Peroxisome proliferator-activated receptor α deficiency modifies glucose handling by isolated mouse adipocytes

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Abstract

Peroxisome proliferator-activated receptor α (PPAR α) is a transcription factor that regulates enzymes involved in fatty acid (FA) utilisation. PPAR α null mice have recently been demonstrated to have increased whole-body glucose turnover *in vivo*. This has been attributed to increased glucose uptake by adipose tissue, but the impact of PPAR α deficiency on the characteristics of glucose handling by isolated adipocytes *ex vivo* is unknown. To determine directly the impact of PPAR α deficiency on adipocyte glucose handling, thereby excluding any influence of humoral/neuronal factors, we examined total glucose metabolism as well as glucose disposition towards alternative fates in epididymal adipocytes isolated from wild-type and PPAR α null mice. Total glucose metabolism (oxidation, incorporation into FA and glycerol moieties of triglyceride

(TAG) and conversion to lactate) was measured under basal conditions (low glucose) and 'stimulated lipogenic' conditions (high glucose + insulin). Adipocytes from PPAR α null mice had higher rates of glucose metabolism under both basal and stimulated lipogenic conditions, with increased glucose utilisation both for oxidation and entry into the synthesis of the FA and glycerol components of lipid. In particular, the capacity of adipocytes from PPAR α -deficient mice to utilise glucose for synthesis of the glycerol backbone of TAG was greatly enhanced under stimulated (high glucose + insulin) conditions. The increased use of glucose for the glycerol moiety of adipocyte TAG may therefore contribute to, and provide explanation for, enhanced glucose turnover in PPAR α null mice.

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Introduction

The ability of adipocytes to store triglyceride (TAG) provides animals with a fuel store for use in time of need. In the fed state, insulin increases adipocyte TAG storage by augmenting adipocyte glucose uptake, allowing the production of glycerol-3-phosphate to form the TAG backbone for fatty acid (FA) esterification. Glucose can also be used for the formation of FA *de novo*. Adipocyte glucose uptake and its conversion to glycerol-3-phosphate are also enhanced by the activation of peroxisome proliferator-activated receptor (PPAR) γ (Guan *et al.* 2002, Picard & Auwerx 2002, Tordjman *et al.* 2003, Li *et al.* 2005), a lipogenic transcription factor.

Contrasting with the role of PPAR γ , PPAR α acts as a lipoxidative transcription factor (Kersten *et al.* 1999). It is highly expressed in tissues with high rates of FA oxidation, particularly liver, where it augments the expression of enzymes involved in FA catabolism (Braissant *et al.* 1996). PPAR α is expressed in white adipose tissue, although not highly (Islam *et al.* 2005). PPAR α deficiency in mice maintained on a high-carbohydrate, low-fat diet results in increased rates of FA synthesis in adipose tissue *in vivo* (measured using ³H₂O *in vivo*), which provides an indication

of total flux through the FA synthetic pathway (Islam *et al.* 2005). Recently, it has been demonstrated that postabsorptive (6 h starved) PPAR α null mice exhibit increased whole-body glucose turnover (production and utilisation), which was attributed to increased rates of glucose uptake/phosphorylation by (gonadal) white adipose tissue (measured using radiolabelled 2-deoxyglucose *in vivo*; Knauf *et al.* 2006). This response is unusual since enhanced whole-body glucose turnover is normally accounted for by enhanced glucose uptake (and storage) by skeletal muscle rather than by enhanced adipocyte glucose uptake, since the skeletal muscle mass is considered to be the major site of glucose disposal *in vivo* and rates of glucose uptake/phosphorylation by white adipose tissue are usually relatively low (Issad *et al.* 1987, Holness 1996). The mechanism by which adipocytes can account for a large increase in whole-body glucose disposal therefore remains unaccounted for.

In this study, we examined the effects of PPAR α deficiency on glucose handling by isolated adipocytes from PPAR α null mice. Our aim was to compare the characteristics of glucose handling *ex vivo* in adipocytes isolated from wild-type and PPAR α null mice to establish whether increased glucose utilisation *in vivo* is dependent on systemic or neuronal

influences. A further objective was to determine the metabolic fate of glucose within the adipocyte.

Materials and Methods

Materials

Laboratory reagents were purchased from Roche Diagnostics, Sigma or Fisher Scientific (Loughborough, UK). U[^{14}C] D-glucose was purchased from Amersham Biosciences. Type I collagenase was purchased from Worthington Biochemical Corporation (Lakewood, NJ, USA). Wild-type SV/129 mice were purchased from Charles River (Margate, Kent, UK).

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 licences (G F G) in accordance with the Home Office Animals (Scientific Procedures) Act 1986. PPAR α null mice, bred onto a SV/129 genetic background, were provided by Drs J Peters and FJ Gonzalez (National Institutes of Health, Bethesda, MD, USA). Wild-type SV/129 mice were used as controls. Male mice (30–40 weeks of age) were housed (four to six per cage) and maintained in a temperature-controlled, air-conditioned environment, subject to a 12 h light:12 h darkness cycle beginning at 2000 h. Mice were allowed free access to water and standard laboratory chow. Mice were sampled between 0900 and 1100 h and were therefore in the absorptive state.

Tissue sampling

Mice were anaesthetised by i.p. injection of sodium pentobarbital (80 mg/kg). Fat pads from the epididymal (EPI), s.c. and perirenal (PR) depots were harvested and weighed and the mice were killed by removal of the heart.

Adipocyte preparation and incubation

EPI fat pads were collected in Krebs–Ringer–Hepes-buffer (KRHB) containing 3% insulin-free BSA, 200 nM adenosine and 5 mM glucose (KRHB pH 7.4) and adipocytes were isolated by previously described methods (Rodbell 1964) with minor modifications as detailed in Walker *et al.* (2006). Adipocyte numbers in the final cell preparation were determined by counting under phase contrast microscopy. Adipocyte size was determined using the program Image J by microscopic measurement of 100–120 cells per preparation using seven preparations. There was no overall difference in mean adipocyte diameter (74 ± 10 cf. 76 ± 4 μm) or the range of adipocyte diameters (32–204 cf. 24–190 μm) between EPI adipocytes prepared from fed wild-type and PPAR α null mice.

Glucose fluxes in isolated adipocytes

Adipocytes (100 μl packed cell volume) were pre-incubated in 1 ml glucose-free KRHB under various incubation conditions. Incubations (4 h) were commenced by the addition of U[^{14}C]D-glucose (4 $\mu\text{Ci/ml}$) with a final glucose concentration of 3 or 20 mM. Glucose oxidation was measured by a previously described method (Rodbell 1964) with minor modifications as detailed in Walker *et al.* (2007a). Incubations, once terminated by the addition of 6 M H_2SO_4 , released $^{14}\text{CO}_2$, which was sequestered by 4 M NaOH, spotted onto filter paper and counted with Optiphase Safe scintillation fluid (Fisher Scientific) in a Beckman L6500 scintillation counter (Beckman Instruments, Urbana, IL, USA). Glucose incorporated into glycerol and the FA fraction of TAG was determined as described previously (Dole & Meinertz 1960). Lactate production was measured spectrometrically.

Statistical analyses

Results are presented as the mean $\pm$ S.E.M. Statistical analyses were performed by ANOVA followed by Fisher's *post hoc* tests for individual comparisons or unpaired (*in vivo*) or paired (*in vitro*) Student's *t*-tests as appropriate. Results were considered significant if $P < 0.05$.

Results

Body weight and adiposity in PPAR α null mice

Body weight and fat pad weight (EPI, PR and s.c. depots) were measured in *ad libitum*-fed mice. Although body weight was unchanged (Fig. 1A), both EPI and s.c. fat pad masses

Figure 1 Body weights and adiposity in PPAR α null mice in the fed state. Body weight (A) and fat pad weight (epididymal (EPI), perirenal (PR) and s.c. (SC) depots) (B) were measured in *ad libitum*-fed wild-type mice (open bars) and PPAR α null mice (closed bars) at a mean age of 34 ± 1 weeks. Results are the mean $\pm$ S.E.M. for 30 wild-type mice and 26 PPAR α null mice. Statistically significant differences between wild-type and PPAR α null mice are indicated by * $P < 0.05$.

were lower in the PPAR α null mice compared with those of wild-type mice when expressed as the percentage of whole body weight (Fig. 1B).

Adipocyte glucose metabolism ex vivo under 'fasting' conditions

We incubated isolated EPI adipocytes with low glucose and in the absence of insulin to mimic the fasting extracellular milieu. Absolute rates of glucose oxidation to CO₂ by adipocytes from the PPAR α null mice under low-glucose conditions were 2.3-fold higher than those of adipocytes from the wild-type mice (Fig. 2A). Adipocytes from the PPAR α null mice also had 2.5-fold increased rates of lactate production compared with those from the wild-type mice (Fig. 2B). Thus, both glucose oxidation and its conversion to lactate are favoured in adipocytes from PPAR α null mice under 'fasting' conditions. PPAR α deficiency was also associated with a 13-fold increase in the use of glucose for FA synthesis compared with wild-type mice under low-glucose conditions (Fig. 3A), indicating preferential utilisation of glucose-derived acetyl-CoA for FA synthesis. For both genotypes, glucose incorporation into the glycerol moiety of TAG was quantitatively of much greater importance than its use for FA synthesis *de novo*. For adipocytes from wild-type mice, incorporation of glucose into the glycerol backbone of TAG was 14.2-fold higher than its use for FA synthesis. Glucose incorporation into glycerol was 2.8-fold higher ($P < 0.05$) for adipocytes from PPAR α null mice compared with wild-type mice (Fig. 3A). It is concluded that PPAR α deficiency causes stable changes in adipocyte glucose handling such that there is increased use of glucose for the synthesis of both glycerol-3-phosphate and FA for TAG synthesis, and that PPAR α deficiency permits significant rates of *de novo* FA synthesis and esterification to occur even under conditions of low extracellular glucose.

Effects of insulin stimulation on adipocyte glucose utilisation and disposition

Using isolated EPI adipocytes, we also measured glucose handling by adipocytes under stimulated lipogenic conditions by incubating in 20 mM glucose with 16 nM insulin. Incubation under glucose + insulin-stimulated conditions resulted in only a modest (34%) non-significant increase in glucose oxidation by adipocytes isolated from wild-type mice, whereas there was a much more marked increase (170%; $P < 0.05$) in glucose oxidation by adipocytes from PPAR α null mice (Fig. 2, compare A and C). As a consequence, the amount of glucose oxidised in adipocytes of the PPAR α null mice was 2.9-fold that of wild-type mice under stimulated incubation conditions (Fig. 2C). Production of lactate was stimulated under the lipogenic incubation conditions by 4.4-fold ($P < 0.05$) in adipocytes of wild-type mice and 3.5-fold ($P < 0.05$) in adipocytes of PPAR α null mice (Fig. 2, compare B and D).

Figure 2 Adipocyte glucose oxidation and production of lactate. Adipocytes (100 μ l packed cell volume) were pre-incubated in 1 ml glucose-free KRHB under various incubation conditions. Incubations were commenced by the addition of U[¹⁴C]D-glucose (4 μ Ci/ml). (A) and (B) Data obtained with EPI adipocytes from *ad libitum*-fed wild-type mice (open bars) and PPAR α null mice (closed bars) incubated at 3 mM glucose in the absence of insulin. (C) and (D) Data obtained with EPI adipocytes from *ad libitum*-fed wild-type mice (open bars) and PPAR α null mice (closed bars) incubated at 20 mM glucose in the presence of 16 nM insulin. Glucose oxidation was measured by previously described methods (Rodbell 1964) with minor modifications as detailed in Walker *et al.* (2007a). Lactate production (B and D) was measured spectrophotometrically. Results are the mean $\pm$ S.E.M. for nine adipocyte preparations from wild-type mice and eight adipocyte preparations from PPAR α null mice. Statistically significant differences between adipocytes from wild-type and PPAR α null mice are indicated by * $P < 0.05$ and † $P < 0.01$.

Whereas glucose incorporation into FA was very low with adipocytes from wild-type mice incubated at low glucose, a 7.3-fold increase ($P < 0.05$) was observed under conditions of stimulation by high glucose + insulin (Fig. 3, compare A and B). Glucose incorporation into FA was less markedly affected by switching from low glucose to high glucose + insulin with adipocytes from PPAR α null mice: a 14% increase only. Switching from low glucose to high glucose + insulin elicited

Figure 3 Glucose incorporation into the FA and glycerol moieties of TAG. Glucose incorporation into the FA and glycerol moieties of TAG are shown for adipocytes isolated from wild-type mice (open bars) and PPAR α null mice (closed bars). Data obtained with EPI adipocytes incubated at 3 mM glucose in the absence of insulin are shown in A. (B) Data obtained with EPI adipocytes incubated at 20 mM glucose in the presence of 16 nM insulin. Glucose incorporation into the FA and glycerol moieties of TAG was determined by a previously described method (Dole & Meinertz 1960). Results are the mean $\pm$ S.E.M. for nine adipocyte preparations from wild-type mice and eight adipocyte preparations from PPAR α null mice. Statistically significant differences between adipocytes from wild-type and PPAR α null mice are indicated by * P < 0.05 and [†] P < 0.01.

a 1.7-fold increase (P < 0.01) in incorporation of glucose into the glycerol backbone of TAG with adipocytes from wild-type mice; an increase of similar magnitude was observed with adipocytes from PPAR α null mice (1.6-fold; Fig. 3). Thus, incorporation of glucose into the glycerol backbone of TAG remained significantly greater than its contribution to the FA moiety of TAG in the stimulated state for both genotypes, but adipocytes from PPAR α null mice were characterised by markedly higher rates of glucose incorporation into the glycerol moiety of TAG (Fig. 3B). A 1.5-fold increase in lipolysis (assessed by glycerol release) was seen in adipocytes from PPAR α null mice compared with wild-type mice (1580 ± 460 cf. 1056 ± 433 nmol glycerol released per 10^6 cells). Thus, increased availability of FA may have allowed the increased formation of TAG, incorporating the glycerol backbone generated from glucose.

Discussion

Recent research has demonstrated that postabsorptive (6 h starved) PPAR α null mice show increased whole-body glucose turnover (production and utilisation; Knauf *et al.* 2006). The latter was attributed to an increased rate of glucose uptake by white adipose tissue (measured using radiolabelled 2-deoxyglucose *in vivo*; Knauf *et al.* 2006). In the present study, adipocyte glucose handling was examined *ex vivo* using isolated adipocytes from age-matched PPAR α null and wild-type mice. We delineated the characteristics of adipocyte glucose handling *ex vivo* in PPAR α null mice, demonstrating that increased glucose utilisation is intrinsic to the adipose tissue itself, either as a direct result of PPAR α deficiency in

the adipocyte or due to conditioning from the whole-body PPAR α phenotype. We also show that PPAR α deficiency allows a much higher rate of glucose incorporation into the glycerol backbone of TAG, both under conditions of insulin stimulation and also under basal conditions.

In the paper by Knauf *et al.* (2006), white adipocytes were found to be larger as were the adipose tissue depots of PPAR α null mice, whereas we have consistently found adipose depots to be smaller in the PPAR α null mice colony that we utilise (Walker *et al.* 2007b). This may be a result of difference in age, as the mice in the present study were 30–40 weeks of age compared with those used in the study by Knauf *et al.* (2006) that were only 14 weeks of age. Nevertheless, our results of increased adipocyte glucose metabolism *in vitro* consolidate the findings of Knauf *et al.* (2006) of increased glucose uptake by adipose tissue *in vivo*. We believe that this indicates that, regardless of adipocyte size, these changes in adipocyte glucose metabolism may be intrinsic to the PPAR α null phenotype and consistent among the colonies of PPAR α null mice. In the fed state, adipocyte TAG storage is promoted by augmenting adipocyte glucose uptake, glycerol-3-phosphate formation and FA synthesis *de novo*. These events are co-ordinated by insulin and PPAR γ activation. PPAR α signalling is regarded as minimal in the well-fed state, and the PPAR α null mouse (a model of PPAR α deficiency), when maintained on standard high-carbohydrate/low-fat diet, does not exhibit any obvious metabolic phenotype. However, when PPAR α null mice are maintained on a high-fat diet, they become more obese than their wild-type counterparts and are protected from the development of dietary lipid-induced insulin resistance (Guerre-Millo *et al.* 2001). The absence of PPAR α , by lowering the rate of FA oxidation in tissues that normally exhibit high rates of FA uptake and oxidation (e.g. liver, skeletal muscles), was proposed to favour glucose oxidation in these tissues and, therefore, glucose clearance when a high-fat diet was provided. PPAR α is expressed in white adipose tissue from *ad libitum*-fed wild-type mice (Islam *et al.* 2005) and here we demonstrate that glucose utilisation, both for oxidation and entry into lipid synthesis, is augmented in white adipocytes prepared from fed PPAR α -deficient mice.

The mechanism by which glucose metabolism, in particular lipogenesis, is up-regulated in the PPAR α null mice is not clear. Although rates of flux through the fatty acid pathway (measured *in vivo* by ^3H incorporation from $^3\text{H}_2\text{O}$, which measures total lipogenesis from all available substrates) are greatly increased in EPI adipose tissue of *ad libitum*-fed PPAR α null mice, gene expression of the lipogenic transcription factor SREBP-1c and lipogenic gene acetyl-CoA carboxylase has previously been shown to be lower (Islam *et al.* 2005). Previous studies have also demonstrated increased GLUT4 expression in adipose tissue of PPAR α null mice in the fasting (but not in the fed) state (Knauf *et al.* 2006). Thus, altered GLUT4 expression is unlikely to underlie the changes observed in glucose metabolism in the present experiments when adipocytes prepared from fed mice

are stimulated with insulin. However, as changes in GLUT4 responsiveness to insulin can be manipulated within 20 min in isolated adipocytes (Haruta *et al.* 1995), it is possible that PPAR α status may have altered GLUT4 recruitment during the incubation, contributing to the observed changes in glucose metabolism.

While re-expression of PPAR α in the livers of null mice does not lower whole-body glucose turnover (and by implication adipocyte glucose utilisation) *in vivo*, infusion of the PPAR α agonist WY14,643 into the lateral ventricle of the brain for 3 h specifically lowers adipose tissue glucose uptake/phosphorylation *in vivo* (Knauf *et al.* 2006). It was proposed that the effect of PPAR α activation in the brain to lower glucose uptake might be exerted through regulation of cerebral neuropeptide expression by PPAR α activation. Although we did not directly investigate the impact of altered neuro/hormonal stimulation on adipocyte function *in vitro*, our data show that increased glucose metabolism in adipocytes from PPAR α null mice is stable to adipocyte preparation and incubation, and thus does not require a sustained neuro/hormonal stimulus.

In summary, our data demonstrate that glucose utilisation, both for oxidation and entry into lipid synthesis, is augmented in white adipocytes from PPAR α null mice. In particular, the capacity of adipocytes from PPAR α -deficient mice to utilise glucose for synthesis of the glycerol backbone of TAG is greatly enhanced under stimulated (high glucose + insulin) conditions. The increased use of glucose for the glycerol moiety of adipocyte TAG may therefore contribute to, and provide explanation for, enhanced glucose turnover in PPAR α null mice.

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