Depressed Levels of Prostaglandin F$_{2\alpha}$ in Mice Lacking Akr1b7 Increase Basal Adiposity and Predispose to Diet-Induced Obesity

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Negative regulators of white adipose tissue (WAT) expansion are poorly documented in vivo. Prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) is a potent antiadipogenic factor in cultured preadipocytes, but evidence for its involvement in physiological context is lacking. We previously reported that Akr1b7, an aldo-keto reductase enriched in adipose stromal vascular fraction but absent from mature adipocytes, has antiadipogenic properties possibly supported by PGF$_{2\alpha}$ synthase activity. To test whether lack of Akr1b7 could influence WAT homeostasis in vivo, we generated Akr1b7$^{-/-}$ mice in 129/Sv background. Akr1b7$^{-/-}$ mice displayed excessive basal adiposity resulting from adipocyte hyperplasia/hypertrophy and exhibited greater sensitivity to diet-induced obesity. Following adipose enlargement and irrespective of the diet, they developed liver steatosis and progressive insulin resistance. Akr1b7 loss was associated with decreased PGF$_{2\alpha}$ WAT contents. Cloprostenol (PGF$_{2\alpha}$ agonist) administration to Akr1b7$^{-/-}$ mice normalized WAT expansion by affecting both de novo adipocyte differentiation and size. Treatment of 3T3-L1 adipocytes and Akr1b7$^{-/-}$ mice with cloprostenol suggested that decreased adipocyte size resulted from inhibition of lipogenic gene expression. Hence, Akr1b7 is a major regulator of WAT development through at least two PGF$_{2\alpha}$-dependent mechanisms: inhibition of adipogenesis and lipogenesis. These findings provide molecular rationale to explore the status of aldo-keto reductases in dysregulations of adipose tissue homeostasis.

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Obesity is the result of genetic predisposition and environmental factors that confer an imbalance between energy intake and energy expenditure (1). Resulting energy excess is stored as triglycerides in white adipose tissue (WAT). Adult obesity is typically characterized by adipocyte hypertrophy when triglyceride synthesis exceeds breakdown (lipolysis). However, a concomitant adipocyte hyperplasia may also occur as a result of increased recruitment and differentiation of precursor cells (adipogenesis) (2,3).

In addition to its role in energy storage, WAT is also a highly active metabolic and endocrine organ that regulates energy homeostasis by secreting a variety of adipokines (4). These factors may act at local (auto/paracrine) or systemic (endocrine) levels and enable WAT to modulate its own metabolic activities or that of other tissues. So, excess or deficiency of adipose tissue may generate metabolic disorders such as liver steatosis, insulin resistance, type 2 diabetes, and cardiovascular diseases.

The use of well-characterized preadipocyte cell lines, 3T3-L1 or 3T3-F442A, allowed us to draw a network of transcription factors regulating differentiation of fibroblast-like preadipocytes to lipid-loaded adipocytes (5,6). Peroxisome proliferator-activated receptor γ and CCAAT/enhancer-binding proteins emerged as master regulators of the transcriptional cascade that drives adipogenesis (7,8). Expression and activity of these transcriptional factors are controlled by various upstream stimuli (hormonal, paracrine, or nutritional), leading to the regulation of adipocyte-specific genes. Therefore, study of the balance between negative and positive regulators of adipogenesis is essential for understanding the early mechanisms involved in the establishment of obesity.

Prostaglandins (PG) are lipid paracrine mediators that were shown to play opposite and sometimes conflicting actions on cultured adipocytes. Therefore, until the recent development of genetically engineered mice models, their physiological importance in adipose tissue homeostasis remained unclear. PGE$_2$ and PGD$_2$/PGJ$_2$ were shown to promote adiposity in mice through inhibition of lipolysis and induction of adipogenesis, respectively (9-11). By contrast, PGF$_{2\alpha}$ is a potent inhibitor of adipocyte differentiation in vitro (12-14). PGF$_{2\alpha}$, via its PGF (FP) receptor, activates downstream signaling pathways involving mitogen-activated protein kinase and/or calcineurin. This leads to inhibition of expression or activity of the proadipogenic transcription factors peroxisome proliferator-activated receptor γ and CCAAT/enhancer-binding protein α (12,15,16). Although the molecular action of PGF$_{2\alpha}$ has been well-described in cell cultures, its role in adipose tissue homeostasis in vivo has not been elucidated so far as the PGF synthases (PGFS) responsible for its production were only recently identified.

Indeed, we showed that some aldo-keto reductase 1B (AKR1B) members exhibited PGFS activity in vitro. Human AKR1B1 and murine Akr1b3 and Akr1b7 catalyse the synthesis of PGF$_{2\alpha}$ from its precursor PGH$_2$ through

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their 9,11-endoperoxide reductase activity (17). Although a recent study indicated that the PGFS activity of Akr1b3 can influence adipogenesis in 3T3-L1 cells (18), no defect in adipose tissue homeostasis was reported in Akr1b3-deficient mice (19). Contrasting with the ubiquitous Akr1b3 protein, Akr1b7 isoform is expressed in a restricted set of tissues (i.e., adrenal glands, reproductive organs, and intestine) (20–23) and was found more recently in the adipose tissue (24). Interestingly, in fat depots, Akr1b7 expression is limited to the preadipocyte-enriched stromal fraction and downregulated during the differentiation into adipocytes (24). Consistently, overexpression of Akr1b7 in 3T3-L1 preadipocytes inhibits their differentiation, whereas Akr1b7 knockdown accelerates the adipogenic program. All of these data show that Akr1b7 exerts an antiadipogenic action in cell culture, although the mechanisms involved and the potential role of its PGFS activity remain to be established. We recently demonstrated that Akr1b7 is able to regulate the stress-induced response of adrenal gland by generating a PGF2α signature in vivo (25). Hence, we hypothesized that the enrichment of Akr1b7 in the adipose stromal fraction could have a key role in preventing adiposity in vivo by controlling PGF2α content. To firmly demonstrate the antiadipogenic action of Akr1b7 and the possible involvement of PGF2α in this process, we generated Akr1b7−/− mice and characterized their phenotype under standard or obesogenic diet conditions. We report that ablation of Akr1b7 increases adiposity under regular diet and decreases metabolic rate. This predisposes mice to develop severe obesity under a high-fat diet (HFD). These phenotypes result from deregulation of both adipogenesis and lipogenesis through a PGF2α-dependent mechanism.

**RESEARCH DESIGN AND METHODS**

*Generation of Akr1b7-null mice. Akr1b7 genomic vector was created using PCR-amplified fragments from a phage λ genomic clone containing the whole Akr1b7 gene. The targeted construct was generated by inserting hygromycin resistance cassette flanked by two loxP sites and a third loxP to flank exons 4–6 (Fig. 1A). The 11-kb targeting fragment was linearized and electroporated into 129/Sv embryonic stem cells. Hygromycin-resistant clones were screened for homologous recombination by Southern blot of HindIII-digested DNA with the 5′ untranslated region probe of the Akr1b7 gene. Chimeric mice, containing a targeting construct integrated into 129/Sv WT mice were crossed with 129/Sv wild-type (WT) females to screen for germline transmission. Homozygous animals for Akr1b7fl/fl allele were crossed with mice of the MeuCre40 transgenic line (26) to obtain recombination between loxP sites and to delete exons 4–6 were detected by Southern blotting. They were crossed with 129/Sv WT animals to generate Akr1b7−/− mice. Details on vector construction or genotyping are available on request. Akr1b7−/− mice were born viable at Mendelian frequency and showed no defects in reproductive functions as reported for a previous model of Akr1b7 knockout (KO) mice (27).

*Mice, diet, and treatments. All animal studies were approved by the Anzures Ethics committee and conducted in agreement with international standards for animal welfare. WT and mutant litterate (KO) males were fed ad libitum a standard diet (Harlan Diet) or a high-fat diet (HFD) (63% kcal fat, 19% carbohydrate, 16% protein) (USFS) for either 12 or 5 weeks, respectively. Food intake was measured daily over a 6-day period. For nephrotic syndrome studies, Akr1b7−/− mice were intraperitoneally injected daily with vehicle PBS or cloprenol (0.1 mg/kg/day; Sigma-Aldrich) throughout the duration of HFD protocols. Mice were killed by decapitation, and trunical blood was collected.*

*Ambulatory activity. Ambulatory activity of 3-, 9-, and 18-month-old mice was recorded with the Actiview hardware (IntelliBio). Mice were housed individually in cages containing an activity wheel (IntelliBio). The recordings of locomotor activity were performed at least on six mice of each genotype. Two recordings of 24 h were performed per mouse. Indirect calorimetry. Respiratory gas exchanges were measured continuously using whole-body calorimetric chambers (TSE Systems, Bad Homburg v.d.h., Germany). Energy expenditure was calculated using Weir’s equation (28). Respiratory quotient was calculated from the ratio between CO2 production and O2 consumption.*

**RESULTS**

*Generation of Akr1b7−/− mice. The strategy to generate Akr1b7−/− mice is depicted in Fig. 1A–C. In WT mice, Akr1b7 was strongly detected in the adrenal gland and to a lesser extent in WAT. In contrast, it was undetectable in brown adipose tissue (BAT), muscle, or liver (Fig. 1D). Absence of Akr1b7 signal in KO tissues confirmed complete inactivation of the Akr1b7 gene. Mouse also expresses two other members of Akr1b family, Akr1b9 and Akr1b8, plasma and intratissular metabolites. Blood samples were collected after an overnight fasting. Insulin/leptin and adiponectin/resistin (Thermo Fisher Scientific) levels were measured by ELISA kits (BioVendor, Assaypro; both Thermo Fisher Scientific). Plasma concentration of cholesterol, triglycerides (Thermo Fisher Scientific), glyceral, and free fatty acids (Randox) were determined using commercial kits. For intratissular lipids assays, lipids were extracted from livers as previously described (29).**
but their expression pattern in adipose tissue has never been characterized. Using specific antibodies (25), we showed that Akr1b3 was expressed in all tissues tested, whereas Akr1b8 was absent from all adipose depots and muscle but abundant in adrenal and liver. Importantly, accumulation of both Akr1b3 and Akr1b8 isoforms was not upregulated in Akr1b7$^{-/-}$ tissues.

Akr1b7 ablation results in increased adipogenesis and lipogenesis. Under standard diet, Akr1b7$^{-/-}$ mice gained weight faster than WT mice starting at 7 to 8 weeks. This difference was exacerbated with time (Fig. 2A). Six-month-old Akr1b7$^{-/-}$ mice exhibited a significant excess of major WAT depots, such as gonadal, peri-adrenal, and inguinal WAT (Fig. 2B) and an increased liver mass. In contrast, weight of other organs including heart, kidney, and testis was not affected. Therefore, lack of Akr1b7 led to a significant overweight, correlated with expansion of WAT. This was confirmed by higher plasma leptin levels in KO mice (Table 1). In contrast, there was no alteration of adiponectin or resistin levels (Table 1).

Measurement of cross-sectional areas of adipocytes revealed clear adipocyte hypertrophy in all Akr1b7$^{-/-}$ white adipose depots compared with WT (Fig. 2C). In agreement with this observation, mRNA levels of the lipogenic genes Scd1 and Fasn were found increased in mutant gonadal WAT (Fig. 2D), suggesting enhancement of the lipogenic capacity, although no change was recorded for expression of Srebp-1c and genes related to triglyceride synthesis. In addition, the DNA content of the fat depots was significantly higher in Akr1b7$^{-/-}$ than in WT mice, demonstrating increased cellularity (Fig. 2E). Altogether, these results suggest that under standard diet, deletion of Akr1b7 leads to expansion of adipose tissue, which may be the result of improvement of both lipid storage, leading to adipocyte hypertrophy and adipogenesis, which leads to adipocyte hyperplasia.

The increased adiposity of Akr1b7$^{-/-}$ mice relative to their WT counterparts was not attributable to differences in food consumption and ambulatory activity. Indirect calorimetry revealed that Akr1b7$^{-/-}$ mice exhibited decreased energy expenditure (1.510 ± 0.055 vs. 1.358 ± 0.025 kJ/24 h/g).
and respiratory exchange ratio (0.91 ± 0.02 vs. 0.86 ± 0.02) (Supplementary Fig. 1). These data indicate that Akr1b7−/− mice have a lower metabolic rate and a switch to relatively more fat oxidation.

Lack of Akr1b7 predisposes to diet-induced obesity. To test whether lack of Akr1b7 gene could predispose to diet-induced obesity, we fed 5-week-old WT and KO mice with an HFD for 12 weeks and monitored growth.
In contrast with WT HFD mice, Akr1b7−/− HFD mice gained weight rapidly and became obese. This was associated with a fivefold increase in plasma leptin levels and reduced food intake (Fig. 3). The exacerbated obese phenotype observed in Akr1b7−/− mice fed with an HFD compared with regular diet was accompanied by a marked increase in plasma levels of triglycerides, glycerol, and free fatty acids, which remained unchanged under a standard diet (Tables 1 and 2). We concluded that lack of Akr1b7 predisposed mice to develop severe obesity on an HFD.

Akr1b7−/− mice display liver steatosis and progressive insulin resistance. Oil Red O staining and triglycerides assays revealed that Akr1b7−/− liver accumulated a large amount of lipid droplets and contained twice as many triglycerides than WT (Supplementary Fig. 2B), confirming that Akr1b7−/− mice fed with a standard diet developed liver steatosis from 6 months of age.

To determine the respective roles of WAT lipolysis and liver lipogenesis in steatosis, plasmatic pools of lipids were measured, and mRNAs of genes involved in lipid and glucose metabolism were quantified in the liver. Compared with WT mice, Akr1b7−/− mice showed unchanged fasted plasma levels of free fatty acids and glycerol (Table 1), suggesting that lack of Akr1b7 did not increase basal WAT lipolysis. In contrast, the significant increase in Scd1 expression in the liver of KO mice was correlated to the presence of steatosis (Supplementary Fig. 2B). However, this link remains unclear because expression of upstream lipogenic genes and triglyceride synthesis genes was unchanged. Finally, mRNA expression of genes related to β-oxidation and gluconeogenesis was unaltered in mutant mice.

Excess of adipose tissue and liver steatosis are frequently associated with altered glucose homeostasis and insulin resistance. Although fasting plasma glucose tended to be more elevated in 6-month-old Akr1b7−/− than in WT mice (Fig. 4A, right), both exhibited similar responses to the glucose tolerance test (Fig. 4A, left). However, Akr1b7−/− mice showed an attenuated response to insulin during the insulin tolerance test (Fig. 4B, left). The absence of significant fasted hyperglycemia in Akr1b7−/− mice along with basal hyperinsulinemia (Fig. 4B, right) suggest a resistance to the insulin-mediated action on hepatic glucose output.

To examine time-dependent changes in insulin sensitivity, we quantified Akt phosphorylation on Ser473 in response to insulin surge in peripheral tissues of 3-, 6-, and 9-month-old mutant and WT mice. Insulin-induced Akt phosphorylation remained unchanged in muscle, liver, or WAT of 3-month-old Akr1b7−/− mice (Fig. 4C) in agreement with their normal response to the insulin tolerance

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**FIG. 3.** Akr1b7−/− mice are more sensitive to diet-induced obesity. A: Body weight gain of WT and Akr1b7−/− mice fed with HFD. *P < 0.05, **P < 0.01, ***P < 0.001 using two-way ANOVA and Bonferroni posttest. B: Plasma leptin levels of 17-week-old WT and Akr1b7−/− mice fed with HFD. C: Food intake measured at 16 weeks of age every day over a 6-day period during HFD. n = 6 to 7/group for each experiment. *P < 0.05 using Student t test.

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**TABLE 1.** Plasma parameters of 24-week-old WT and Akr1b7-null mice

| Variables                  | Standard diet | HFD | P value* |
|----------------------------|---------------|-----|----------|
| Blood metabolites          |               |     |          |
| Triglycerides (g/L)        | 1.04 ± 0.1    | 14  | 1.07 ± 0.2 | 14  | 0.65   |
| Cholesterol (g/L)          | 1.22 ± 0.2    | 14  | 1.18 ± 0.3 | 14  | 0.65   |
| Free fatty acid (mmol/L)   | 0.93 ± 0.2    | 14  | 0.93 ± 0.3 | 14  | 0.73   |
| Glycerol (μmol/L)          | 346 ± 62      | 14  | 365 ± 70  | 14  | 0.46   |
| Glucocorticoid (ng/mL)     | 31.4 ± 17     | 5   | 37.1 ± 14 | 5   | 0.6    |
| Adipokines                 |               |     |          |
| Leptin (ng/mL)             | 6.70 ± 2.1    | 6   | 10.34 ± 2.3 | 6   | 0.04†  |
| Adiponectin (ng/mL)        | 9.94 ± 2.3    | 6   | 11.11 ± 2.9 | 6   | 0.46   |
| Resistin (ng/mL)           | 1.08 ± 0.1    | 6   | 1.15 ± 0.2 | 6   | 0.51   |

*P values are the results of nonpaired Student t test. †P ≤ 0.05.
test (not shown). In contrast, insulin-dependent Ser473 phosphorylation was reduced by 42% in liver from mutant mice of 6 months of age, whereas both muscle and WAT retained normal insulin response. Finally, Akt phosphorylation was reduced by at least 40% in all three tissues from 9-month-old mutant mice (Fig. 4C). Thus, whole-body insulin resistance observed in mutant mice of 6 months of age is at least linked to a defect in insulin-induced Akt phosphorylation in liver. In these mice, unaffected insulin responsiveness in other peripheral tissues implies normal muscle (and WAT) glucose disposal and likely explains the unaffected postchallenge glucose levels (Fig. 4A). Importantly, progressive insulin resistance of Akr1b7 mice of 6 months of age is at least linked to a defect in insulin-induced Akt phosphorylation in liver. In these mice, unaffected insulin responsiveness in other peripheral tissues implies normal muscle (and WAT) glucose disposal and likely explains the unaffected postchallenge glucose levels (Fig. 4A). Importantly, progressive insulin resistance of Akr1b7 mice occurs from the age of 6 months secondarily to establishment of the overweight phenotype.

**Expansion of Akr1b7**<sup>−/−</sup> **adipose tissue is linked to decreased PGF<sub>2α</sub> levels.** Our observations suggest that Akr1b7 is acting on adipose tissue by regulating both adipocyte differentiation and lipogenesis. We previously showed that Akr1b7 is endowed with a PGFS activity both in vitro and in living cells (17,25). When we compared the levels of various PGs in adipose tissue from Akr1b7<sup>−/−</sup> and WT mice (Fig. 5A), we found that PGF<sub>2α</sub> contents were specifically reduced by 45% in Akr1b7-deficient adipose tissue, whereas PGF<sub>2β</sub> and PGE<sub>2</sub> were unaltered. Importantly, these changes occurred without any alteration in Akr1b3 gene expression (Fig. 5B), which has been reported to affect adipocyte differentiation in vitro (18). We next examined expression of the receptors for PGF<sub>2α</sub> (FP<sub>A</sub> and FP<sub>B</sub>) in various mouse tissues and found that adipose depots were major sites of FP protein expression (Fig. 5C).

To confirm that the alteration of adipose tissue homeostasis resulted from decreased PGF<sub>2α</sub> production, 4-week-old Akr1b7<sup>−/−</sup> mice were treated daily for 3 months with cloprostenol, a synthetic PGF<sub>2α</sub>. Plasma leptin levels of Akr1b7<sup>−/−</sup> mice were significantly decreased by cloprostenol (Fig. 6A), reflecting a reduction of WAT mass. Indeed, compared with Akr1b7<sup>−/−</sup> control mice, mice injected with cloprostenol showed a significant reduction in adipocyte areas and lower Scd1 gene expression in gonadal WAT (Fig. 6B and C). Hence, in good agreement with the PGFS activity of Akr1b7, cloprostenol treatment was able to correct increased adiposity observed in Akr1b7-deficient mice.

Our in vivo data (Figs. 2C–E and 6A–C) showed that lack of Akr1b7, by lowering PGF<sub>2α</sub> levels, led to hypertrophy of adipocytes in which high levels of FP receptors were detected (Fig. 5C). To test a direct impact of PGF<sub>2α</sub> on lipogenesis, we treated 3T3-L1 adipocytes with various concentrations of cloprostenol. Cloprostenol reduced mRNA levels of Scd1 and Fasn genes in a dose-dependent manner, whereas neither Srebp-1c transcripts nor mature protein levels were affected (Fig. 6D). This result demonstrated that in addition to inhibiting adipocyte differentiation, PGF<sub>2α</sub> could also repress lipogenic genes in mature adipocytes in an Srebp-1c-independent manner.

To test the possibility that expansion of WAT in Akr1b7<sup>−/−</sup> mice could also be the result of an improved recruitment of new adipocytes, mice were exposed to HFD for 5 weeks in the presence or absence of cloprostenol and treated with BrdU for the last 3 weeks. In agreement with the sensitivity of Akr1b7-deficient WAT to expand in response to long-term HFD (Fig. 3), a 5-week exposure to HFD was sufficient to impart a 7.7-fold increase of gonadal WAT mass in Akr1b7<sup>−/−</sup> mice compared with the 1.7-fold increase observed in WT mice (Fig. 6E). Concomitant cloprostenol treatment eventually reduced the expansion of mutant WAT mass to values similar to those of mutant mice fed with a standard diet. Quantification of new adipocyte production during HFD exposure revealed a 3.5-fold increase in BrdU-positive adipocyte-associated nuclei in gonadal WAT from Akr1b7<sup>−/−</sup> compared with WT mice (Fig. 6F). Cloprostenol treatment of Akr1b7<sup>−/−</sup> mice reduced production of new adipocytes during HFD-induced adipose tissue expansion to values similar to those of WT mice fed with HFD. Altogether, these data supported in vivo evidence that the PGFS activity of Akr1b7 regulates adipose tissue expansion by repressing both adipocyte hypertrophy and adipocyte recruitment.

**DISCUSSION**

**Akr1b7 has antiadipogenic and antilipogenic activities.** We investigated the physiological function of Akr1b7, a protein enriched in stromal vascular fraction of WAT, by generating a KO mouse model. Our results showed for the first time the direct contribution of a protein producing the antiadipogenic paracrine factor PGF<sub>2α</sub> in the regulation of adipose tissue expansion. Indeed, even under standard diet, Akr1b7<sup>−/−</sup> mice exhibited an increased number and size of white adipose cells. This increased adiposity was correlated with a specific decrease in PGF<sub>2α</sub> concentrations in WAT. The ability of chronic administration of a PGF<sub>2α</sub> agonist to reverse the adipose phenotype of Akr1b7<sup>−/−</sup> mice shows that antiadipogenic activity of Akr1b7 is essentially resulting from its PGFS activity.

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**TABLE 2**

| Variables       | WT          | Akr1b7<sup>−/−</sup> | P value* |
|-----------------|-------------|-------------------|----------|
| Blood metabolites |            |                   |          |
| Triglycerides (g/L) | 0.83 ± 0.12 | 1.2 ± 0.24         | 0.01†    |
| Cholesterol (g/L)  | 1.45 ± 0.33 | 1.41 ± 0.24        | 0.79     |
| Free fatty acid (mmol/L) | 0.63 ± 0.11 | 0.75 ± 0.10        | 0.06     |
| Glycerol (μmol/L)  | 276 ± 58    | 427 ± 94           | 0.009†   |
| Adipokines       |            |                   |          |
| Leptin (ng/mL)   | 4.22 ± 3.37 | 21.6 ± 12.8        | 0.03‡    |
| Adiponectin (ng/mL) | 9.31 ± 2.56 | 15.12 ± 2.33       | 0.02‡    |
| Resistin (ng/mL)  | 2.09 ± 1.0  | 1.50 ± 0.61        | 0.25     |

*P values are the results of nonpaired Student t test. †P ≤ 0.01, ‡P ≤ 0.05.
FIG. 4. Lack of Akr1b7 leads to insulin resistance. A and B: Left, glucose tolerance test (GTT) and insulin tolerance test (ITT). Six-month-old WT and Akr1b7 null mice (n = 7/group) were either fasted for 16 h before receiving an intraperitoneal injection of 2 g/kg glucose or 5 h before receiving an intraperitoneal injection of 0.75 units/kg insulin. Blood glucose concentration was measured at the indicated time points. Right, fasting plasma glucose and insulin levels of the WT and Akr1b7 KO mice fasted overnight. C: Right, Western blot analysis of phospho-Akt (P-Akt) at Ser473 and total Akt levels in WAT, liver, and muscle of 3- (top), 6- (middle), and 9-month-old (bottom) WT and Akr1b7 KO mice after insulin injection. Left, quantifications of insulin-induced Akt Ser473 phosphorylation reported to total Akt levels in WT and Akr1b7 KO mice normalized with β-actin signal. *P < 0.05, **P < 0.005 using Student t test.
This is the first demonstration that PGF$_{2\alpha}$ is indeed an antiadipogenic molecule in vivo. Moreover, we showed that the antiadipogenic function of PGF$_{2\alpha}$ was not only resulting from inhibition of the recruitment of preadipocytes but also from inhibition of adipocyte lipogenesis in Akr1b7$^{-/-}$ mice. This direct effect of PGF$_{2\alpha}$ on lipogenesis was confirmed in 3T3-L1 adipocytes.

Akr1b7$^{-/-}$ mice showed exacerbated sensitivity to obesity in a 129/Sv genetic background. This phenotype is associated with metabolic disorders such as hepato-steatosis and insulin resistance even under standard diet. Therefore, Akr1b7$^{-/-}$ mouse is a powerful model to study obesity and its complications.

The phenotype of Akr1b7$^{-/-}$ mice is linked to Akr1b7 function in adipose tissue. In the adrenal cortex, Akr1b7 expression was shown to limit glucocorticoids release under stress conditions through an internal-feedback loop (25). Glucocorticoids are powerful regulators of adipose tissue homeostasis (30). Nevertheless, we showed that basal plasma corticosterone levels were not affected in Akr1b7$^{-/-}$ mice (Table 1). This excludes an adrenal contribution to the adipose phenotype.

In the intestine, Akr1b7 expression is regulated by several nuclear receptors and is mainly implicated in detoxification processes by metabolizing bile acids or lipid peroxidation products (23,31,32). This function could influence nutrient intake and disrupt energy balance. However, we did not find any alterations of food consumption, fecal production, or fat content (Supplementary Fig. 1) and intestine histology (not shown) in Akr1b7$^{-/-}$ mice. Therefore, it seems unlikely that lack of Akr1b7 in the intestine could account for increased adiposity in Akr1b7$^{-/-}$ mice.

Akr1b14 mRNA, the rat ortholog of Akr1b7, is undetectable in the liver until induction by growth hormone...
FIG. 6. Phenotypic rescue by injection of PGF\_2\_a agonist. A–C: Akr1b7-null mice were daily injected with 0.1 \( \mu \)g/g body weight of cloprostenol, a PGF\_2\_a agonist, or with vehicle from 4–16 weeks of age (n = 7–9/group). A: Fasted plasma leptin levels from vehicle WT and KO mice and from KO mice treated with cloprostenol (KO + clopro.). Statistical significance was assessed by Mann–Whitney test. B: Analysis of adipocyte proportion according to their size. Adipocyte area was measured on paraffin sections from Akr1b7 KO mice treated with vehicle (KO) or with cloprostenol (KO + clopro.). C: Expression of lipogenic genes Scd1 and Fasn in adipose tissue from Akr1b7 KO mice treated with vehicle (KO) or with cloprostenol (KO + clopro.) analyzed by RTqPCR. D: RTqPCR of lipogenic genes Scd1, Fasn, Acc1, and Srebp-1c (left) and Western blot analysis of Srebp-1 precursor and mature cleaved forms in 3T3-L1 adipocytes (right). Differentiated adipocytes were treated 24 h with various concentrations of cloprostenol or with vehicle. E and F: Cloprostenol effect on adipose tissue expansion induced by 5-week HFD in Akr1b7 KO mice. E: Weight of gonadal WAT from WT mice, KO mice, and KO mice treated with cloprostenol (KO + clopro.) after standard diet (SD) or HFD. F: Detection of new adipocyte production in vivo by BrdU labeling. Confocal immunofluorescence analysis was performed on paraffin section taken from WT and KO animals exposed to BrdU over the last 3 weeks of the 5-week HFD with or without cloprostenol treatment. Nuclei were stained using Hoechst, and autofluorescence from the green channel was used to detect tissue structures. Quantification of BrdU-positive adipocyte-associated nuclei was carried out from n = >900 nuclei/group (n = 4/group). *P < 0.05, **P < 0.01, ***P < 0.005 using Student t test. (A high-quality digital representation of this figure is available in the online issue.)
or oxidative stress (33,34). In mouse, Akr1b7 expression is hardly detected in the liver under basal conditions (21) but is inducible by farnesoid X receptor/pregnane X receptor agonists (31,32). Recently, it was shown that forced expression of Akr1b7 in the liver using adenoviral vector significantly lowered hepatic lipid contents in genetically obese mice, suggesting a protective effect against hepatosteatosis (35). Hence, we cannot completely exclude that hepatosteatosis observed in Akr1b7−/− mice could also result from the chronic lack of a very faint hepatic expression of the enzyme. However, several arguments suggest that this metabolic complication is rather the consequence of altered adipose tissue physiology in Akr1b7-null mice. First, using our antibody designed to discriminate Akr1b isoforms (25,36), we never managed to detect Akr1b7 in WT mouse liver (Fig. 1D). Second, we demonstrated that hepatosteatosis and insulin resistance were established from the age of 6 months, far after the increase of adiposity in Akr1b7−/− mice. Finally, it is well-established that excessive development of adipose tissue can lead to hepatosteatosis and insulin resistance through several interconnected pathways (37). Thus, the overall phenotype of Akr1b7−/− mice can mainly be attributed to Akr1b7 function in the adipose tissue.

Akr1b7-null mice phenotype does not rely on defects in detoxification of lipid peroxidation by-products. Besides its PGF2α synthase activity, Akr1b7 has other reported enzymatic activities with possible impacts on adipose tissue, such as reduction of 4-hydroxynonenal (4-HNE) (17,25,38). Increased 4-HNE levels in response to oxidative stress promote and perpetuate the obese state (39).}

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**Akr1b7-dependent production of PGF2α regulates adipose tissue homeostasis.** For decades, PGF2α has been implicated in the inhibition of adipogenesis (12–14), but its function on adipocyte differentiation was never established in vivo or tested on already differentiated tissues. We demonstrate in this study that a decrease in intradipose tissue PGF2α levels following Akr1b7 ablation leads to increased adiposity. This phenotype results from alleviation of the repression exerted on both adipocyte precursors recruitment and on lipid storage in mature adipocytes and can be reverted by exogenous PGF2α injections. Using 3T3-L1 adipocytes, we confirmed that PGF2α inhibited expression of lipogenic enzymes. This unsuspected result is consistent with the high expression of the FP receptor in cultured adipocytes (18) and in vivo (Fig. 5C). The mechanism leading to PGF2α-mediated inhibition of lipogenic genes remains to be explored. We showed in this paper that it occurs independently of Srebp-1c, in agreement with previous findings indicating that Srebp-1c does not transactivate lipogenic genes in adipocytes (43).

**Akr1b family members: novel actors in adipose tissue homeostasis?** In mouse, aldose reductases Akr1b3 and Akr1b7 are so far the only members of the Akr1b family that are expressed in adipose tissue (Fig. 1D). A recent study indicated that PGFS activity of Akr1b3 could influence adipogenesis. Indeed, siRNA-mediated Akr1b3 knockdown decreased the concentrations of PGF2α in 3T3-L1 medium, which resulted in enhanced adipogenesis (18).

The presence of Akr1b3 could thus account for maintenance of a low level of PGF2α in the adipose tissue of Akr1b7−/− mice (Fig. 5A). However, this isoform was not able to compensate for the lack of Akr1b7. Moreover, no defect in adipose tissue homeostasis was reported in Akr1b3−/− mice but careful in vivo investigations will be needed to definitively conclude (19).

Among human members of the AKR family, AKR1B1 and AKR1C3 exhibited a PG synthase activity in vitro. AKR1C3 is expressed in human adipocytes and was for a long time considered as the only PGFS in humans (44,45). However, AKR1B1 was recently shown to have a higher PGFS activity than AKR1C3 in vitro (17) and to be highly active in human endometrium (46). The role of AKR1B1 has been extensively studied in the pathogenesis of diabetes complications (47), but its role in the physiology of adipose tissue is an almost virgin field of investigation. One could hypothesize that AKR1B1 could act as an antiadipogenic factor through local PGF2α production.

In conclusion, our mouse model provides functional evidence for novel mechanisms linking prostanoid metabolism with adipose tissue homeostasis. It also opens up new perspectives on the potential contribution of Akr1b isoforms to physiological control of adipose tissue expansion and pathogenesis of obesity in patients.

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F.E.V. and J.-C.P. conceived and designed the study, analyzed and interpreted the data, and drafted and revised the manuscript. E.P. and B.M. analyzed and interpreted the data, provided technical assistance, and revised the manuscript. B.S., G.H., M.G., and R.C. provided technical assistance, and revised the manuscript. F.E.V. and J.-C.P. conceived and designed the study, provided technical assistance, and revised the manuscript. B.S., G.H., M.G., and R.C. provided technical assistance and advice. A.-M.L.-M. and A.M. conceived and designed the study, analyzed and interpreted the data, and drafted, wrote, and revised the manuscript. A.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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