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Deficiency of PPARα disturbs the response of lipogenic flux and of lipogenic and cholesterogenic gene expression to dietary cholesterol in mouse white adipose tissue

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Abstract

PPARα-deficiency in mice fed a high-carbohydrate, low-cholesterol diet was associated with a decreased weight of epididymal adipose tissue and an increased concentration of adipose tissue cholesterol. Consumption of a high (2% w/w) cholesterol diet resulted in a further increase in the concentration of cholesterol and a further decrease in epididymal fat pad weight in PPARα-null mice, but had no effect in the wild-type. These reductions in fat pad weight were associated with an increase in hepatic triacylglycerol content, indicating that both PPARα-deficiency and cholesterol altered the distribution of triacylglycerol in the body. Adipose tissue de novo lipogenesis was increased in PPARα-null mice and was further enhanced when they were fed a cholesterol-rich diet; no such effect was observed in the wild-type mice. The increased lipogenesis in the chow-fed PPARα-null mice was accompanied paradoxically by lower mRNA expression of SREBP-1c and its target genes, acetyl-CoA carboxylase and fatty acid synthase. Consumption of a high-cholesterol diet increased the mRNA expression of these genes in the PPARα-deficient mice but not in the wild-type. De novo cholesterol synthesis was not detectable in the adipose tissue of either genotype despite a relatively high expression of the mRNA's encoding SREBP-2 and 3-hydroxy-3-methylglutaryl Coenzyme A reductase. The mRNA expression of these genes and of the LDL-receptor in adipose tissue of the PPARα-deficient mice was lower than that of the wild-type and was not downregulated by cholesterol feeding. The results suggest that PPARα plays a role in adipose tissue cholesterol and triacylglycerol homeostasis and prevents cholesterol-mediated changes in de novo lipogenesis.

Keywords: Adipose tissue; PPARα; Cholesterol; Lipogenic flux; mRNA expression; SREBP

1. Introduction

The liver plays a central role in whole-body cholesterol homeostasis and it has been shown recently that the normal hepatic regulation of cholesterogenic flux and cholesterogenic gene expression is dependent upon functional PPARα [1]. Recent studies [2,3] have highlighted an important role for cholesterol in the regulation of lipid metabolism in white adipocytes. In particular, abnormalities in the regulation of cholesterol metabolism and in its distribution within the adipocyte are associated with adipocyte enlargement and consequent disturbances in insulin sensitivity and leptin secretion [4]. Cholesterol-induced changes in the molecular architecture of membrane lipid microdomains may underlie such disturbances in insulin signal transduction [5,6]. The

Abbreviations: NEFA, non-esterified fatty acid; TAG, triacylglycerol; FAS, fatty acid synthase; ACC, acetyl CoA carboxylase; SREBP, sterol regulatory binding protein; PPAR, peroxisome proliferator-activated receptor; LXR, liver-X receptor; LDL, low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl Coenzyme A

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possibility that PPARα can modulate cholesterol metabolism in adipose tissue in the same way as it does in liver has not been investigated. Despite its relatively low expression in white adipose tissue, PPARα plays an important role in the leptin-mediated regulation of body fat content in rodents [7] by altering the balance of fat synthesis and oxidation. In this connection, it has previously been shown that PPARα-deficiency in the liver is associated with a decreased mRNA expression of SREBP-lc, its target genes fatty acid synthase (FAS) and acetyl CoA carboxylase (ACC) and of carbon flux through the lipogenic pathway [8,9]. It is not known whether similar changes occur in white adipose tissue in response to PPARα-deficiency. The present study was designed to provide information about several aspects of the role of PPARα in adipose tissue. First, the extent to which PPARα is involved in the regulation of lipogenic gene expression and lipogenesis. Second, whether PPARα is capable of modifying the response of lipogenic and cholesterogenic genes to a challenge presented by a high-cholesterol diet. Finally, we sought to define the effects of PPARα-deficiency and a high cholesterol diet on the metabolic and physiological integration of white adipose tissue with liver, the efficiency of which is essential for the effective maintenance of whole body lipid balance.

2. Materials and methods

2.1. Materials

RNAzol kits were purchased from Biogenesis (Poole, Dorset, UK). Tritiated water, glycerol [^{14}C]trioleate, and [^{14}C]cholesterol were obtained from Amersham Pharmacia Biotech (Little Chalfont, Bucks, UK). Organic solvents were of analytical grade and were obtained from BDH (Poole, Dorset, UK). General laboratory reagents were from Roche Diagnostics (Lewes, East Sussex, UK) or from Sigma (Poole, Dorset, UK). Mouse insulin assay kits were obtained from Mercodia AB (Uppsala, Sweden). Glucose assay kits were obtained from Randox Labs Ltd. (County Antrim, UK).

2.2. Animals

All procedures performed in this study were approved by the Local Ethics Review Committee of the University of Oxford and were carried out under the authority of the appropriate Home Office (UK) personal and project licenses in accordance with the Home Office Animals (Scientific Procedures) Act 1986.

PPARα-null mice [10], bred onto a SV/129 genetic background, were provided by Dr. J. Peters and Dr. F.J. Gonzalez (National Institutes of Health, Bethesda, MD). Wild-type SV/129 mice (Charles River, UK) were used as controls. Male mice (14–20 weeks old) were housed (4 per cage) and maintained in a temperature controlled, air-conditioned environment, subject to a 12-h light/dark cycle with the dark period beginning at 0300 h. Mice were fed ad libitum with either a standard diet [1] or with the standard diet supplemented with non-esterified cholesterol (2% w/w; 99% purity). The cholesterol-supplemented diet was fed for 7 days.

Mice were anaesthetized by intraperitoneal (i.p.) injection of a solution of sodium pentobarbital in saline. Plasma was obtained from a blood sample derived from the vena cava. Livers and epididymal fat pads were quickly removed, freeze-clamped and stored in liquid nitrogen.

2.3. Measurements of rates of hepatic and adipose tissue cholesterol and fatty acid synthesis in vivo

Mice were injected i.p. with tritiated water (37 MBq) at the mid-point of the dark-phase of the cycle (0900 h), returned to their cages, and killed 2 h later. The specific radioactivity of plasma water 2 h after the injection of tritiated water was determined by scintillation counting. This value was used for determination of the molar incorporation of $^{3}H_2O$ into fatty acids and cholesterol. The labelled hepatic fatty acid fraction was obtained as described by de Vasconcelos et al. [11] following the addition of a known amount of glycerol [^{14}C]trioleate as internal standard to account for losses. Adipose tissue labelled fatty acids were obtained following a total lipid extraction [12] of frozen tissue, ground under liquid nitrogen. Again, a known amount of glycerol [^{14}C]trioleate was added as internal standard. The resulting total lipid fraction was saponified with ethanolic potassium hydroxide and the non-saponifiable lipid fraction was extracted using hexane [13]. The remaining aqueous layer containing the labelled fatty acids was acidified to pH 2.0 and the fatty acid-containing fraction was isolated by extraction with hexane [11]. Cholesterol was purified from the non-saponifiable fraction by thin-layer chromatography as described by Marco de la Calle et al. [14].

2.4. Measurement of mRNA

Frozen adipose tissue was ground to a powder under liquid nitrogen and total RNA was extracted from portions of powder using the RNAzol kit. The amount of mRNA was assayed by reverse transcription followed by real-time PCR using the same methods and the same primers and probes as described previously [1,15] All values were related to a standard adipose tissue extract and were corrected for β-actin mRNA content.

2.5. Measurement of plasma and tissue lipids

Plasma non-esterified fatty acids (NEFA), triacylglycerol (TAG), total cholesterol and non-esterified cholesterol were measured enzymically using commercial kits adapted for use in a “Monarch” analyser as described previously [1].