Original Article

MOLECULAR METABOLISM

The Peroxisome Proliferator-Activated Receptor α is dispensable for cold-induced adipose tissue browning in mice

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ABSTRACT

Objective: Chronic cold exposure causes white adipose tissue (WAT) to adopt features of brown adipose tissue (BAT), a process known as browning. Previous studies have hinted at a possible role for the transcription factor Peroxisome Proliferator-Activated Receptor alpha (PPARα) in cold-induced browning. Here we aimed to investigate the importance of PPARα in driving transcriptional changes during cold-induced browning in mice.

Methods: Male wildtype and PPARα−/− mice were housed at thermoneutrality (28 °C) or cold (5 °C) for 10 days. Whole genome expression analysis was performed on inguinal WAT. In addition, other analyses were carried out. Whole genome expression data of livers of wildtype and PPARα−/− mice fasted for 24 h served as positive control for PPARα-dependent gene regulation.

Results: Cold exposure increased food intake and decreased weight of BAT and WAT to a similar extent in wildtype and PPARα−/− mice. Except for plasma non-esterified fatty acids, none of the cold-induced changes in plasma metabolites were dependent on PPARα genotype. Histological analysis of inguinal WAT showed clear browning upon cold exposure but did not reveal any morphological differences between wildtype and PPARα−/− mice. Transcriptomics analysis of inguinal WAT showed a marked effect of cold on overall gene expression, as revealed by principle component analysis and hierarchical clustering. However, wildtype and PPARα−/− mice clustered together, even after cold exposure, indicating a similar overall gene expression profile in the two genotypes. Pathway analysis revealed that cold upregulated pathways involved in energy usage, oxidative phosphorylation, and fatty acid β-oxidation to a similar extent in wildtype and PPARα−/− mice. Furthermore, cold-mediated induction of genes related to thermogenesis such as *Ucp1*, *Elovl3*, *Cox7a1*, *Cox8*, and *Cidea*, as well as many PPAR target genes, was similar in wildtype and PPARα−/− mice. Finally, pharmacological PPARα activation had a minimal effect on expression of cold-induced genes in murine WAT.

Conclusion: Cold-induced changes in gene expression in inguinal WAT are unaltered in mice lacking PPARα, indicating that PPARα is dispensable for cold-induced browning.

Keywords Cold; Adipose tissue browning; PPARα; Transcriptomics

1. INTRODUCTION

Cold exposure in mice causes specific white adipose tissue (WAT) depots to adopt features of brown adipose tissue (BAT), a process known as browning [1]. Browning is characterized by the appearance of adipocytes with multiple lipid droplets and by the acquisition of a thermogenic capacity, the latter of which is dependent on an increase in uncoupling protein (UCP) 1 [2]. Concurrent with the changes in cell morphology, the transcription of numerous genes involved in thermogenesis and lipid catabolism is strongly activated [3]. Recently, it was shown that human white adipose tissue is also capable of browning under condition of prolonged and severe adrenergic stress [4]. Nowadays, browning is considered as a potential molecular target to promote weight loss and improve metabolic risk factors, prompting investigation into the molecular mechanisms that drive browning.

One of the transcription factors that has been implicated in browning is PPARα [5]. PPARα is a member of the family of Peroxisome Proliferator-Activated Receptors, which play key roles in the regulation of lipid homeostasis and oxidative metabolism [6–8]. Three different PPAR subtypes exist in mammals: PPARα, PPARδ (also referred to as PPARβ), and PPARγ, each with a distinct tissue expression profile and set of functions. PPARs regulate gene expression in response to binding small lipophilic ligands and function as a heterodimeric complex with the retinoid X receptor RXR [9–11]. The ligands for PPARs include a variety of synthetic and endogenous compounds

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ranging from industrial chemicals to specific drug classes, fatty acids, eicosanoids, and other lipid species [12].

The PPARα subtype is expressed in several tissues, with the highest expression levels in mice found in brown adipose tissue (BAT) and liver, followed by kidney, heart, skeletal muscle and intestine [13,14]. At the physiological level, PPARα is mainly known as the master regulator of lipid metabolism in the liver during fasting. This notion is based on the finding that fasted whole-body or liver-specific PPARα−/− mice have a severe metabolic phenotype characterized by hypoglycemia, hyperketonemia, elevated plasma non-esterified fatty acids (NEFAs), and a fatty liver [15–17]. These metabolic defects are rooted in reduced expression of hundreds of genes involved in numerous metabolic pathways covering nearly every aspect of hepatic lipid metabolism [18].

The PPARγ subtype is predominantly expressed in colonocytes, macrophages, and adipocytes [13]. It is mainly known as the target for the insulin-sensitizing thiazolidinedione drugs [19]. In addition, PPARγ is essential for the differentiation of brown and white adipocytes [20]. In mice, loss of PPARγ fails to yield viable offspring [21], while in humans homozygosity for loss-of-function mutations in PPARγ leads to a form of lipodystrophy [22].

Both PPARα and PPARγ have been implicated in browning [5]. With respect to PPARγ, treatment of mice with synthetic PPARγ agonists promotes adipose tissue browning, as shown by the appearance of multilocular adipocytes and the increased expression of thermogenic genes such as *Ucp1*, triggering thermogenic capacity in WAT [23–25]. Conversely, mice carrying a functionally impaired mutant form of PPARγ have reduced adipose tissue browning and an impaired thermogenic capacity after treatment with the β3-adrenergic agonist CL316,243 [26]. Furthermore, activation of PPARγ in human preadipocytes and adipocytes increased expression of *UCP1* and other browning-related genes [27,28].

The role of PPARα in browning is less clear. Although a number of studies have implicated PPARα in CL316,243-induced browning [27,29], no studies have carefully examined the transcriptional role of PPARα during cold-induced browning. Based on the reported attenuation of browning by PPARα ablation after CL316,243 treatment [27,29], and considering the marked induction of PPARα mRNA and protein during cold-induced browning [30,31], we hypothesized that PPARα is essential for stimulating gene expression in inguinal WAT in response to cold. The objective of this study was to verify this hypothesis and investigate the importance of PPARα in cold-induced browning in mice.

2. METHODS

2.1. Animals and diet

Male and female wildtype and PPARα−/− mice that had been backcrossed on a pure C57Bl/6J background for more than 10 generations were acquired from Jackson Laboratories (no. 000664 and 008154, respectively). The mice were further bred at the animal facility of Wageningen University under specific pathogen free conditions to generate the number of mice necessary for the experiments. For the chronic cold experiment, 40 three-to four-month-old male wildtype and PPARα−/− mice were placed at thermo-neutral temperature (28 °C) for 5 weeks. Thereafter, 20 wildtype and 20 PPARα−/− mice were randomly distributed across 2 groups: half of the mice of each genotype were kept at thermo-neutral temperature and half of the mice of each genotype was placed at 21 °C for one week followed by a period of 10 days in the cold (5 °C) (n = 10 per group). For cold exposure, mouse cages were placed in a cold cabinet that was kept at 5 °C (ELDG800, VDW Coolsystems, Geldermalsen). Mice had ad libitum access to chow feed and water. Body weight and body temperature were monitored daily during the cold exposure period. Body temperature of the cold-exposed mice was monitored via read-out of transponders (IPTT-300) that were injected subcutaneously prior to the experiment (Bio Medic Data Systems, Seaford, USA). For the acute cold experiment, three-to four-month-old male wildtype and PPARα−/− mice were placed at thermo-neutral temperature (28 °C) for 5 weeks. Thereafter, the mice were placed in the cold (5 °C) for 24 h (n = 10 per group). For the fasting experiment, three-to four-month-old male wildtype and PPARα−/− mice were either fasted for 24 h or had food available ad libitum (n = 10–11 per group). For PPAR agonist treatment, Sv129 male mice were placed on a high fat diet (formula D12451 Research Diets, Inc., manufactured by Research Diet Services, Wijk bij Duurstede) for 21 weeks with the addition of Wy14,643 (0.1% w/w of feed) or rosiglitazone (0.01% w/w of feed) during the last week. All mice were housed at the animal facility of Wageningen University. Mice were housed in individual cages with normal bedding and cage enrichment on a 12 h light–dark cycle. They had visual and auditory contact with littermates. At the end of the study, between 9.00h and 11.00h, blood was collected via orbital puncture under isoflurane anesthesia into EDTA tubes. Mice were euthanized via cervical dislocation, after which tissues were excised and directly frozen in liquid nitrogen or prepared for histology. All studies were approved by the Animal Ethics Committee of Wageningen University and by the Central Animal Testing Committee (CCD, AVD104002015236).

2.2. RNA isolation and quantitative PCR

Total RNA was extracted from cells using TRIzol reagent (Life Technologies, Bleiswijk, The Netherlands). For tissues, total RNA was isolated using the RNeasy Micro kit from Qiagen (Venlo, The Netherlands). Subsequently, 500 ng RNA was used to synthesize cDNA using iScript cDNA synthesis kit (Bio-Rad Laboratories, Veenendaal, The Netherlands). Messenger RNA levels of selected genes were determined by reverse transcription quantitative PCR using SensiMix (Bioline; GC Biotech, Alphen aan den Rijn, The Netherlands) on a CFX384 real-time PCR detection system (Bio-Rad Laboratories, Veenendaal, the Netherlands). The housekeeping gene *36b4* was used for normalization.

2.3. Histology/immunohistochemistry

Fresh WAT tissues were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Hematoxilin & Eosin staining was performed using standard protocols.

2.4. Plasma measurements

Plasma concentrations of glucose (Sopachem, Ochten, the Netherlands), triglycerides, cholesterol, ketone bodies (Instruchemie, Delfzijl, the Netherlands), glycerol (Sigma–Aldrich, Houten, the Netherlands), and non-esterified fatty acids (Wako Chemicals, Neuss, Germany; HR(2) Kit) were determined following the manufacturers' instructions.

2.5. Liver triglycerides

Liver triglyceride levels were determined in 10% liver homogenates prepared in buffer containing Sucrose 250 mM, EDTA 1 mM, Tris–HCl 10 mM pH 7.5 using a commercially available kit from Instruchemie (Delfzijl, The Netherlands).

2.6. Microarray analysis

Microarray analysis was performed on inguinal adipose tissue samples from 5 to 6 mice of each group and liver samples from 5 mice per group. RNA was isolated as described above and subsequently purified using

