Bars indicate the mean ± SEM of 6 to 10 mice per group coming from 3 different litters. Significant differences between feeding and starvation are shown by * $p<0.05$ and ** $p<0.001$. Comparison between wild-type respect to PPARα-KO by # $p<0.05$.

Figure 2. UCP3 mRNA levels in skeletal muscle and heart from control and PPARα-KO mice just after birth (0h, no feeding) or 8 and 16 hours after birth. Quantitative RT-PCR was performed to assess the amount of UCP3 from total mRNA from hindlimb or heart of neonates. Results are the mean ± SEM of 4 to 6 mice per group coming from at least 3 different litters. Significant differences between wild-type and PPARα-KO mice are shown by * $p<0.05$ and ** $p<0.001$. Comparison between wild-type respect to PPARα-KO mice is shown by # $p<0.05$.

Figure 3. Transient transfection of 2mUCP3-Luc performed in L6E9 rat skeletal muscle cell line. A construct containing 2 kb of the promoter region of mouse UCP3 gene was fused to luciferase reporter gene. Experiments were performed as indicated in Materials and Methods. When indicated 0.3 μg of MyoD expression vector was co-transfected and also, when indicated, with 0.3 μg of PPARα, δ or γ and RXRα expression vectors. All treatments with the different agonists were performed for 24 h. Results are the mean ± SEM of at

least three independent experiments performed in triplicate. All samples co-transfected with MyoD-expression vectors show significantly higher differences than non co-transfected ones ($p<0.001$). Significant differences due to addition of the agonists respect to their relative controls are shown as ♦ $p<0.05$ or ♦♦ $p<0.01$.

Figure 4. Relative amount of PPARδ mRNA in skeletal muscle and heart in newborn and adults of wild-type and PPARα–KO mice. Bars indicate the mean ± SEM of 4 to 10 mice per group coming from at least 3 different litters. Significant differences between fed and fasted are shown by * $p<0.05$.

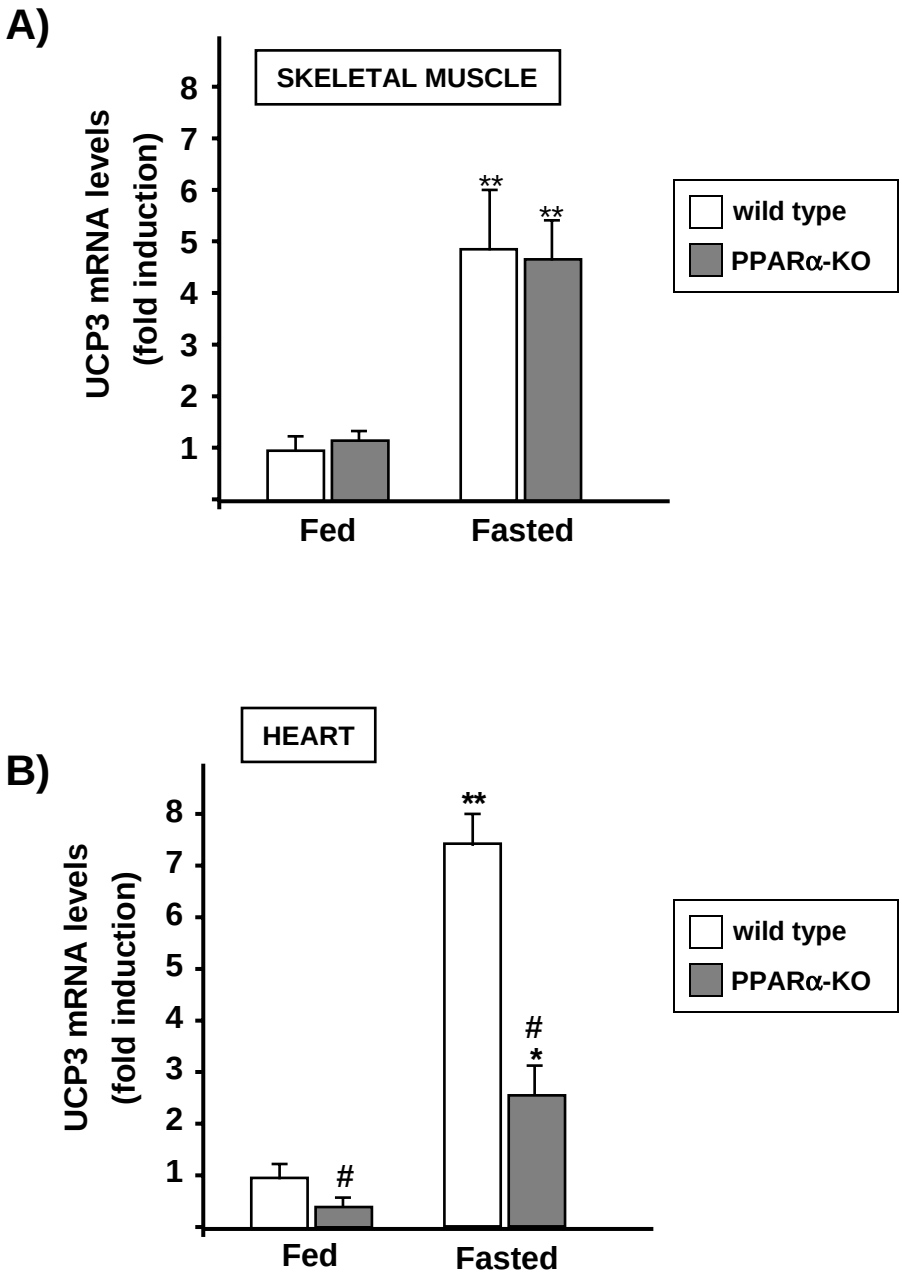
	FED		FASTED	
	wild type	PPARα-KO	wild type	PPARα-KO
Glucose (mM)	9,8±1,1	8,5±1	4,2 ±0,6**	2,8 ± 0,3 ** #
FFA (μM)	650±85	770±20	825±27 *	1558±102 ** #
β-hydroxybutyrate (mM)	0,2±0,01	0,2±0,04	1,2±0,1 *	0,39±0,14 #

Table I. Plasma levels of glucose, free fatty acids and ketone bodies from wild type and PPARα-KO mice in basal conditions and under 30h of fasting. Results indicate the mean ± SEM of 6 to 10 samples per group. Significant differences between feeding and fasting are shown by * $p<0.05$ and ** $p<0.001$. Comparison between wild type and PPARα-KO by # $p<0.05$.

	16 hours	
	wild type	PPARα–KO
Glucose (mM)	1,45 ± 0,42	1,31 ± 0,43
FFA (μM)	746 ± 103	946 ± 245
β-hydroxybutyrate (mM)	0,70 ± 0,38	0,18 ± 0,09 **

Table II. Plasma levels of glucose, free fatty acids and ketone bodies from wild type and PPARα–KO mice 16 hours after birth, allowing them to suck. Results indicate the mean ± SEM of 4 to 6 samples per group. Significant differences between wild type and PPARα-KO are shown by ** $p<0.001$.

Fig. 1


A)
SKELETAL MUSCLE
UCP3 mRNA levels
(fold induction)
8
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UCP3 mRNA levels
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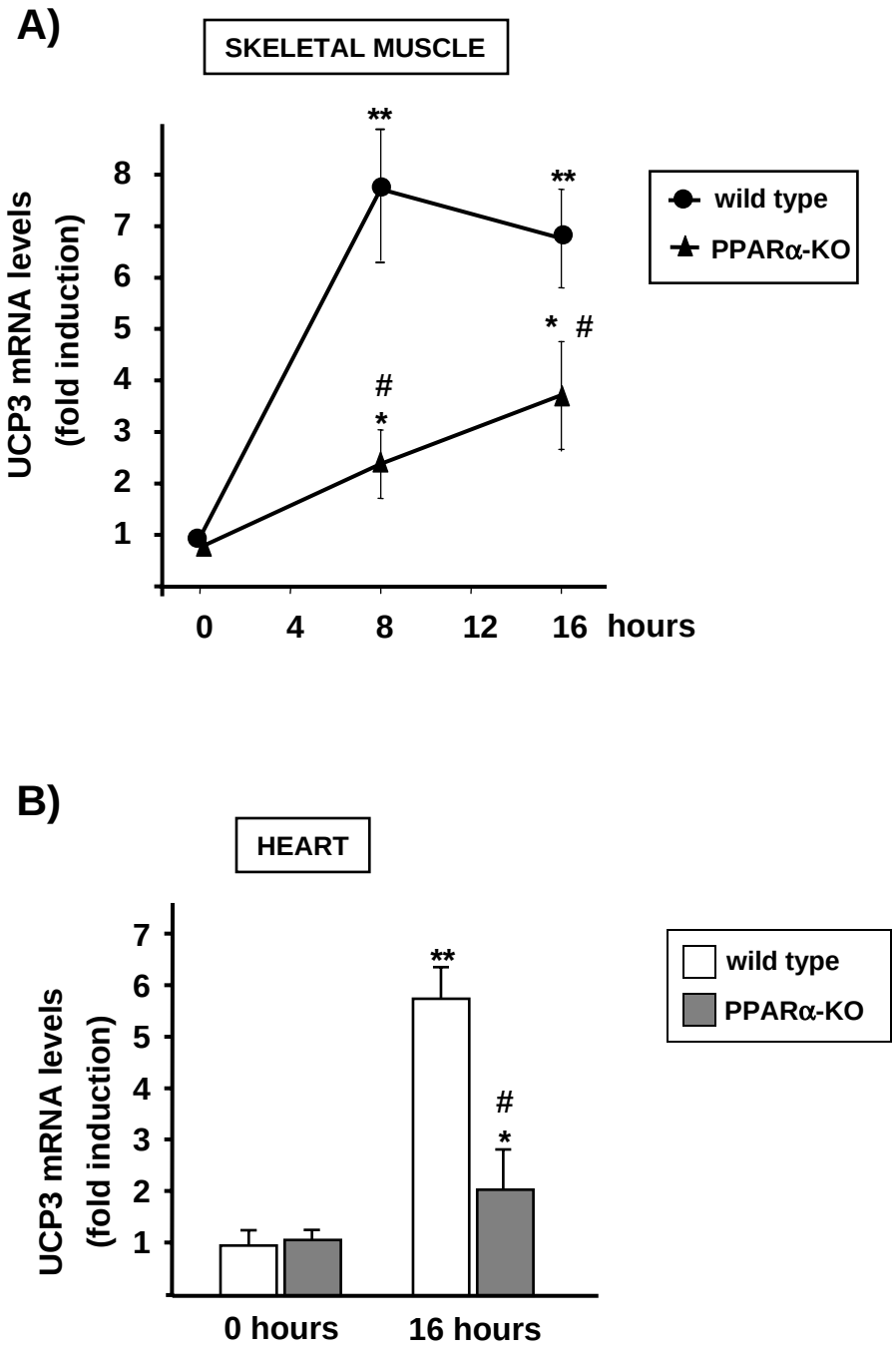
Fig. 2


A)
SKELETAL MUSCLE
UCP3 mRNA levels (fold induction)
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12
16
hours
B)
HEART
UCP3 mRNA levels (fold induction)
1
2
3
4
5
6
7
**
*
wild type
PPARα-KO
0 hours
16 hours

Fig. 3

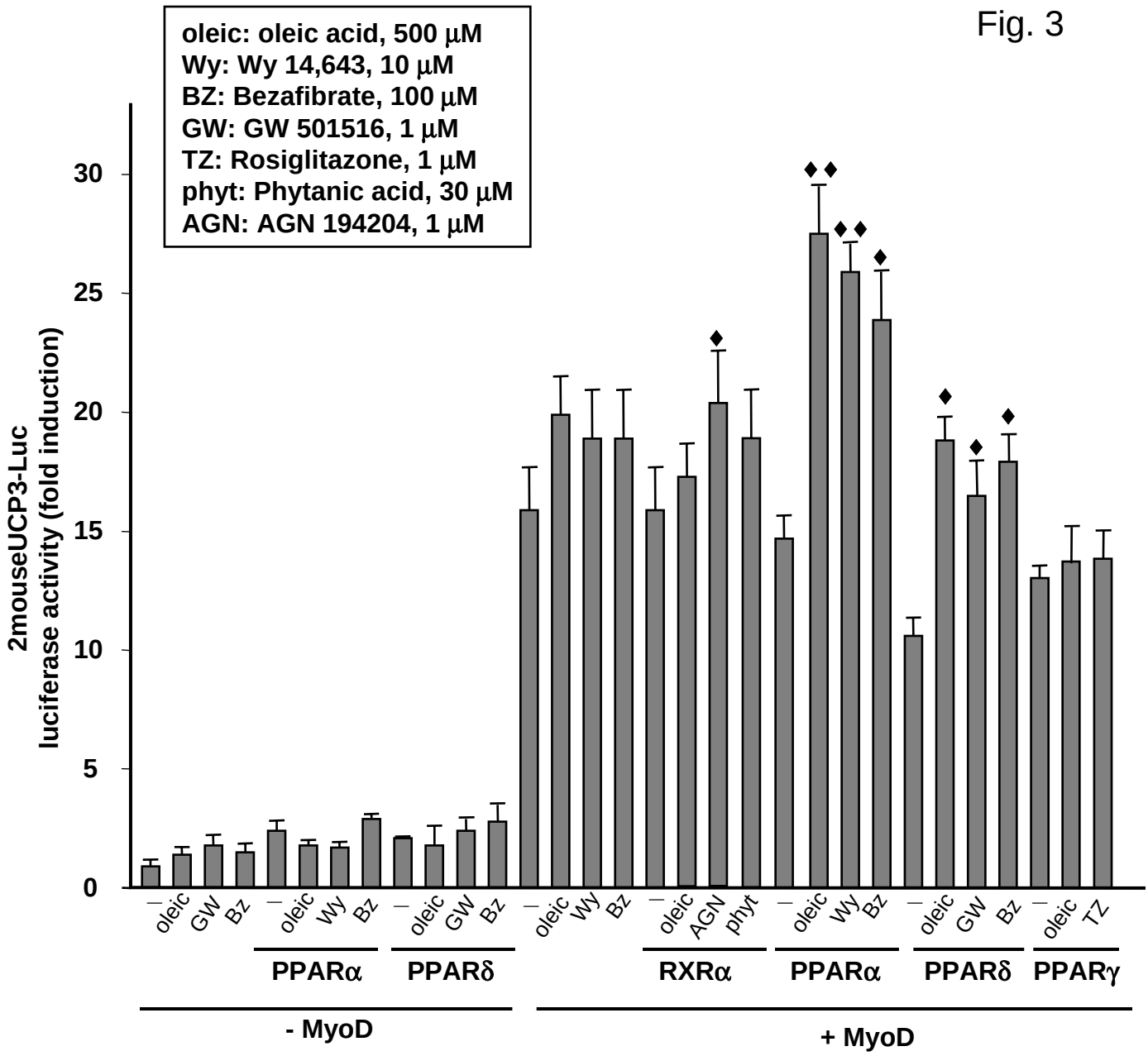
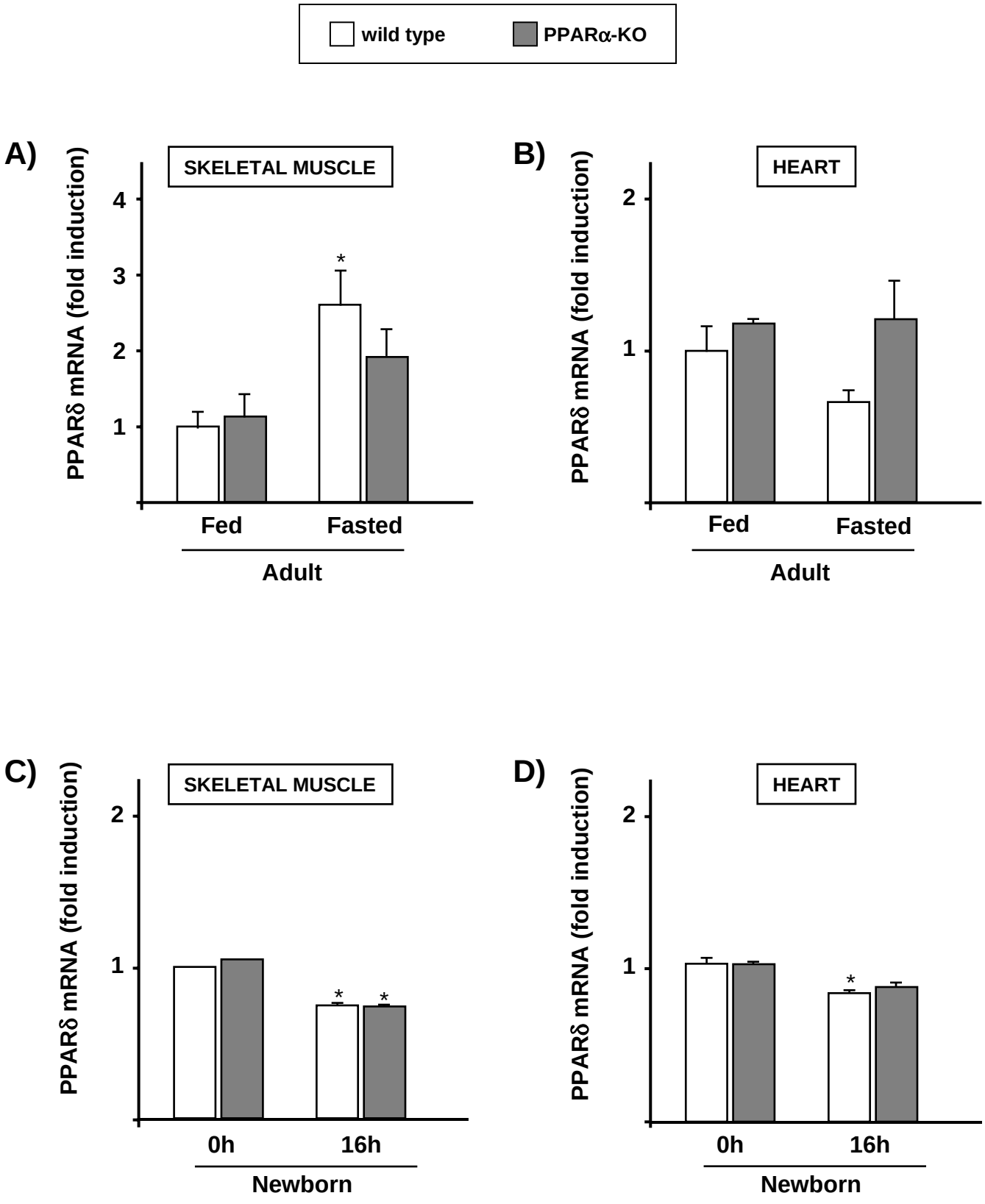

oleic: oleic acid, 500 μM
Wy: Wy 14,643, 10 μM
BZ: Bezafibrate, 100 μM
GW: GW 501516, 1 μM
TZ: Rosiglitazone, 1 μM
phyt: Phytanic acid, 30 μM
AGN: AGN 194204, 1 μM
2mouseUCP3-Luc luciferase activity (fold induction)
0
5
10
15
20
25
30
—
oleic
GW
Bz
—
oleic
Wy
Bz
—
oleic
GW
Bz
—
oleic
Wy
Bz
—
oleic
AGN
phyt
—
oleic
Wy
Bz
—
oleic
GW
Bz
—
oleic
TZ
PPARα
PPARδ
RXRα
PPARα
PPARδ
PPARγ
- MyoD
+ MyoD

Fig. 4


wild type
PPARα-KO
A)
SKELETAL MUSCLE
PPARδ mRNA (fold induction)
4
3
2
1
*
Fed
Fasted
Adult
B)
HEART
PPARδ mRNA (fold induction)
2
1
Fed
Fasted
Adult
C)
SKELETAL MUSCLE
PPARδ mRNA (fold induction)
2
1
*
*
0h
16h
Newborn
D)
HEART
PPARδ mRNA (fold induction)
2
1
*
0h
16h
Newborn