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Peroxisome proliferator-activated receptor-α modulates insulin gene transcription factors and inflammation in adipose tissues in mice

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Abstract We have recently reported that PPARα deficiency leads to hypoglycaemia and hypoinsulinemia in mice (Yessoufou et al. Endocrinology 147:4410–4418, 2006). Besides, these mice exhibited high adiposity with an inflammatory state. We, therefore, assessed, in this study, the effects of PPARα deficiency on the expression of mRNA encoding for the insulin gene transcription factors in pancreatic β-cells along with those implicated in inflammation in adipose tissues. On fasting, the adult PPARα-null mice were hypoglycemic. Serum insulin concentrations and its pancreatic mRNA transcripts were downregulated in PPARα-null mice, suggesting that PPARα gene deletion contributes to low insulin gene transcription. The PPARα gene deletion downregulates the mRNA expression of insulin gene transcription factors, i.e., Pdx-1, Nkx6.1, and MafA. Besides, the pancreatic function was diminished by PPARα deficiency as PPARα-null mice expressed low pancreatic Glut2 and glucokinase mRNA. PPARα-null mice also expressed high adiponectin and leptin mRNA levels compared to wild type animals. Adipose tissues of PPARα-null mice exhibited upregulation of CD14 and CD68 mRNA, generally expressed by macrophages. PPARα gene deletion downregulates the adipocyte mRNA of certain pro-inflammatory agents, like MCP-1, TNF-α, IL-1β, IL-6, and RANTES, though pro-inflammatory TLR-2 and TLR-4 mRNAs were upregulated in the adipose tissues. Our results suggest that PPARα deficiency, in mice, is implicated in the modulation of insulin gene transcription and inflammatory status in adipose tissues.

Keywords PPARα · Insulin · Adipose tissue · Inflammation

Abbreviations

GK	Glucokinase
Nkx6.1	NK6 transcription factor related-locus-1
Pdx-1	Pancreatic and duodenal homeobox-1
PPARα	Peroxisome proliferator-activated receptor-α
MCP-1	Monocyte-chemoattractant protein-1
RANTES	Regulated on activation of normal T cell expressed and secreted
SREBP1c	Sterol response element-binding protein 1c
TG	Triglyceride
TLR	Toll-like receptor
FFA	Free fatty acids
WAT	White adipose tissue
PBS	Phosphate buffered saline
WT	Wild type

Introduction

Peroxisome proliferator-activated receptors (PPARs) play key roles as lipid/nutritional state sensors and transcriptional

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regulators of lipid metabolism [1, 2]. So far, three isotypes of PPARs have been well-identified in lower vertebrates and mammals [2]. PPARα, PPARβ (δ), and PPARγ exhibit different tissue distribution as well as different ligand specificities and functions [3]. PPARα is principally expressed in organs with a high capacity for fatty acid oxidation, e.g., heart, skeletal muscle, liver, and kidney [2, 4]. In a recent study [5], we have reported that, after overnight fasting, PPARα-null mice were hypoglycemic and their serum insulin concentration was lower than that of their counterpart wild type (WT) animals. Besides, PPARα-null mice exhibited a high adiposity and high lipidemia. The offspring of PPARα-null mice were larger than their homologous age-matched WT animals, both at birth and during their growth [5]. Moreover, the adult offspring of WT diabetic dams developed hyperglycemia, hyperinsulinemia, and hyperlipidemia as compared to their corresponding controls. Although the adult offspring of PPARα-null diabetic mice were hyperglycemic and hyperlipidemic, they were, in contrast, hypoinsulinemic as compared to their corresponding controls [5]. All these observations prompted us to conduct the present study, since PPARα has been reported to play a key role in the regulation of insulin resistance and inflammation [6].

It has been generally accepted that glucose stimulates both insulin release, and the transcription of insulin gene and translation of the nascent mRNA in pancreatic β-cells [7–9]. Glucose also enhances the stability of insulin mRNA in rodent β-cells through a mechanism involving the binding of polypyrimidine tract-binding protein to the 3′ untranslated region of the insulin mRNA [10]. Therefore, we examined the expression of mRNA encoding for insulin gene in the pancreas and assessed, in the β-cells, the levels of Glut2 and glucokinase mRNA. For insulin gene transcription, some factors like pancreatic and duodenal homeobox (Pdx)-1, NK6 transcription factor related-locus-1 (Nkx6.1) and MafA are required. Pdx-1 transcriptionally regulates insulin, glucokinase, and islet amyloid polypeptide [11, 12]. Nkx6.1 is important for the terminal differentiation of β-cells [11] and is known to affect glucose-induced insulin secretion [13]. It is noteworthy that Pdx-1 and MafA can exert their positive actions separately on the promoter and their effects are additive [14].

Since adipose tissue, which secrets adipokines, plays crucial role in obesity [15], we determined the mRNA encoding for leptin and adiponectin in adipose tissues. Macrophages have been shown to infiltrate adipose tissues in obesity [16]. We, therefore, detected the expression of mRNA of CD14, CD68, and F4/80 in the adipose tissues of these animals. CD14 has been shown to bind to lipopolysaccharide (LPS) and to interact with Toll-like receptor (TLR)-4 [17], and this spurred us to examine the expression of TLR-2 and TLR-4 mRNA in adipocytes. The inflammatory agents like MCP-1, IL-6, and TNF-α are also known to be secreted by adipocytes [18].

PPARα-null mice are known to develop overweight compared to corresponding WT animals [19]. The status of the formers seems to be a permanent inflammatory state. Since PPARα ligands are known to have immunosuppressive and anti-inflammatory effect, it was thought worthwhile to assess the implication of this receptor in the regulation of the mRNA expression of these inflammatory markers (MCP-1, TNF-α, IL-6, CD14, CD68, TLR-2, and TLR-4) along with that of IL-1β and TCRα, and regulated on activation of normal T cell expressed and secreted (RANTES) and its receptor CCR5 in adipose tissues. Moreover, PPARα is needed to ensure appropriate insulin secretion by pancreatic β-cells [20]. Hence, we determined the pancreatic mRNA of the transcription factors in order to respond the question whether these factors are involved in the β-cell adaptation in response to deletion of PPARα gene.

Materials and methods

Animals, blood, liver, pancreas, and adipose tissue samples

The study was performed on male adult WT and homozygous PPARα-null (PPARα-knockout) mice of C57BL/6J genetic background ([19], the Jackson Laboratory, Bar Harbour, ME, USA). Mice were housed in wood chip-bedded plastic cages at constant temperature (25°C) and humidity (60 ± 5%) with a 12-h light–dark cycle.

After overnight fasting, 15 male mice at age of 3 months, in each group, were anesthetized with pentobarbital (60 mg/kg body weight). The abdominal cavity was opened, and whole blood was drawn from the abdominal aorta. Serum was obtained by low-speed centrifugation (1,000g× 20 min) and immediately used for glucose and lipid determinations. Some aliquots were stored at −80°C for insulin determination. The pancreas, liver, and epididymal white adipose tissues were removed, washed with cold saline and immediately frozen in liquid nitrogen and used for total RNA extraction. The general guidelines for the care and use of laboratory animals, recommended by the council of European Economic Communities, were followed. The experimental protocol was approved by the Regional Ethical Committee.

Glucose and insulin determination

The determination of insulin was performed in samples that were stored at −80°C. Serum insulin was determined using

an ELISA kit (LINCO Research Inc, St. Charles, MO, USA), according to the manufacturer's instructions. Serum glucose was determined by the glucose oxidase method using a glucose analyser (Beckman Instruments, USA).

Determination of serum and liver lipids

Serum triglyceride (TG) content was measured using enzymatic methods. After liver lipid extraction according to the method of Folch et al. [21], liver TG content was measured by using a commercially available kit (Boehringer, France). Free fatty acids (FFA) were separated on silica gel by thin layer chromatography (TLC) using the following solvent: hexane/diethyl ether/acetic acid/methanol at 90:20:2:3 (v/v/v/v). The purified fractions of FFA and TG were quantified by gas liquid chromatography [22] using an internal standard, C17:0 for FFA and TriC15:0 for TG, with a Becker gas chromatograph (Becker instruments, Downers Grove, IL).

Real-time RT-PCR quantification assay

Total RNA was prepared from the liver, pancreas, and adipose tissue using Trizol reagent (Invitrogen Life Technologies, Groningen, The Netherlands) according to the manufacturer's instructions. The integrity of RNA was electrophoretically checked by ethidium bromide staining and by the OD absorption ratio $OD_{260\ nm}/OD_{280\ nm}$ more than 1.9. One microgram of total RNA was reverse transcribed with Superscript II RNAse H-reverse transcriptase using oligo (dT) according to the manufacturer's instructions (Invitrogen Life Technologies, France).

Real-time PCR was performed on an iCycler iQ real-time detection system (Bio-Rad, Hercules, CA, USA), and amplification was done by using SYBR Green I detection (SYBR Green JumpStart, Taq ReadyMix for Quantitative PCR, Sigma-Aldrich, St. Louis, MO, USA). Oligonucleotide primers, used for mRNA analysis, were based on the sequences of mice gene in the GeneBank database. All mice RT-PCR primer sets used to amplify the genes in these studies are presented in Table 1. Forward and reverse primers used to amplify β-actin message in mouse were 5′-AGAGGGAAATCGTGCGTGAC-3′ and 5′-CAATAGT GATGACCTGGCCGT-3′.

Results were evaluated by iCycler iQ software including standard curves, amplification efficiency (E) and threshold cycle (Ct). Relative quantitation of mRNA expression of a large number of signaling factors in different groups was determined using the ΔΔCt method, in which ΔΔCt = ΔCt of gene of interest − ΔCt of β actin. ΔCt = Ct of interest group − Ct of control group. Relative quantity (RQ) was calculated as follows: $RQ = (1 + E)^{(-\Delta\Delta Ct)}$.

Table 1 Gene regions amplified and their corresponding primer sequences used for RT-PCR

Genes amplified	Primer sequences
Mouse RANTES	F: 5′-GCAGTCGTGTTTGTCACTCG-3′
	R: 5′-TAGGACTAGAGCAAGCGATGAC-3′
Mouse CCR5	F: 5′-GCCTAAACCCTGTCATCTATGC-3′
	R: 5′-ATATTTCCCGGCCCTGATAAAAG-3′
Mouse MCP-1	F: 5′-GAGAGCCAGACGGGAGGAAG-3′
	R: 5′-TGAATGAGTAGCAGCAGGTGAG-3′
Mouse CD68	F: 5′-TTCAGGGTGGAAGAAAGGTAAAGC-3′
	R: 5′-CAATGATGAGAGGCAGCAAGAGG-3′
Mouse IL-6	F: 5′-CCGCTATGAAGTTCCTCTCTGC-3′
	R: 5′-ATCCTCTGTGAAGTCTCCTCTCC-3′
Mouse TCRα	F: 5′-CCTCTACAGCAGCGTTCTCATCC-3′
	R: 5′-GGGTAGGTGGCGTTGGTCTCTTTG-3′
Mouse CD14	F: 5′-GCGTGTGCTTGGCTTGTTG-3′
	R: 5′-CAGGGCTCCGAATAGAATCCG-3′
Mouse F4/80	F: 5′-TCCAGCACATCCAGCCAAAGC-3′
	R: 5′-CCTCCACTAGCATCCAGAAGAAGC-3′
Mouse IL-1β	F: 5′-TGTTCTTTGAAGTTGACGGACCC-3′
	R: 5′-TCATCTCGGAGCCTGTAGTGC-3′
Mouse TLR2	F: 5′-CTACAGTGAGCAGGATTCC-3′
	R: 5′-CAGCAAAACAAGGATGGC-3′
Mouse TLR4	F: 5′-GCAGCAGGTGGAATTGTATCG-3′
	R: 5′-GCTTAGCAGCCATGTGTTCC-3′
Mouse TNF-α	F: 5′-CTCTTCTCATTCCTGCTTGTGG-3′
	R: 5′-AATCGGCTGACGGTGTGG-3′
Mouse SREBP-1c	F: 5′-CATCAACAACCAAGACAGTC-3
	R: 5′-CCAGAGAAGCAGAAGAGAAG-3′
Mouse FAT/CD36	F: 5′-TGCTCTCCCTTGATTCTGCTGC-3′
	R: 5′-TTTGCTGCTGTTCTTTGCCACG-3′
Mouse adiponectin	F: 5′-GCCGCTTATGTGTATCGCTCAG-3′
	R: 5′-GCCAGTGCTGCCGTCATAATG-3′
Mouse leptin	F: 5′-ACACACGCAGTCGGTATCC-3′
	R: 5′-GAGTAGAGTGAGGCTTCCAGG-3′
Mouse PDX-1	F: 5′-CTACTGCCTTCGGGCCTTAG-3′
	R: 5′-TTGGAACGCTCAAGTTTGTACC-3′
Mouse glucokinase	F: 5′-AGAAGGCTCAGAAGTTGGAGAC-3′
	R: 5′-GGATGGAATACATCTGGTGTTTCG-3′
Mouse insulin	F: 5-GTGGCTTCTTCTACACACCCAT-3′
	R: 5′-CTCCAGTGCCAAGGTCTGAA-3′
Mouse Glut 2	F: 5′-TGTGGTGTCGCTGTTTGTTG-3′
	R: 5′-AATGAAGTTTGAGGTCCAGTTGG-3′
Mouse C/EPB-β	F: 5′-AGCTGAGCGACGAGTACAAG-3′
	R: 5′-AGCTGCTCCACCTTCTTCTG-3′
Mouse MafA	F: 5′-ATCACTCTGCCCACCATCAC-3′
	R: 5′-CGCCAACTTCTCGTATTTCTCC-3′
Mouse Nkx6-1	F: 5′-GGGTCTTCCTCCTCCTCCTC-3′
	R: 5′-GGTCTGGTGTGTTTTCTCTTCC-3′

Statistical analysis

Results are shown as means ± SEM. The significance of the differences between mean values was determined by two-way ANOVA (STATISTICA, Version 4.1, Statsoft, Paris, France), followed by the least significant difference (LSD) test. Differences were considered significant at $P < 0.05$.

Results

Serum glucose concentration and body weight, and serum insulin concentrations and pancreatic insulin mRNA expression

PPARα-null mice were hypoglycemic compared to WT animals (Fig. 1). PPARα-null mice also gained higher weight than the WT animals. Insulin concentrations were decreased in PPARα-null mice as compared to WT animals. In order to link protein secretion and gene transcription, we determined the expression of insulin mRNA in these animals. We noticed that PPARα-null mice had lower expression of insulin mRNA than WT animals (Fig. 1).

Expression of mRNA of the genes implicated in the transcriptional regulation in the pancreas

In order to gain insight into functional aspects of β-cells, we performed quantitative analysis of β-cell transcription factors. We noticed that the pancreas of PPARα-null mice expressed lower levels of mRNA of Pdx-1, MafA, and Nkx6-1 than WT animals (Fig. 2a). However, C/EBP-β mRNA was slightly expressed in pancreas of WT and PPARα-null animals without any differences in both the strains of mice. We have also investigated the expression of glucokinase (GK) and Glut2 mRNA as they are implicated in the transcriptional regulation in β-cells by Pdx-1. The expression of Glut2 and GK mRNA was downregulated

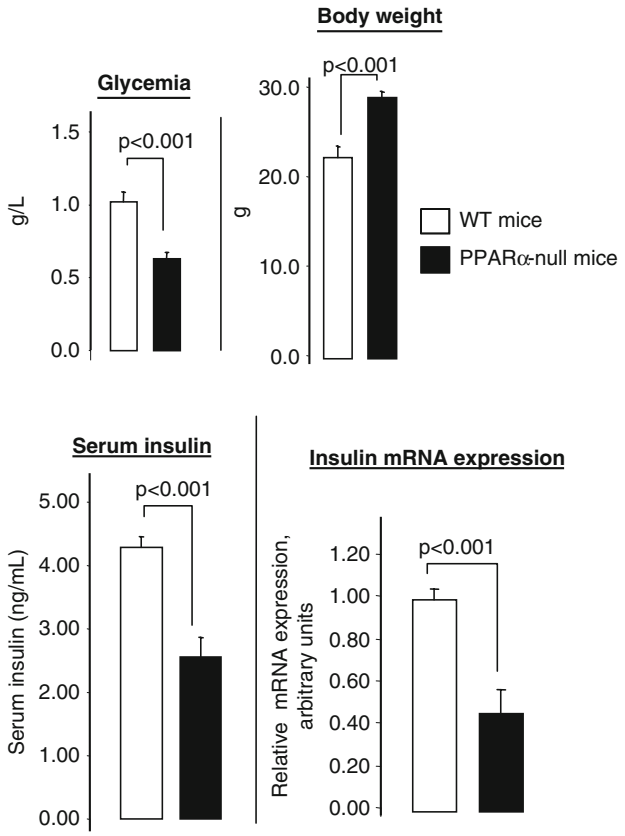

Fig. 1 Serum glycemia and body weight, and serum insulin concentration and insulin mRNA expression in the pancreas of wild type and PPARα-null mice. The parameters were determined as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA

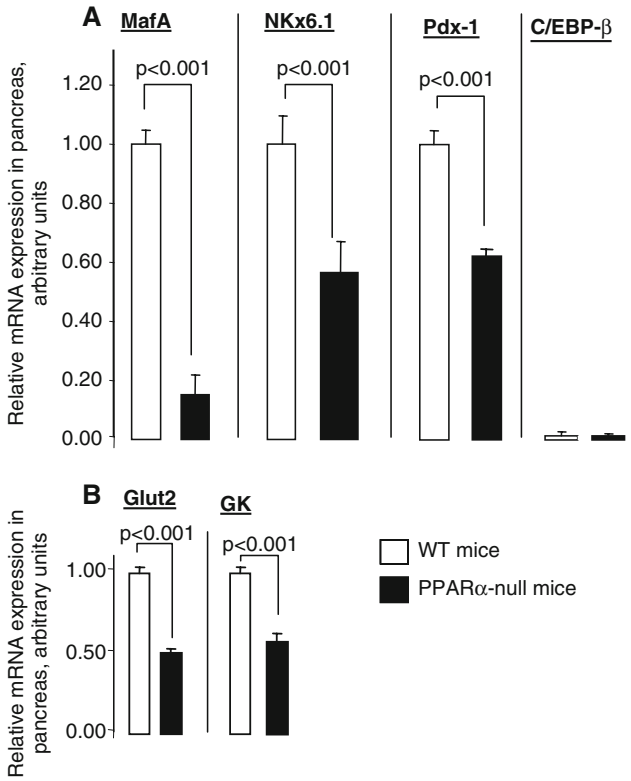

Fig. 2 C/EBP-β, PDX-1, MafA, Nkx6-1, Glut2, and glucokinase (GK) mRNA expression in the pancreas of wild type and PPARα-null mice. The expression of mRNA was quantitatively analyzed by employing real-time RT-PCR as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA

in PPARα-null mice in comparison with WT animals (Fig. 2b).

Serum and hepatic triglyceride (TG) and free fatty acids (FFA)

It is commonly known that obesity is associated with high fat contents in adipose tissue, serum, and liver [5, 19, 23]. Since it has been suggested that free fatty acids may regulate β-cell functions via PPARα [24], we determined the levels of triglyceride and free fatty acids in serum and liver of these animals. Hepatic TG and FFA were higher in PPARα-null mice as compared to WT animals (Fig. 3). However, serum FFA levels were not significantly different in WT and PPARα-null mice, though serum TG levels were lower in the latters as compared to the formers (Fig. 3).

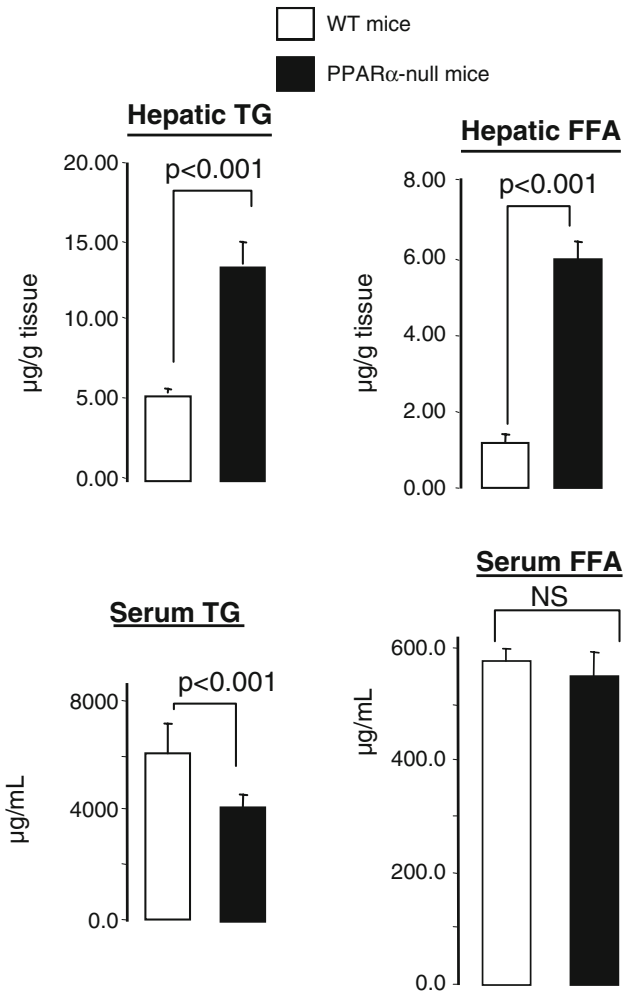

Fig. 3 Serum and hepatic triglyceride (TG) and free fatty acids (FFA) in wild type and PPARα-null mice. The lipid parameters were determined as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA. *NS* insignificant differences

FAT/CD36 and SREBP-1c mRNA expression in the liver

Since FAT/CD36 is actively implicated in the uptake of lipids and hence may contribute to high lipid contents in the liver, we assessed the mRNA expression of the FAT/CD36. The hepatic expression of FAT/CD36 was upregulated in PPARα-null mice as compared to their corresponding controls (Fig. 4). However, SREBP-1c mRNA expression was downregulated in PPARα-null mice in comparison with their corresponding WT animals (Fig. 4).

Adipose tissue weight, adiponectin, and leptin mRNA expression

PPARα knockout mice are known to rapidly gain weight and adiposity compared to their age-matched WT animals [19]. Since obesity has been linked to high adiposity and hyperlipidemia [5, 19, 23], we assessed in these mice the adipose tissue quantity and the obesity-related parameters such as adiponectin and leptin. We observed that PPARα-null mice had significantly more adipose tissues than WT mice (Fig. 5). The expression of adiponectin and leptin mRNA in adipose tissues was also upregulated in PPARα-null mice as compared to WT animals (Fig. 5).

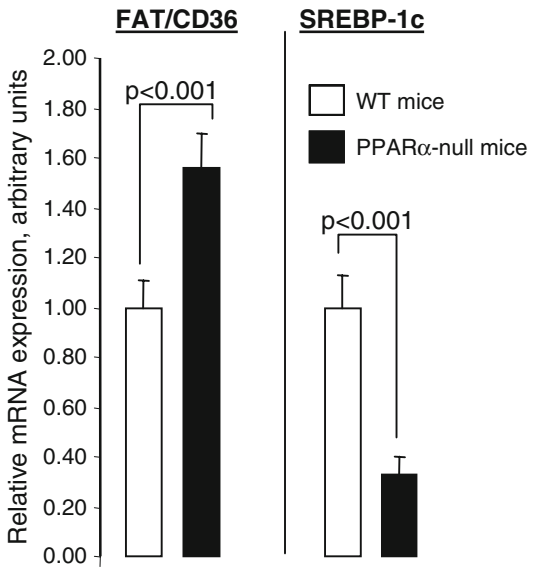

Fig. 4 FAT/CD36 and SREPB-1c mRNA expression in liver of wild type and PPARα-null mice. The expression of FAT/CD36 and SREPB-1c mRNA was quantitatively analyzed by employing real-time RT-PCR as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA

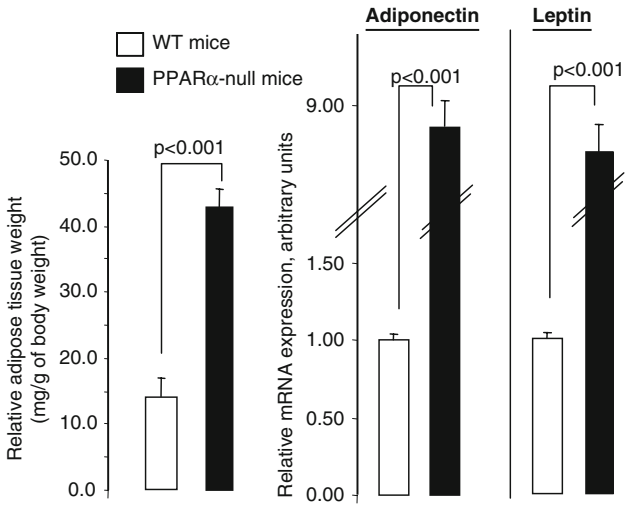

Fig. 5 Adipose tissue weight and adiponectin and leptin mRNA expression in white adipose tissue of wild type and PPARα-null mice. The expression of adiponectin and leptin mRNA was quantitatively analyzed by employing real-time RT-PCR as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA

CD14, CD68, F4/80, IL-1β, and TCRα mRNA expression in the adipose tissue

Obesity has been associated with the accumulation macrophages [16] and T cell infiltration [18] in adipose tissue. We, therefore, determined the presence of macrophage and T cell markers in the adipose tissue of these animals. The expression of CD14 and CD68 mRNA in adipose tissues was upregulated in PPARα-null mice as compared to WT animals (Fig. 6). On the other hand, the expression of IL-1β and F4/80 mRNA was decreased in PPARα-null mice as compared to WT animals (Fig. 6). However, expression TCRα mRNA was not evident in WT and PPARα-null mice (Fig. 6).

Expression of mRNA of other pro-inflammatory agents in adipose tissue

Chronic inflammation has been associated with the development of obesity [24]. In order to investigate the effect of the PPARα gene deletion, we examined the expression of some key inflammatory markers in adipose tissue of mice as PPARα-null mice developed high overweight [5, 19]. The expression of MCP-1 and RANTES mRNA was lower in PPARα-null mice than WT animals (Fig. 7). Interestingly, CCR5 mRNA expression was not apparent in adipose tissues of both the groups of animals. We did not observe any significant differences in the expression of TNF-α and IL-6 mRNA in the liver of these animals

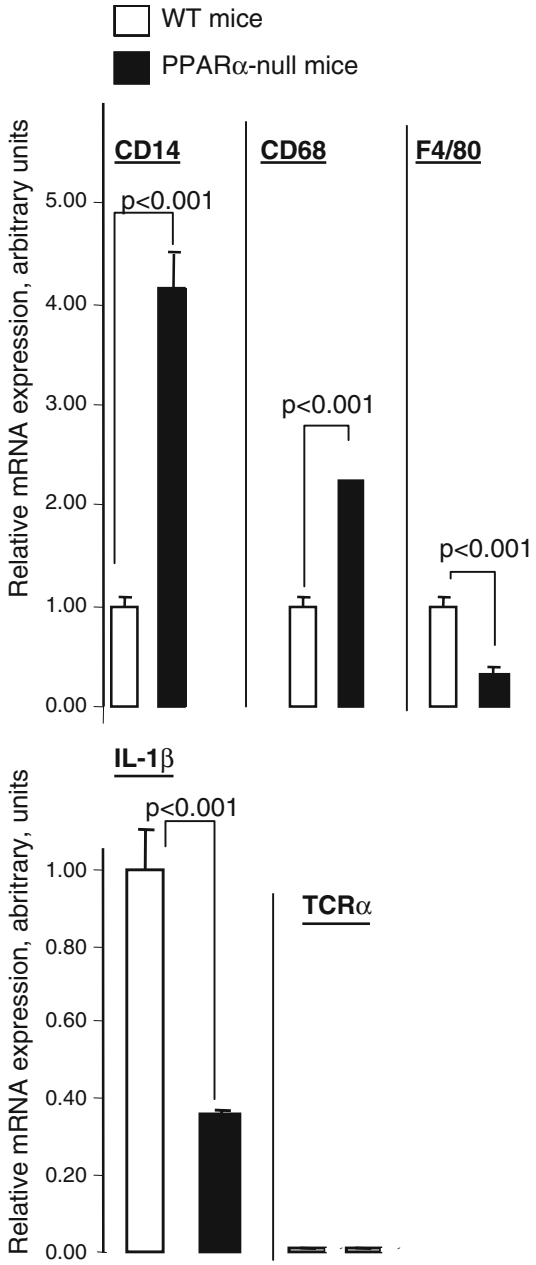

Fig. 6 CD14, CD68, F4/80, IL-1β, and TCRα mRNA expression in white adipose tissue of wild type and PPARα-null mice. The expression of CD14, CD68, F4/80, IL-1β, and TCRα mRNA was quantitatively analyzed by employing real-time RT-PCR as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA

whether they were WT and PPARα-null. However, the expression of TNF-α and IL-6 mRNA was downregulated in the adipose tissues of PPARα-null mice as compared to WT animals (Fig. 8). On the other hand, PPARα-null mice had higher expression of TLR-2 and TLR-4 mRNA than WT animals (Fig. 9).

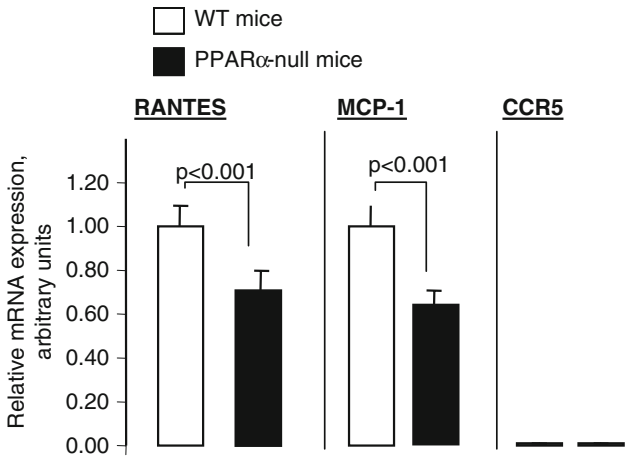

Fig. 7 RANTES, MCP-1, and CCR5 mRNA expression in white adipose tissue of wild type and PPARα-null mice. The expression of RANTES, MCP-1, and CCR5 mRNA was quantitatively analyzed by employing real-time RT-PCR as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA

Discussion

It has been reported that PPARα plays a key role in the regulation of insulin secretion and inflammation [5, 20]. Hence, in order to shed light on the implication of PPARα in the modulation of insulin gene transcription and adipocyte inflammation, we conducted the present study on WT and PPARα-null mice. The first and foremost question is how PPARα, in fasted conditions, modulates β-cell functions. As reported by other investigators [25], we also observed, in our study that, under fasting conditions, PPARα-null mice developed hypoglycemia which might be related to impaired fatty acid oxidation and increased reliance on glucose as an energy source [26]. Furthermore, the PPARα-null mice exhibited low insulin concentrations and mRNA transcripts as compared to WT mice. These observations corroborate the study of Bihan et al. [20] who have reported that PPARα is required to ensure correct insulin secretory response and for the induction of insulin mRNA, which is a part of the β-cell responses to hyperglycemia.

Pancreatic β-cells produce and store insulin in response to physiological demand; hence, hyperglycemia, within 15 min, results in the activation of a complex network of intracellular signaling pathways that trigger insulin release [27]. The positive transcription regulators are Pdx-1 and MafA, which bind to the insulin promoter to provide a synergistic effect [28]. Nkx6.1 also affects glucose-induced insulin secretion [13]. Hypoinsulinemic state in PPARα-null mice might be due to diminished expression of Pdx-1, MafA, and Nkx6.1 mRNA in their pancreas. Again, highly downregulated insulin gene transcription in PPARα-null mice might be due to lower expression of Pdx-1 mRNA in these animals than the control mice. Indeed, the deletion of Pdx-1 gene in the pancreas results in abnormally low insulin concentrations [29]. In our study, the action of PPARα deletion on hypoinsulinemia does not seem to be

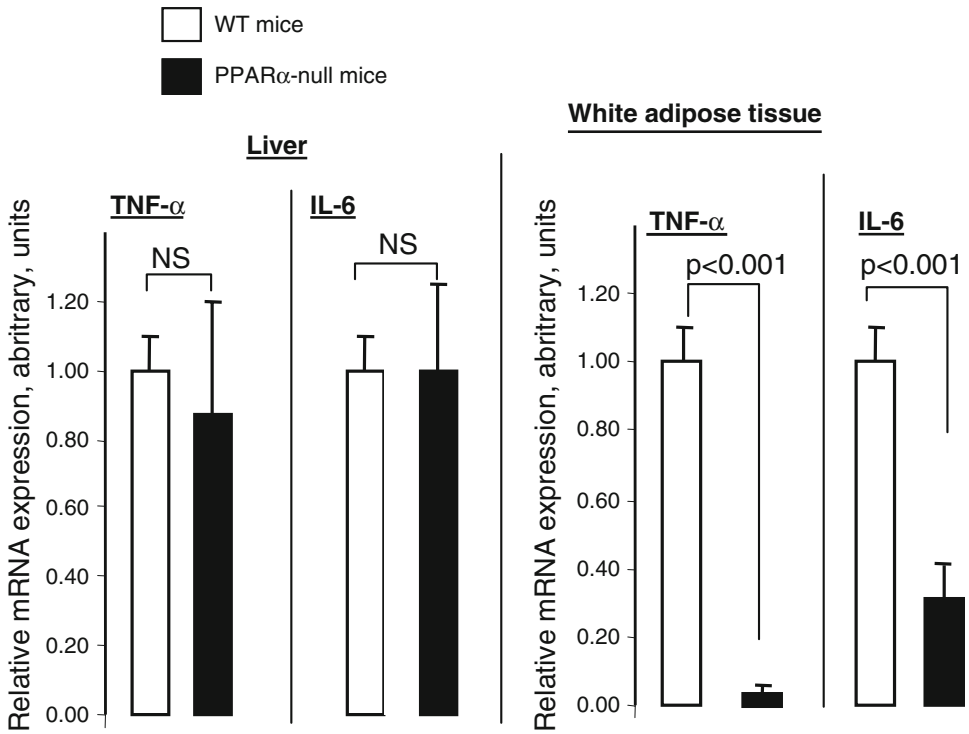

Fig. 8 TNF-α and IL-6 cytokines mRNA expression in liver and white adipose tissue of wild type and PPARα-null mice. The expression of TNF-α and IL-6 mRNA cytokine was quantitatively analyzed by employing real-time RT-PCR as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA. *NS* insignificant differences

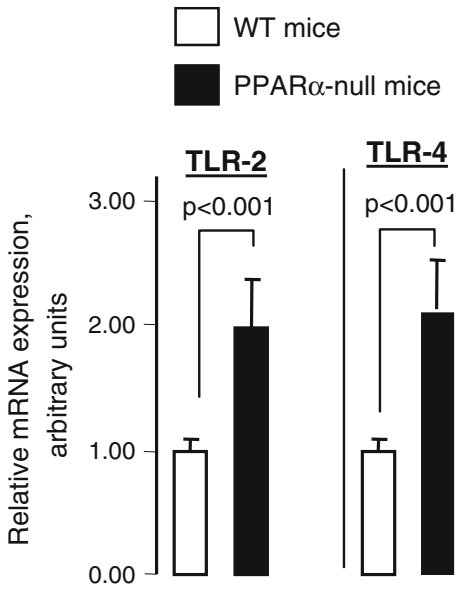

Fig. 9 TLR-2 and -4 mRNA expression in white adipose tissue of wild type and PPARα-null mice. The expression of TLR2 and TLR4 mRNA was quantitatively analyzed by employing real-time RT-PCR as described in Research Methods and procedures section. Values are means ± SEM, $n = 15$ per group of animals. Data were analyzed by two-way ANOVA

mediated by C/EBP-β as the expression of this repressor was not significantly altered in none of the groups. However, it is still not clear how PPARα is responsible for the inhibition of the expression of Pdx-1, MafA, and Nkx6.1 mRNA. Whether this phenomenon is directly controlled by PPARα or whether the lipotoxicity, caused by PPARα-deficiency, is responsible for it.

In the present study, the WT and PPARα-null mice are of C57BL/6J genetic background. The specificity related to this mouse strain is that, at the same age, the weight gain is greater in PPARα-null mice than in their counterpart WT animals [19]. In this study, the PPARα-null mice exhibited a high adiposity and high lipidemia. In the liver, we observed that PPARα-null mice abundantly accumulated not only free fatty acids but also TG. In contrast, these animals exhibited low levels of serum TG or no difference in FFA. This inverse correlation between liver and serum levels of lipids might be due to the fact that PPARα controls positively the mitochondrial β-oxidation of fatty acids. PPARα deficient mice [19] exhibit a reduced capacity to metabolize long-chain fatty acids [25] in the serum, which likely contributes to dyslipidemia [30] and larger adipose stores observed in these mice with aging [31]. Campbell et al. [32] have explained this phenomenon by demonstrating that there is a marked increase in malonyl-CoA, a potent inhibitor of fatty acid oxidation, in the hearts of PPARα-null mice. Our observations corroborate the findings of several investigators [33] who have shown that PPARα-null mice accumulate increased hepatic TG in response to feeding and during fasting. Djouadi et al. [34] have reported that PPARα-null mice develop massive accumulations of myocardial lipids under conditions that increase fatty acid flux. We would like to recall that PPARα agonists lower TG levels by increasing lipoprotein lipase gene expression via a PPAR response element (PPRE) in the LPL promoter [35] and decreasing apo C-III levels [36]. Besides, PPARα-null mice, in our study, exhibit high levels of CD36/FAT which will again participate in high uptake of lipids by liver and ultimately contribute to liver steatosis in these animals. Furthermore, the mRNA of SREBP-1c was downregulated in PPARα-null mice. SREBP1c controls the transcription of lipogenic genes. Hence, a high accumulation of TG and FFA in the liver, due to high expression of FAT/CD36, might be related to the PPARα gene deletion in these animals. On the other hand, insulin is a major regulatory factor which increases markedly and rapidly SREBP-1c in hepatocytes [37]. In this study, the low levels of insulin may explain, at least in part, the downregulation of hepatic SREBP-1c gene expression in PPARα-null mice [38].

As far as the mechanistic aspect is concerned, it is well-known that acute elevation of free fatty acids stimulates insulin release, both under normal and high glucose concentrations [39, 40]. However, chronic exposure to fatty acids inhibits insulin secretion [41]. The mechanism of this reciprocal effect of fatty acids on insulin release needs to be elucidated; indeed, the present study showed that PPARα-null mice accumulated high FFA and TG in their liver. In fact, it is well-known that the lack of PPARα leads to lipid accumulation [5, 19]. PPARα-null mice also showed low insulin contents, at protein and mRNA levels. Our study revealed a double importance of PPARα which is not probably, physiologically, independent to each other. First, the lipid accumulation in PPARα-null mice may be due to chronic exposure to fatty acids which contribute to diminished insulin release [24]. Second, PPARα is needed to ensure insulin secretion in response to glucose exposure [20]. In summary, PPARα is required to ensure appropriate insulin secretion through its ability to maintain lipid homeostasis [20, 24, 41]. Furthermore, PPARα deficiency leads to low GK mRNA level in PPARα-null mice as compared to WT animals. Pancreatic β-cell function is associated to glucokinase (GK) activity [42]. Hence, the diminished GK mRNA in PPARα-null mice may further contribute to reduced insulin β-cell function, thereby, causing reduced insulin secretion [43]. Our results also suggest that PPARα may be implicated in the control of Glut2 or GK mRNA expression in the pancreas. Indeed, PPARα has been considered as one of the transcription factors involved in the upregulation of Glut2 or GK mRNA [44]. Yoshikawa et al. [24] have shown that long-term

cultures of pancreatic cells with palmitate were associated to reduced expression of PPARα mRNA and, consequently, to low expression of Glut2, GK, or preproinsulin mRNA probably through the inhibition of Pdx-1 mRNA. Our results, on the importance of PPARα in Glut2 or GK mRNA expression in the pancreas, corroborate the recent study of Lalloyer et al. [45]. These authors have shown that, in the context of genetic obesity, PPARα-deficiency in ob/ob mice affected pancreatic β-cell size and, thereby, resulted in the decreased insulin secretion in response to glucose in vitro and in vivo [45].

As far as the adipokines are concerned, we observed a concomitant increase in adiponectin and leptin mRNA in PPARα-null mice and this increase might be due to larger adipose tissues in these animals as compared to WT mice. As far as inflammation is concerned, high adiponectin may counterbalance the effects of leptin as the former is anti-atherogenic and anti-inflammatory whereas the latter is pro-inflammatory [46]. We clearly demonstrated that PPARα plays an important role in insulin secretion by pancreatic β-cells and in inflammation [5, 20]. Moreover, the inflammation has been established as a link between insulin resistance and obesity, associated with lipid accumulation [47]. In order to determine inflammation in adipocytes, we quantified the mRNA of well-known markers of macrophages. Surprisingly, we observed that the expression of mRNA of CD14 and CD68, but not F4/80, was upregulated in PPARα-null mice. These observations suggested that the adipocytes of PPARα-null mice seemed to be differentiated into macrophage-like cells, as they expressed upregulated mRNA transcripts of CD14 and CD68 antigens, generally expressed by macrophages. However, there was no peripheral macrophages infiltration into adipose tissues, as the mRNA expression of F4/80, a true macrophage marker, was downregulated. Our observations are in close agreement with the results of Khazen et al. [48] who have reported that murine and human adipose tissues express CD14 and CD68, but not F4/80, both at protein and mRNA levels. Besides, Cousin et al. [49] have demonstrated that preadipocytes can be differentiated into macrophage-like cells which are stained with MOMA-2, a marker of monocyte-macrophage lineage, but are negative for F4/80.

MCP-1 has been known to be secreted by adipose tissues and this factor favors infiltration and the differentiation of the macrophages [16]. We observed that PPARα-null mice express lower MCP-1 mRNA than WT animals, suggesting that the deletion of PPARα gene accounts for the diminution of the population of macrophages in the adipose tissues. Consequently, the adipose tissues of PPARα-null mice express lower IL-1β, IL-6, and TNF-α mRNA than those of WT animals.

RANTES has been considered as an adipokine and its receptor CCR5 has been expressed principally on infiltrated T-cells [18]. In our study, we could not detect both the T-cells (as evidenced by the absence of TCRα mRNA) and CCR5 mRNA in adipose tissues of the mice. However, we observed that RANTES mRNA expression was diminished in adipose tissues of PPARα-null mice. Adiponectin has been shown to regulate RANTES expression [18]. High adiponectin mRNA in adipose tissues of these mice might be responsible for the downregulation of RANTES mRNA. Our hypothesis can be supported by the study of Wu et al. [18] who have shown that RANTES mRNA levels were negatively correlated with adiponectin in mouse adipose tissues.

In vitro differentiated adipocytes have been shown to express TLR-2 and TLR-4 [50]. Since TLRs are the mediators of the cellular response to bacterial lipopolysaccharide (LPS), it possible that during an antigenic (microbial) challenge, the adipocytes may play a role in immunomodulation [51]. High expression of TLRs is associated with increased inflammation. The upregulation of TLR-2 and -4 mRNA in PPARα-null mice suggests that PPARα might be implicated in the modulation of endotoxemia. As far as the mechanism in the induction of TLR expression is concerned, we would like to state that leptin has been recently shown to induce the expression of TLR1-9 in adipocytes [52]. In our study, high levels of leptin mRNA in PPARα-null mice might be responsible for the upregulation of TLR-2 and -4 mRNA.

To sum up, our study demonstrates that PPARα controls the transcription of insulin gene and modulates inflammation by downregulating the mRNA expression of certain pro-inflammatory agents like mRNA of MCP-1, TNF-α, IL-6 and RANTES though the mRNA of other pro-inflammatory mediators like TLR-2 and TLR-4 are upregulated in the absence of PPARα. In the light of our results in PPARα-null mice, we can state that one should be careful while interpreting the scientific data on these animals. However, further studies are required to clarify the role of PPARα, in detail, in hyperglycemic conditions.

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References

1. Issemann I, Green S (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347:645–650. doi:10.1038/347645a0
2. Wahli W (2002) Peroxisome proliferator-activated receptors (PPARs): from metabolic control to epidermal wound healing. Swiss Med Wkly 132:83–91
3. Braissant O, Foufelle F, Scotto C et al (1996) Differential expression of peroxisome proliferator-activated receptors

(PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology 137:354–366. doi:10.1210/en.137.1.354
4. Zambon A, Gervois P, Pauletto P et al (2006) Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPAR-{alpha} activators: clinical and experimental evidence. Arterioscler Thromb Vasc Biol 26:977–986. doi:10.1161/01.ATV.0000204327.96431.9a
5. Yessoufou A, Hichami A, Besnard P et al (2006) PPARα deficiency increases the risk of maternal abortion and neonatal mortality in murine pregnancy with or without diabetes mellitus: modulation of T cell differentiation. Endocrinology 147:4410–4418. doi:10.1210/en.2006-0067
6. Guerre-Millo M, Rouault C, Poulain P et al (2001) PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. Diabetes 50:2809–2814. doi:10.2337/diabetes.50.12.2809
7. Guillausseau PJ (2003) Pathogenesis of type 2 diabetes mellitus. Rev Med Interne 24:730–737. doi:10.1016/S0248-8663(03)00244-3
8. German K, Bedwani J, Davies J et al (1994) An assessment of the contribution of visco-elastic factors in the aetiology of poor compliance in the human neuropathic bladder. Br J Urol 74:744–748
9. Wicksteed B, Alarcon C, Briaud I et al (2003) Glucose-induced translational control of proinsulin biosynthesis is proportional to preproinsulin mRNA levels in islet beta-cells but not regulated via a positive feedback of secreted insulin. J Biol Chem 278:42080–42090. doi:10.1074/jbc.M303509200
10. Tillmar L, Carlsson C, Welsh N (2002) Control of insulin mRNA stability in rat pancreatic islets. Regulatory role of a 3′-untranslated region pyrimidine-rich sequence. J Biol Chem 277:1099–1106. doi:10.1074/jbc.M108340200
11. Johnson JD, Ahmed NT, Luciani DS et al (2003) Increased islet apoptosis in PDX1 +/− mice. J Clin Invest 111:1147–1160
12. Sander M, Sussel L, Conners J et al (2000) Homeobox gene Nkx6.1 lies downstream of Nkx2.2 in the major pathway of beta-cell formation in the pancreas. Development 127:5533–5540
13. Schisler JC, Jensen PB, Taylor DG et al (2005) The Nkx6.1 homeodomain transcription factor suppresses glucagon expression and regulates glucose-stimulated insulin secretion in islet beta cells. Proc Natl Acad Sci USA 102:7297–7302. doi:10.1073/pnas.0502168102
14. Docherty HM, Hay CW, Ferguson LA et al (2005) Relative contribution of PDX-1, MafA and E47/beta2 to the regulation of the human insulin promoter. Biochem J 389:813–820. doi:10.1042/BJ20041891
15. Waki H, Tontonoz P (2007) Endocrine functions of adipose tissue. Annu Rev Pathol 2:31–56. doi:10.1146/annurev.pathol.2.010506.091859
16. Weisberg SP, McCann D, Desai M et al (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808
17. Triantafilou M, Triantafilou K (2002) Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. Trends Immunol 23:301–304. doi:10.1016/S1471-4906(02)02233-0
18. Wu H, Ghosh S, Perrard XD et al (2007) T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. Circulation 115:1029–1038. doi:10.1161/CIRCULATIONAHA.106.638379
19. Lee SS, Pineau T, Drago J et al (1995) Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol Cell Biol 15:3012–3022
20. Bihan H, Rouault C, Reach G et al (2005) Pancreatic islet response to hyperglycemia is dependent on peroxisome proliferator-activated receptor alpha (PPARalpha). FEBS Lett 579:2284–2288. doi:10.1016/j.febslet.2005.03.020
21. Folch J, Lees M, Stanley Sloane GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509
22. Slover HT, Lanza E (1979) Quantitative analysis of food fatty acids by capillary gas chromatography. J Am Oil Chem Soc 56:933–943. doi:10.1007/BF02674138
23. Xu H, Barnes GT, Yang Q et al (2003) Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 112:1821–1830
24. Yoshikawa H, Tajiri Y, Sako Y et al (2001) Effects of free fatty acids on beta-cell functions: a possible involvement of peroxisome proliferator-activated receptors alpha or pancreatic/duodenal homeobox. Metabolism 50:613–618. doi:10.1053/meta.2001.22565
25. Kroetz DL, Yook P, Costet P et al (1998) Peroxisome proliferator-activated receptor alpha controls the hepatic CYP4A induction adaptive response to starvation and diabetes. J Biol Chem 273:31581–31589. doi:10.1074/jbc.273.47.31581
26. Kersten S, Seydoux J, Peters JM et al (1999) Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Invest 103:1489–1498. doi:10.1172/JCI6223
27. Leibiger B, Moede T, Uhles S et al (2002) Short-term regulation of insulin gene transcription. Biochem Soc Trans 30:312–317. doi:10.1042/BST0300312
28. Lawrence MC, McGlynn K, Park BH et al (2005) ERK1/2-dependent activation of transcription factors required for acute and chronic effects of glucose on the insulin gene promoter. J Biol Chem 280:26751–26759. doi:10.1074/jbc.M503158200
29. Ahlgren U, Jonsson J, Jonsson L et al (1998) Beta-cell-specific inactivation of the mouse Ipf1/PDX1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev 12:1763–1768. doi:10.1101/gad.12.12.1763
30. Peters JM, Hennuyer N, Staels B et al (1997) Alterations in lipoprotein metabolism in peroxisome proliferator-activated receptor alpha-deficient mice. J Biol Chem 272:27307–27312. doi:10.1074/jbc.272.43.27307
31. Costet P, Legendre C, More J et al (1998) Peroxisome proliferator-activated receptor alpha-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. J Biol Chem 273:29577–29585. doi:10.1074/jbc.273.45.29577
32. Campbell FM, Kozak R, Wagner A et al (2002) A role for peroxisome proliferator-activated receptor alpha (PPARalpha) in the control of cardiac malonyl-CoA levels: reduced fatty acid oxidation rates and increased glucose oxidation rates in the hearts of mice lacking PPARalpha are associated with higher concentrations of malonyl-CoA and reduced expression of malonyl-CoA decarboxylase. J Biol Chem 277:4098–4103. doi:10.1074/jbc.M106054200
33. Park CW, Kim HW, Ko SH et al (2006) Accelerated diabetic nephropathy in mice lacking the peroxisome proliferator-activated receptor alpha. Diabetes 55:885–893. doi:10.2337/diabetes.55.04.06.db05-1329
34. Djouadi F, Weinheimer CJ, Saffitz JE et al (1998) A gender-related defect in lipid metabolism and glucose homeostasis in peroxisome proliferator-activated receptor alpha-deficient mice. J Clin Invest 102:1083–1091. doi:10.1172/JCI3949
35. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM et al (1996) PPARα and PPAR activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. EMBO J 15:5336–5348
36. Staels B, Vu-Dac N, Kosykh VA et al (1995) Fibrates down-regulate apolipoprotein C-III expression independent of induction

of peroxisomal acyl coenzyme A oxidase. J Clin Invest 95:705–712. doi:10.1172/JCI117717
37. Hansmannel F, Mordier S, Iynedjian PB (2006) Insulin induction of glucokinase and fatty acid synthase in hepatocytes: analysis of the roles of sterol-regulatory-element-binding protein-1c and liver × receptor. Biochem J 399:275–283. doi:10.1042/BJ20060811
38. Hebbachi AM, Knight BL, Wiggins D et al (2008) PPARalpha-deficiency abolishes the response of lipogenic gene expression to re-feeding. Restoration of the normal response by activation of LXRalpha. J Biol Chem 283:4866–4876. doi:10.1074/jbc.M709471200
39. Crespin SR, Greenough WB 3rd, Steinberg D (1969) Stimulation of insulin secretion by infusion of free fatty acids. J Clin Invest 48(10):1934–1943. doi:10.1172/JCI106160
40. Malaisse WJ, Malaisse-Lagae F (1968) Stimulation of insulin secretion by noncarbohydrate metabolites. J Lab Clin Med 72(3):438–448
41. Zhou YP, Grill VE (1994) Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest 93(2):870–876. doi:10.1172/JCI117042
42. Im SS, Kim SY, Kim HI et al (2006) Transcriptional regulation of glucose sensors in pancreatic beta cells and liver. Curr Diabetes Rev 2:11–18. doi:10.2174/157339906775473581
43. Liu YQ, Nevin PW, Leahy JL (2000) Beta-cell adaptation in 60% pancreatectomy rats that preserves normoinsulinemia and normoglycemia. Am J Physiol Endocrinol Metab 279:E68–E73
44. Wang MY, Koyama K, Shimabururo M (1998) Overexpression of leptin receptors in pancreatic islets of Zucker diabetic fatty rats restores GLUT-2, glucokinase, and glucose-stimulated insulin secretion. Proc Natl Acad Sci USA 95:11921–11926. doi:10.1073/pnas.95.20.11921
45. Lalloyer F, Vandewalle B, Percevault F et al (2006) Peroxisome proliferator-activated receptor alpha improves pancreatic adaptation to insulin resistance in obese mice and reduces lipotoxicity in human islets. Diabetes 55:1605–1613. doi:10.2337/db06-0016
46. Diez JJ, Iglesias P (2003) The role of the novel adipocyte-derived hormone adiponectin in human and possible biological roles. Eur J Endocrinol 148:293–300. doi:10.1530/eje.0.1480293
47. Dandona P, Aljada A, Bandyopadhyay A et al (2004) Inflammation: the link between insulin resistance, obesity and diabetes. Trends Immunol 25:4–7. doi:10.1016/j.it.2003.10.013
48. Khazen W, M'bika JP, Tomkiewicz C et al (2005) Expression of macrophage-selective markers in human and rodent adipocytes. FEBS Lett 579:5631–5634
49. Cousin B, Munoz O, Andre M et al (1999) A role for preadipocytes as macrophage-like cells. FASEB J 13:305–312
50. Lin Y, Lee H, Berg AH et al (2000) The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. J Biol Chem 275:24255–24263. doi:10.1074/jbc.M002137200
51. Creely SJ, McTernan PG, Kusminski CM et al (2007) Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Physiol Endocrinol Metab 292:E740–E747. doi:10.1152/ajpendo.00302.2006
52. Batra A, Pietsch J, Fedke I et al (2007) Leptin-dependent toll-like receptor expression and responsiveness in preadipocytes and adipocytes. Am J Pathol 170:1931–1941. doi:10.2353/ajpath.2007.060699