Protein kinase Cβ deficiency attenuates obesity syndrome of ob/ob mice by promoting white adipose tissue remodeling

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Abstract To explore the role of leptin in PKCβ action and to determine the protective potential of PKCβ deficiency on profound obesity, double knockout (DBKO) mice lacking PKCβ and ob genes were created, and key parameters of metabolism and body composition were studied. DBKO mice had similar caloric intake as ob/ob mice but showed significantly reduced body fat content, improved glucose metabolism, and elevated body temperature. DBKO mice were resistant to high-fat diet-induced obesity. Moreover, PKCβ deficiency increased β-adrenergic signaling by inducing expression of β1- and β3-adrenergic receptors (β-ARs) in white adipose tissue (WAT) of ob/ob mice. Accordingly, p38MAPK activation and expression of PGC-1α and UCP-1 were increased in WAT of DBKO mice. Consistent with results of in vivo studies, inhibition of PKCβ in WAT explants from ob/ob mice also increased expression of above β-ARs. In contrast, induction of PGC-1α and UCP-1 expression in brown adipose tissue of DBKO mice was not accompanied by changes in the expression of these β-ARs. Collectively, these findings suggest that PKCβ deficiency may prevent genetic obesity, in part, by remodeling the catabolic function of adipose tissues through β-ARs dependent and independent mechanisms.—Huang, W., R. R. Bansode, N. Bal, M. Mehta, and K. D. Mehta. Protein kinase Cβ deficiency attenuates obesity syndrome of ob/ob mice by promoting white adipose tissue remodeling. J. Lipid Res. 2012. 53: 368–378.

Supplementary key words protein kinase Cβ • adipose tissue remodeling • β-adrenergic receptors • thermogenesis

Leptin is an adipocyte-derived hormone that is required for normal energy homeostasis (1–3). It plays a key role in the control of body weight by suppressing food intake through actions on hypothalamic receptors and by increasing energy expenditure via activation of sympathetic activity and brown adipose tissue (BAT) thermogenesis. This is best illustrated by loss of function mutations in genes encoding leptin or the leptin receptor, which result in severe obesity in rodents and humans. Leptin is also known to play a dual role in glucose metabolism and insulin signaling, acting as an insulin sensitizer and as an antagonist. In vivo, leptin has been reported to enhance insulin action in inhibiting hepatic glucose output while antagonizing insulin action on the expression of metabolic genes (4). The insulin and leptin signaling pathways are known to share downstream targets such as Janus kinase-2, insulin receptor substrates, phosphatidyl-inositol 3-kinase, protein kinase B, mitogen-activated protein kinase, and protein kinase C (PKC). Recent data provide evidence that PKC is activated by leptin via increasing calcium concentration and stimulating inositol triphosphate (IP-3) production (5). PKC-dependent phosphorylation of Ser-318 in insulin receptor substrate-1 has been implicated in mediating the inhibitory signal of leptin on the insulin-signaling cascade (6). Several other interactions in different physiological systems have been described between PKC and leptin (7–10).

PKCs comprise a large family of serine/threonine protein kinases that plays a key role in signal transduction and regulation of gene expression (11–14). Twelve distinct members have been discovered in mammalian cells, and these have been subdivided into three distinct subfamilies as follows: conventional PKCs (α, β, and γ), novel PKCs (δ, ε, ν, and θ), and atypical PKCs (ε and η/λ). These PKC isoforms are unique not only with respect to their primary structures but also in their expression patterns, subcellular localization, in vitro activation, and responsiveness to

Abbreviations: β-AR, beta-adrenergic receptor; BAT, brown adipose tissue; DBKO, ob/ob mice deficient in PKCβ; HFD, high-fat diet; ob/ob, leptin-deficient mice; p38MAPK, stress-activated mitogen-activated protein kinase; PGC-1α, peroxisome-proliferator-activated receptor γ coactivator-1α; PKC, protein kinase C; PKCβ, protein kinase Cbeta; PKCβ2−/−, PKCβ-deficient mice; p38MAPK, stress-regulated mitogen-activated protein kinase; SIRT, sirtuin; TG, triglyceride; UCP-1, uncoupling protein-1; WAT, white adipose tissue; iWAT, inguinal WAT; eWAT, epididymal WAT.
extracellular signals. Most importantly, these isoforms show differences in cofactor dependence and responsiveness to calcium and phospholipid metabolites. Conventional PKCs bind to and are activated by sn-1,2-diacylglycerol, which increases the specificity of the enzyme for phosphatidylserine and its affinity for calcium. Novel PKCs are also activated by DAG and require phosphatidylserine as a cofactor but have lost the requirement for calcium. Atypical PKCs do not respond to DAG or calcium but apparently still require phosphatidylserine as a cofactor. Recent studies have shown that DAG-PKC signaling is activated in diabetic conditions, and the induction appears to be restricted to a few “diabetic-related” isoforms (15, 16). PKCB is one isoform that has been most directly linked to important aspects of hyperglycemia in in vivo and in vitro. PKCB was also one of the earliest isoforms recognized in insulin signaling and appears to play dual roles in insulin signaling pathways (17–22). PKCB does not appear to regulate glucose-induced insulin secretion in vivo (23), even though it has been reported to undergo translocation to the plasma membrane subsequent to stimulation by glucose in primary islet cells (24). We recently showed that PKCB is markedly elevated in white adipose tissue (WAT) of leptin-deficient (ob/ob) mice and is significantly induced by intake of high-fat diet (HFD) (25, 26). We also assessed the impact of PKCB deficiency on glucose and lipid homeostasis in vivo and found that deficiency of PKCB signaling resulted in adipocyte atrophy, hypoleptinemia, hyperphagia, and altered expression of genes involved in energy homeostasis in the adipose tissue. The lean phenotype of PKCB−/− mice was associated with reduced serum leptin and compensatory increased food intake (26). Furthermore, adiposity is not increased when PKCB-deficient (PKCB−/−) mice are challenged with a HFD. These studies identified the PKCB signaling pathway as a novel modulator of adipose tissue homeostasis.

Unlike PKCB−/− mice, ob/ob mice exhibit marked obesity, hyperphagia, insulin resistance, hypothermia, and increased food efficiency. To explore the protective potential of PKCB deficiency on profound obesity and to better understand the regulatory pathways that govern energy metabolism, we examined the effects of PKCB gene disruption in genetically obese ob/ob mice on diverse elements of energy balance, focusing particularly on the β-AR signaling. We report that deletion of PKCB in ob/ob mice (DBKO) decreases food efficiency through increasing energy expenditure and thermogenesis and through enhanced insulin sensitivity, thus improving the energy balance of ob/ob mice. A significant component of the effect of PKCB deficiency on energy expenditure is independent of leptin and involves signaling through β-ARs in WAT. In fact, enhanced β-adrenergic signaling may account for hypoleptinemia in PKCB−/− mice.

MATERIALS AND METHODS

Animals and diet

A double knockout mouse simultaneously lacking the leptin and PKCB genes was generated by intercrossing male ob/ob−/− mice with female PKCB−/− mice on a C57BL/6J background (Jackson Laboratories, Bar Harbor, ME) to generate ob+/− x PKCB−/−. These leptin heterozygous and PKCB homozygous mice were used to generate double knockout ob/ob x PKCB−/− mice. Genotyping for ob/ob and PKCB were performed as previously described (25). Unless indicated, all experiments were performed on male animals.

Male mice were weaned at 21 days of age, genotyped, and maintained at a room temperature of 22 ± 2°C on a 12:12 light-dark cycle with a relative humidity of 50%. Animals had free access to water and were fed ad libitum. Body weight and food intake were registered weekly. Body temperature was assessed by measuring rectal temperature using a rectal thermometer.

Seven- to eight-week-old ob/ob and DBKO mice were fed ad libitum for the indicated period continuously either on a HFD (D12492; Research Diets, New Brunswick, NJ) in which 60% of the total calories were derived from fat (soybean oil and lard) or a standard diet containing 17% kcal from fat (7912 rodent chow; Harlan Laboratories, Inc., Indianapolis, IN) (25). All procedures on mice followed guidelines established by the Ohio State University College of Medicine Animal Care Committee. Unless indicated, all experiments were performed on mice starved for approximately 16 h.

Blood and tissue collection

Eighteen-week-old mice were fasted for 6 h and euthanized by CO2 inhalation. Blood samples were obtained by submandibular bleeding, and plasma or sera were collected after centrifugation (4°C) at 12,000 rpm for 15 min and stored at −20°C. Epididymal, inguinal, and retroperitoneal white adipose tissue, together with brown fat from the interscapular depot, and livers were carefully excised. Tissue samples were weighed and then immediately frozen in liquid nitrogen. For morphological assessment, parts of adipose tissue was fixed in 4% buffered formaldehyde overnight and then dehydrated in graded ethanols and embedded in paraffin. Sections (10 μm) were cut and mounted on slides and stained with hematoxylin and eosin or UCP-1 antibody (1:500)-HRP according to standard protocols. Plasma concentrations of triglycerides, total cholesterol, and serum-free fatty acids were measured by enzymatic methods using commercially available kits. Serum insulin and adiponectin were determined by ELISA.

Glucose tolerance test and insulin tolerance test

A glucose tolerance test and insulin tolerance test were performed on fasted (16 h) mice. Mice were weighed and injected intraperitoneally with glucose (1.5 mg/kg body weight) or insulin (0.8 U/kg body weight). Blood samples were collected via tail bleeds, and glucose concentrations were measured before and 15, 30, 60, 90, and 120 min after the challenge. Glucose was determined by glucometer.

Oxygen consumption measurements

Oxygen consumption, CO2 production, and spontaneous physical movement were measured simultaneously over 24 h for each mouse using a computer-controlled, open-circuit Oxymax/CLAMS System (Columbus Instruments, Columbus, OH). Each mouse was measured individually in a resting state at 22°C in the presence of food and water.

Western blot studies

Tissues were homogenized in buffer containing 20 mM Tris, 50 mM NaCl, 250 mM sucrose, 1% Triton X-100, and phosphatase and protease inhibitors cocktail, and protein content was measured as described earlier (25). Equal amounts of protein were run in 12% SDS-PAGE, transferred to nitrocellulose membranes, and blocked in Tris-buffered saline with Tween 20 containing 5%
nonfat dry milk or BSA for 1 h at room temperature. Blots were incubated overnight at 4°C with primary antibodies against UCP-1 (Abcam, Cambridge, MA) at 1:2,000; or P-p38MAPK (Cell Signaling Technology, Inc., Boston, MA) at 1:1,000, or p38MAPK (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:2,000. The antigen-antibody complexes were visualized using peroxidase-conjugated anti-rabbit antibodies (1:5,000) and the enhanced chemiluminescence ECL detection system (Life Technologies, Grand Island, NY). All assays were performed in duplicate.

**In vitro analysis of isolated inguinal WAT (explants)**

Inguinal, epididymal WAT, or interscapular BAT were surgically removed from ob/ob and DBKO mice (n = 3 per group). Freshly dissected fat pads were minced to a size of 2–3 mm³ for iWAT and 1–2 mm³ for BAT on an ice-cold Petri dish containing Krebs-Ringer HEPES buffer (5 mM D-glucose, 2% BSA, 135 mM NaCl, 2.2 mM CaCl₂, 1.25 mM MgSO₄, 0.45 mM KH₂PO₄, 2.17 mM Na₂HPO₄, and 10 mM HEPES) and then rinsed twice with Kreb-Ringer HEPES buffer and once with DMEM containing 10% BSA. Samples were passed through a 200-μm mesh. The tissue explants were transferred to 6-well plate with an equal amount of the tissue in each well. The explants were allowed to stabilize in DMEM medium for 1 h before the treatment. All procedures were performed under sterile conditions. LY333,531 from Alexius Biochemicals was added to each well as indicated for 16 h. Explants were transferred to a 2 ml tube and rinsed with PBS twice before being snap-frozen in liquid nitrogen for later analysis of gene expression.

**Gene expression**

Total RNA was extracted from iWAT and BAT samples by homogenization using TRIzol reagent. Samples were treated with a DNA-free kit (Life Technologies, Grand Island, NY). For first-strand cDNA synthesis, constant amounts of 2 μg of total RNA were reverse transcribed in a 20 μl final volume using random hexamers as primers and 50 units of MultiScribe™ Reverse Transcriptase (High-capacity cDNA Reverse Transcription Kit, Life Technologies, Grand Island, NY) (25, 26). The transcript levels for indicated genes were quantified as described earlier. Relative mRNA expression was expressed as fold expression over the control mice. All samples were run in triplicate, and the average values were calculated.

**Statistical analysis**

The results are shown as means ± SEM. All statistical analysis was performed by Student t-test or ANOVA in Excel; P < 0.05 was considered significant.

**RESULTS**

**Ablation of PKCβ reduces the positive energy balance of ob/ob mice**

To explore the effects of PKCβ deficiency on ob/ob phenotype, we intercrossed ob<sup>−/−</sup> with PKCβ<sup>−/−</sup> mice to generate DBKO mice. Body weight and fat content were compared between ob/ob and DBKO mice. Compared with the ob/ob mice of the same age, DBKO mice have lower body weight (Fig. 1A–C). Noteworthy differences in body weights were apparent by as early as 7 weeks of age and became even more pronounced with aging. At 18 weeks of age, weight was reduced by 34% in female mice and by 29% in male mice. It was accompanied by significantly reduced inguinal and retroperitoneal white fat depots per body weight as compared with ob/ob mice in male (Fig. 1D, E) and female mice (results not shown). The wet weight of inguinal white adipose tissue (iWAT) was reduced by ~41% in DBKO in comparison to ob/ob mice. The weights

**Fig. 1.** Physical appearance, growth curves, adipose stores and livers of ob/ob and DBKO mice. At 7 week of age, male (A) and female (B) mice fed standard chow diet were weighed weekly. C: Gross representative images of 18-week-old male and female ob/ob and DBKO mice on a C57BL/6J background. D–G: Representative pictures and weights of iWAT, rWAT, BAT, and liver for 18-week-old male mice fed chow diet. Values represent the means ± SE of ob/ob and DBKO mice (n = 12). *P < 0.05; **P < 0.01.
Adiposity is influenced by the rate of food consumption and the rate at which energy is expanded metabolically. To determine whether change in food intake and/or energy expenditure was responsible for decreased obesity of DBKO mice relative to ob/ob mice, we compared their rates of food intake and oxygen consumption. With or without normalization for body weight, Fig. 3A and 3B show that male DBKO mice consumed a similar level of food as male ob/ob mice; this rate of food intake was also seen with female mice (results not shown), suggesting that the decrease in food intake cannot account for the failure of DBKO mice to gain weight. Interestingly, basal oxygen consumption at night was significantly higher in male DBKO mice than in male ob/ob mice (Fig. 3C, D). Thus, increased metabolic rate may help to normalize energy balance in DBKO mice. We cannot rule out whether the differences in body weight contribute to increased oxygen consumption of DBKO mice compared with ob/ob mice (27, 28). The increased oxygen consumption could also be due in part to the maintenance of a higher body temperature of DBKO mice (Fig. 3E). These data strongly suggest that PKCβ deficiency may increase energy expenditure in ob/ob mice.

DBKO mice exhibit improved insulin sensitivity

Changes in adiposity are often associated with alterations in glucose and insulin homeostasis. The ob/ob mice develop a form of diabetes similar to human type-2 diabetes, a condition commonly associated with obesity. To evaluate the effect of PKCβ deficiency on glucose metabolism in chow-fed mice, we first evaluated plasma glucose and
PKCβ deficiency protects ob/ob mice from HFD-induced obesity

We also compared responses of ob/ob and DBKO mice under conditions of severe dietary stress. Both genotypes were fed HFD beginning at 8 weeks of age. Body weights and food intake were monitored weekly, and at the end we examined weights of adipose tissues and various metabolic parameters. A significant increase in ob/ob mice body weights were evident even after 2 weeks on HFD, and this trend continued throughout the dietary protocol (Fig. 5A, B). DBKO mice fed HFD gained less weight than ob/ob mice and exhibited an obese-resistant phenotype (Fig. 5B).

Fig. 3. Food intake, energy expenditure, and body temperature of male ob/ob and DBKO mice. A, B: Average daily food consumption. Food intake was determined by measuring 1 day or 7 day intake of chow diet by 12 week-old male mice; n = 12 for each genotype. C and D: Metabolic rate and oxygen consumption of 12- to 14-week-old male mice on chow diet (n = 5 each genotype) as measured by indirect calorimetry. Mice were monitored for 24 h continuously, from 10:00 AM to 10:00 AM the next day. To allow for acclimation, data from the initial 5 h are omitted. E: Average rectal temperatures for both genotypes. All values are given as means ± SE. *P < 0.05 compared with ob/ob mice.

Fig. 4. Increase in vivo insulin sensitivity of DBKO mice compared with ob/ob mice. A: Blood glucose and serum insulin levels were measured in 12-week-old male ob/ob and DBKO mice and then glucose to insulin ratio was calculated accordingly. Data are expressed as means ± SE (n = 6). B: Changes in glucose levels in glucose-tolerance tests (GTT) and insulin-tolerance tests (ITT). Male mice fasted for approximately 16 h were injected with a bolus of glucose (1.5 mg/kg body weight) or insulin (0.8 U/kg body weight), and blood glucose levels were analyzed with a glucometer. Data are expressed as mean ± SE (n = 6). *P < 0.05.

insulin levels between genotypes. Both were significantly lower in fasted DBKO mice compared with those in ob/ob mice (Fig. 4A). Moreover, glucose to insulin ratios in DBKO mice was also significantly elevated (Fig. 4A), indicating improved insulin sensitivity. Meanwhile, DBKO mice showed a significant decrease in plasma glucose compared with ob/ob mice after intraperitoneal injection of glucose or insulin, indicating improved glucose metabolism by PKCβ deficiency in ob/ob mice (Fig. 4B). In agreement with the above findings, DBKO mice showed a significant increase in the whole body glucose uptake, further supporting improved glucose metabolism (Huang et al., unpublished results).
PKCβ deficiency promotes upregulation of brown adipocyte function markers in WAT and BAT of ob/ob mice

To gain further insight into mechanisms underlying improved energy expenditure of DBKO mice, gene expression levels of key molecules involved in the regulation of mitochondrial function and thermogenesis were analyzed. The elevated energy expenditure in DBKO mice led us to first study BAT, which is responsible for adaptive thermogenesis in response to diet or cold (29–31). Diet-induced thermogenesis reduces obesity in humans and animals (32, 33). Leptin deficiency is always associated with a reduction of PGC-1α transcript levels, together with a tendency toward a decrease in sirtuin (SIRT)-1 transcript levels, without changes in expression levels of SIRT-3. On the contrary, DBKO mice showed a significant upregulation of PGC-1α, UCP-1, SIRT-1, and SIRT-3, as compared with the ob/ob group (Fig. 8A). The protein expression of UCP-1 in BAT exhibited a pattern similar to that observed in the gene expression analyses for DBKO mice on chow or on the HFD diet (Fig. 8B, C).

A formalin-fixed, paraffin-embedded section from iWAT for UCP-1 protein stain appears to indicate a morphological change toward a BAT-like phenotype in DBKO (Fig. 8D). To determine the extent of shifting of the physiology and gene expression profiles of iWAT to BAT in DBKO mice, we checked expression levels of BAT-specific genes and several transcriptional regulators. Indeed, mRNA and protein levels of brown fat-specific gene UCP-1 were significantly elevated in the iWAT of DBKO mice compared with that of ob/ob mice (Fig. 8A, B). In addition, the mRNA level of PGC-1α was also increased (Fig. 8A).

The difference in body weights between ob/ob and DBKO mice was further reflected by marked reduction in iWAT and rWAT mass in DBKO mice, indicating that these mice are strongly protected from HFD-induced obesity (Fig. 5C, D). Histological analysis of iWAT revealed smaller adipocytes than those from ob/ob mice fed HFD (Fig. 5C). The weights of DBKO BAT and livers were almost comparable between these genotypes (Fig. 5E, F). Liver tissue appearance in ob/ob male mice was more whitish in color than that in DBKO mice. Similarly, ob/ob mice had higher hepatic TG content than DBKO mice (Fig. 5G) and exhibit increased glucose/insulin ratio (Fig. 5H). It is again clear that the decreased body weight was not a result of reduced food intake of DBKO mice compared with ob/ob mice (Fig. 6). Indirect calorimetry measurements revealed a significant increase in oxygen consumption (Fig. 7A, B) in HFD-fed DBKO mice compared with ob/ob mice. In the DBKO mice, the increase in energy expenditure was more prominent at night when mice actively took food (Fig. 7A). The respiratory quotient was comparable in the ob/ob and DBKO mice (Fig. 7C, D), indicating similar utilization of carbohydrates and fat as energy sources.

**Fig. 5.** DBKO mice are resistant to HFD-induced obesity. A: Growth curves of male mice fed HFD for the indicated period. Eight-week-old ob/ob and DBKO mice fed this diet were weighed weekly. Values represent mean ± SE (n = 8 of each genotype). B: Gross representative images of male ob/ob and DBKO mice before euthanization. C–F: Representative pictures and weights of iWAT, rWAT, BAT, and liver of HFD-fed male mice. G: Hematoxylin and eosin (H+E) staining and TG content of liver (magnification ×20) from ob/ob and DBKO mice. H: Glucose/insulin ratio. Values are the means ± SE of ob/ob and DBKO mice (n = 6). *P < 0.05; **P < 0.01.
To explore the potential relationship between PKCβ and β-AR expression, iWAT explants were prepared from ob/ob and DBKO mice. Treatment with a specific PKCβ inhibitor, LY333,531, increased expression of β1- and β3-ARs, but not β2-AR, of ob/ob mice but not of DBKO mice (Fig. 9B). Also, explants from BAT of ob/ob or DBKO mice did not show alterations in the expression of β-ARs, which is consistent with the above in vivo observation (results not shown).

Consistent with an increased β-adrenergic signaling, greater p38 MAPK phosphorylation was observed in iWAT of DBKO mice compared with ob/ob mice (Fig. 10A). Inhibition of p38 MAPK by using a specific inhibitor, SB202,190, significantly reduced β3-AR agonist (CL341,243)-induced PGC-1α expression (Fig. 10B), suggesting the requirement of p38 MAPK in the induction process.

**DISCUSSION**

Our findings show that PKCβ deficiency in ob/ob mice produces an integrated series of molecular, cellular, and physiological responses that have a profound impact on energy expenditure without affecting the energy intake component of the energy balance equation. The net effect of these responses is a decrease in metabolic efficiency and a corresponding decrease in fat accretion in adipose tissue of DBKO mice compared with ob/ob mice. These results support the view that PKCβ plays an essential role in energy homeostasis by repressing metabolic genes involved in energy expenditure.

The substantial increase in energy expenditure induced by PKCβ deficiency in ob/ob mice is associated with elevated expression of UCP-1 in two distinct populations of
PKCβ regulates β-adrenergic signaling

Our findings also support the view that a significant component of the mechanism engaged by PKCβ/11002/11002 mice to increase energy expenditure involves signaling through β-AR. Accordingly, p38 MAPK activation and the expression of PGC-1α, a key marker of brown fat cell function, were increased in WAT of DBKO mice. PGC-1α is an important factor in mitochondrial function and energy homeostasis and controls several aspects of mitochondrial biogenesis. It plays an essential role in brown fat thermogenesis through activation of UCP-1 (44). In addition, increases in the expression of SIRT-1 and SIRT-3 genes can influence energy homeostasis in the adipose tissues of DBKO mice. SIRT-1 is known to positively act on the activation of metabolic genes through direct deacetylation of PGC-1α (45, 46). Also, growing evidence supports a novel role for SIRT-3 in enhancing the expression of mitochondrial-related genes, participating in adaptive thermogenesis (47).

Brown adipocytes. First, there was improvement in the function of brown adipocytes in BAT itself. On histological analysis, large unilocular lipid droplets were observed in BAT of ob/ob mice, whereas in DBKO mice the characteristic macroscopic, microscopic, and molecular features of BAT are partially restored. Second, expression of UCP-1 protein, which is normally restricted to brown adipocytes, is induced in what is usually regarded as WAT (iWAT) in DBKO mice. A similar response is seen in rats and mice after cold exposure or treatment with β3-AR agonists (36–39). Moreover, considerable precedent exists to support the association between an increase in UCP-1-dependent energy expenditure and leaness in mouse models (40–43). For example, overexpression of UCP-1 in WAT and BAT using a transgene driven by the fat-specific aP2 promoter resulted in lower adiposity, as did the disruption of the Cidea protein, a mitochondrial component that was shown to suppress UCP-1 activity (42). The common finding in these studies is that the observed increase in cAMP signaling or β-AR sensitivity, which induced UCP-1 expression in WAT, produces a similar lean, obesity-resistant phenotype. This is accomplished by activation of p38MAPK signaling, which leads to the activation of transcription factors, such as ATF2 and CREB, and increased expression and phosphorylation of the nuclear factor cofactor PGC-1α. Our findings also support the view that a significant component of the mechanism engaged by PKCβ−/− mice to increase energy expenditure involves signaling through β-AR. Accordingly, p38MAPK activation and the expression of PGC-1α, a key marker of brown fat cell function, were increased in WAT of DBKO mice. PGC-1α is an important factor in mitochondrial function and energy homeostasis and controls several aspects of mitochondrial biogenesis. It plays an essential role in brown fat thermogenesis through activation of UCP-1 (44). In addition, increases in the expression of SIRT-1 and SIRT-3 genes can influence energy homeostasis in the adipose tissues of DBKO mice. SIRT-1 is known to positively act on the activation of metabolic genes through direct deacetylation of PGC-1α (45, 46). Also, growing evidence supports a novel role for SIRT-3 in enhancing the expression of mitochondrial-related genes, participating in adaptive thermogenesis (47). It is well known that PGC-1α is intimately involved in adaptive thermogenesis via the induction of the mitochondrial inner membrane uncoupling protein UCP-1 (48), thereby providing one potential mechanism contributing to the lean phenotype of DBKO mice. In addition to transcriptional induction of UCP-1 expression by

![Fig. 8. Altered expression levels of crucial metabolic regulators and BAT-specific factors in DBKO mice compare with ob/ob mice. A: Bar graphs show mRNA levels of indicated genes normalized for the expression of β-actin in male mice fed HFD. The expression in WT mice was assumed to be 1. B and C: Levels of UCP-1 protein analyzed with Western blot in the BAT (0.5 µg), iWAT (40 µg), and muscle (40 µg) from 12-week-old male ob/ob and DBKO mice fed chow or HFD for 8 weeks. Protein data were normalized for the expression of p38MAPK. D: UCP-1 staining of iWAT by immunohistochemistry. Brown stains indicate UCP-1 protein that appear in smaller adipocytes in iWAT of DBKO mice. Bar, 5 µM. These figures are representative of three different experiments. Values given are means ± SE. *P < 0.05; **P < 0.01 (n = 6 per group).](http://www.jlr.org)
PGC-1α-dependent mechanisms, elevated UCP-1 protein levels in adipose tissues of PKCβ−/− mice could also be due to increased synthesis or stability of UCP-1 protein. One stabilizing influence might be at the level of mitochondria because PKCβ inhibition has recently been linked to enhanced mitochondrial stability (49). The relevance of this mechanism is underscored by the fact that WAT of PKCβ−/− mice contain more mitochondria and show increased fatty acid oxidation (25). In view of a recent demonstration that PKCβ mediates insulin-induced hepatic SREBP-1c expression (18), it is likely that reduction in fatty acid synthesis contributes to reduced TG contents of DBKO mice compared with ob/ob mice.

Another important aspect of these studies relates to the PKCβ-dependent, adipocyte-specific β3-AR expression in fine-tuning the adrenergic signaling in WAT. Results presented here argue strongly in favor of an inverse relationship between PKCβ and β3-AR expression. In fact, the proposed relationship is consistent with earlier reports showing that sustained PKC activation suppressed β-AR expression at the transcriptional level (50–52). It has also been shown that PKCβ mediates insulin-induced suppression of β3-AR expression, and this regulation could play a key role in the body’s adaptation to antilipolysis, lipogenesis, and thermogenesis that occur during hyperinsulinemic states. Our previous observations that insulin induces PKCβ expression in white adipocytes and that ob/ob WAT has higher PKCβ expression (26), together with current results showing that DBKO mice have higher β3-AR expression, strongly support involvement of this isofrom in the suppression process and in mediating insulin action on β3-AR expression in adipocytes. The proposed relationship may explain significantly reduced β3-AR mRNA levels in adipose tissues of ob/ob mice (53). In fact, other models of congenital obesity, such as db/db, tubby, fat, and Zucker fatty rat, show similar decreases in β3-AR and β1-AR expression, the extent of which tends to mirror the severity of obesity (54, 55).

In addition to the potential direct effects of PKCβ on adipocytes, PKCβ deficiency may exert other, more global effects that affect overall energy balance. In particular, PKCβ is expressed in the brain (56), and it may influence PKCβ-dependent signaling events mediated by leptin at
PKCβ regulates β-adrenergic signaling

Fig. 11. Schematic diagram of the proposed sequence of events whereby PKCβ deficiency leads to a reduction in metabolic efficiency. Elevated adipose PKCβ expression in ob/ob mice suppresses β-adrenergic signaling and thereby reduces UCP-1 expression in iWAT and prevents WAT catabolic activity.

It is not known whether the metabolic effects of leptin or insulin in the hypothalamus are dependent on the activity of PKCβ. Our observation that ob/ob and DBKO mice consumed similar calories [unlike PKCβ−/− mice, which consume more calories than WT mice (25)] suggests that chronic hypoleptinemia in these animals is responsible for stimulating appetite and argues against an effect of PKCβ deficiency at the level of the hypothalamus. In addition, our observation that PKCβ deficiency protected against HFD-induced diabetes even in the ob/ob background argues against involvement of leptin-mediated energy expenditure in PKCβ−/− mice.

Beside its role in regulating adiposity, PKCβ plays an important role in modulating glucose homeostasis. This feature was reflected by lower glucose and insulin levels in DBKO mice compared with those of ob/ob mice, suggesting that the metabolic changes induced by PKCβ deficiency lead to increased insulin sensitivity. Moreover, DBKO mice effectively cleared a glucose bolus that was administered intraperitoneally, whereas ob/ob mice stayed hyperglycemic. This phenotype induced by PKCβ deficiency in ob/ob mice supports the hypothesis of lipotoxicity because a decrease in total body lipid content leads to increased insulin sensitivity (57). In this context, it is interesting to note that altering expression of a single gene results in systemic changes in glucose, insulin, and lipid metabolism in a way that protects against the consequences of obesity. We propose a model in which adipose PKCβ levels determine the metabolic efficiency (Fig. 11). The degree of adipogenic sensitivity, regulated by PKCβ, could be regarded as a cellular “set point” for metabolism in adipocytes. In this way, pronounced PKCβ suppression will act as a defense against increasing adipose tissue mass. Based on this model, PKCβ should be regarded as a candidate target gene for obesity, insulin resistance, and type 2 diabetes.

We thank Dr. Qinghua Sun for UCP-1 staining.

REFERENCES


