A New Role for Lipocalin Prostaglandin D Synthase in the Regulation of Brown Adipose Tissue Substrate Utilization

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In this study, we define a new role for lipocalin prostaglandin D synthase (L-PGDS) in the control of metabolic fuel utilization by brown adipose tissue (BAT). We demonstrate that L-PGDS expression in BAT is positively correlated with BAT activity, upregulated by peroxisome proliferator–activated receptor γ coactivator 1α or 1β and repressed by receptor-interacting protein 140. Under cold-acclimated conditions, mice lacking L-PGDS had elevated reliance on carbohydrate to provide fuel for thermogenesis and had increased expression of genes regulating glycolysis and de novo lipogenesis in BAT. These transcriptional differences were associated with increased lipid content in BAT and a BAT lipid composition enriched with de novo synthesized lipids. Consistent with the concept that lack of L-PGDS increases glucose utilization, mice lacking L-PGDS had improved glucose tolerance after high-fat feeding. The improved glucose tolerance appeared to be independent of changes in insulin sensitivity, as insulin levels during the glucose tolerance test and insulin, leptin, and adiponectin levels were unchanged. Moreover, L-PGDS knockout mice exhibited increased expression of genes involved in thermogenesis and increased norepinephrine-stimulated glucose uptake to BAT, suggesting that sympathetically mediated changes in glucose uptake may have improved glucose tolerance. Taken together, these results suggest that L-PGDS plays an important role in the regulation of glucose utilization in vivo. Diabetes 61:3139–3147, 2012

Obesity is a chronic illness that is associated with multiple secondary diseases, including diabetes and cardiovascular disease. Although many ideas have been put forward to explain mechanistically how obesity leads to metabolic complications, this still remains an area of considerable controversy. We and others have suggested that alterations in how lipids are stored and handled may link obesity to metabolic complications through defects in adipose tissue expansion and functional capacity and the process of lipotoxicity (1,2).

An important aspect of appropriate lipid handling is the ability of tissues to switch between carbohydrate and lipid as their major metabolic substrates. Under normal physiological conditions, humans switch from using high levels of carbohydrate during the postprandial state to predominantly utilizing stored lipids during the fasted state. The process of switching from fed to fasted states requires adipose tissue to play an important role in lipid buffering. During the fed state, net lipid flux into adipose tissue increases, whereas in the fasted state net lipid efflux predominates (3). Under pathological conditions where adipose tissue becomes insulin resistant, however, the appropriate fluxes into and out of adipose tissue are blunted (4–6).

The process of being able to switch between using metabolic substrates is termed metabolic flexibility and can be measured by assessing the change in respiratory quotient between fed and fasted states. A reduction in metabolic flexibility has been suggested to be a primary defect leading to insulin resistance. When fed a high-fat diet for 3 days, subjects with a family history of type 2 diabetes showed a lower change in respiratory quotient between fasted and fed states than did subjects without a family history of type 2 diabetes (7). The fact that impairments in metabolic substrate utilization may be a primary cause of insulin resistance suggests the possibility of a direct regulatory mechanism controlling this process; however, what form this mechanism takes is poorly understood.

In addition to the known roles for white adipose tissue (WAT) depots in metabolic health, interest in the role of brown adipose tissue (BAT) in adult humans has recently experienced a resurgence through studies with fluorodeoxyglucose positron emission tomography (8–13). BAT has been demonstrated, at least in rodents, to have a very high capacity for both lipid and glucose uptake and oxidation. In small mammals, such as mice, BAT may be responsible for the oxidation of as much as 90% of the total daily fuel intake. In addition to its high rate of lipid and glucose oxidation, BAT also has very high rates of de novo lipogenesis, suggested to account for as much as 40% of all de novo lipogenesis in a cold-exposed rats (14). Given BAT’s very high metabolic rate, its high levels of lipid and glucose oxidation, and its substantial lipid synthesis, elucidation of how fuel utilization is regulated within BAT is an important question, particularly if it is ever to be used efficiently as a therapy to treat human metabolic disease. In this study we investigate the role of lipocalin prostaglandin D synthase (L-PGDS) in the regulation of carbohydrate and lipid utilization by BAT.

L-PGDS has at least two known functions. It is capable of synthesizing D-series prostaglandins, and it also can act as a carrier of lipophilic molecules (15). It has been
L-PGDS REGULATES BAT FUEL UTILIZATION

RESEARCH DESIGN AND METHODS

Generation of mice. Mice heterozygous for a disruption in exons II–V in the L-PGDS gene were crossed to produce L-PGDS KO mice and wild-type controls (18,19). KO mice for the gene for peroxisome proliferator–activated receptor γ coactivator PGC1β were generated as previously described (20). KO mice for the RIP140 were generated previously as described (21).

Animal care and diets. Mice were housed at a density of four animals per cage in a temperature-controlled room (20–22°C) with 12-h light/dark cycles. All animal protocols used in this study were approved by the U.K. Home Office and the University of Cambridge. Animals were fed a normal chow diet (D12450B; Research Diets) or a high-fat diet; either 45% or 60% calories-from-fat diets (D12451, D12492; Research Diets). For studies of cold acclimation, animals were housed at room temperature (22°C) (Fig. 1A). Conversely, mice housed at thermoneutrality (30°C) for 3 weeks had decreased L-PGDS expression relative to room temperature–housed control mice (Fig. 1B).

Hepatic glycogen assay. Glycogen levels were determined by the anthrone method (28).

Hepatic triglyceride analysis. Lipids were extracted from 100 mg of BAT by solid-phase extraction and quantified by liquid chromatography–mass spectrometry as described previously (27).

Fatty acid methyl ester analysis. The lipid extract was derivatized by acid-catalyzed esterification (29) with 10% BF3 in methanol (Sigma-Aldrich, St. Louis, MO). Enzyme-linked immunosorbent assay kits were used for measurements of leptin (R & D Systems), insulin (DRG; Diagnostics International), and adiponectin (B-Bridge International) according to manufacturer instructions. A Miniscan PT-NR (Brucker Optics) was used to measure body composition.

RESULTS

Regulation of L-PGDS in BAT from states of altered thermogenesis. Two paradigms of BAT activation were investigated: 1) cold exposure and 2) high-fat feeding. L-PGDS expression was physiologically upregulated in the BAT of mice after either cold acclimation (4°C) or high-fat feeding (45% calories from fat) relative to chow-fed mice housed at room temperature (22°C) (Fig. 1A). Conversely, mice housed at thermoneutrality (30°C) for 3 weeks had decreased L-PGDS expression relative to room temperature–housed control mice (Fig. 1B). To identify putative transcriptional regulators of L-PGDS, we focused on key factors implicated in BAT activation. Both PGC1α and PGC1β have been shown to be positive regulators of BAT function, whereas RIP140 is a negative regulator of BAT function (20,32). L-PGDS was found to be downregulated in mice lacking PGC1α (Fig. 1C) or PGC1β (Fig. 1F) but upregulated in mice lacking Rip140 (Fig. 1D). Finally, and consistent with a metabolic role for L-PGDS, L-PGDS targets found to be predominantly expressed in mature brown adipocytes rather than the stromal vascular fraction (Fig. 1F).
Old. All groups consisted of male mice housed at 4°C for 3 weeks before tissue collection fed a chow diet (CE) and mice housed at 22°C fed a 45% calories from fat diet from weaning (HFD); and mice in comparison of mice housed at 22°C fed a chow diet (CTRL); mice housed at 4°C for 3 weeks before tissue collection fed a chow diet (CE).

Reciprocal regulation of L-PGDS by PGCs and RIP140 in vitro. To investigate further the reciprocal regulation of L-PGDS by PGCs and RIP140, cellular models were used. In a BAT cell line, ectopic overexpression of PGC1α caused an increase in endogenous L-PGDS mRNA levels (Fig. 2A). Conversely, increasing expression of Rip140 led to a decrease in L-PGDS expression levels (Fig. 2B). Finally, with Rip140 KO embryonic fibroblasts, we showed that titrating RIP140 back into these cells resulted in a proportional decrease in L-PGDS expression (Fig. 2C).

L-PGDS regulates fuel utilization in vivo. To test whether L-PGDS was required for appropriate BAT function, L-PGDS KO mice were sequentially acclimated to either 4°C or thermoneutrality (30°C) for a period of 3 weeks. At each temperature, both basal and maximal energy expenditure after housing at 4°C (Fig. 3A).

Although changes in energy expenditure in L-PGDS KO mice were modest, there was a large alteration in their respiratory exchange ratio (RER) after norepinephrine stimulation at both 4°C and 30°C. Under unstimulated conditions, L-PGDS KO mice only exhibited an increase in RER when they had been previously acclimated to 4°C (Fig. 3B). The elevated RER of the L-PGDS KO mice relative to wild-type littermates suggests that L-PGDS KO mice met their increased demand for energy at 4°C by utilizing more carbohydrate than did wild-type mice.

Cold-acclimated L-PGDS KO mice exhibit altered lipid metabolism in BAT and liver. After cold acclimation, L-PGDS KO mice had increased lipid content in BAT relative to wild-type mice (Fig. 3C). Lipids were extracted from wild-type and L-PGDS KO mice that had been acclimated for energy at 4°C by utilizing more carbohydrate than did wild-type mice.

![FIG. 1. A: L-PGDS mRNA is induced in active BAT, as shown in comparison of mice housed at 22°C fed a chow diet (CTRL); mice housed at 22°C fed a 45% calories-from-fat diet (HFD); and mice housed at 4°C for 3 weeks before tissue collection fed a chow diet (CE). All groups consisted of male mice n > 7 per group, C57Bl/6 background, 4 months old. B: L-PGDS mRNA levels are reduced in inactive BAT, as shown in comparison of mice housed for 3 weeks before tissue collection at either 22°C or 30°C fed a chow diet. Both groups consisted of female mice, n > 7 per group, mixed sv129/B6 background, 4 months old. C: L-PGDS mRNA levels in BAT are significantly lower in PGC1α KO mice compared with littermate controls (WT). Both groups consisted of male mice, n = 8 per group, C57Bl/6 background, 12 months old. D: Rip140 KO mice (RIP140) have significantly increased mRNA levels of L-PGDS in BAT relative WT. Both groups consisted of male mice, n = 4 per group, C57Bl/6 background, 4 months old. E: L-PGDS mRNA levels are significantly lower in PGC1α KO mice (PGC1B) compared with WT at both 30°C and 4°C. Both groups consisted of male mice, n = 5 per group, C57Bl/6 background, 4 months old. F: L-PGDS mRNA is expressed predominantly in mature adipocytes (Ad) relative to stromal vascular fraction (SVF). Both groups consisted of male mice, n = 8 per group, C57Bl/6 background, 4 months of age. Asterisks indicate P < 0.05.](diabetes.diabetesjournals.org)
there were no significant changes observed in genes regulating brown adipocyte function, fatty acid oxidation, lipid uptake, or brown adipocyte differentiation (Fig. 4C and Supplementary Fig. 1). Taken together, these data suggest that L-PGDS ablation results in active BAT that preferentially utilizes glucose, either directly as a substrate for thermogenesis or for de novo lipogenesis and subsequent β-oxidation.
Similar to the results observed in BAT, L-PGDS KO mice had increased levels of FAS, Elovl6, and Scd1 mRNA expression in liver when compared with wild-type mice (Fig. 4D–E). Despite these changes, no increase in hepatic TG level was detected (Supplementary Fig. 2A). Further to the changes in de novo lipogenesis, L-PGDS KO mice also exhibited a reduction in expression of the key gluconeogenic enzyme PEPCK (Fig. 4D), although no alterations in the glycogen biosynthetic program or glycogen levels were detected (Supplementary Fig. 2B–C). The change in PEPCK suggests that in the liver, products of the citric acid cycle were not driven toward production of glucose but instead were channeled to de novo lipogenesis. This hypothesis was also supported by the lower plasma glucose of cold-acclimated L-PGDS KO mice (Table 1) and by a small but significant reduction in glucose levels in response to a pyruvate tolerance test (Supplementary Fig. 2D).

**L-PGDS-dependent alterations in lipid metabolism are organ specific.** The major sites of de novo lipogenesis in cold-acclimated mice are BAT and liver (14). Consistent with L-PGDS acting principally to regulate fuel availability to and utilization by thermogenic tissues, comparatively few changes in gene expression were found in muscle (Supplementary Fig. 1B–D), epididymal WAT, or subcutaneous WAT (Supplementary Fig. 3). Overall, under conditions of cold exposure, loss of L-PGDS appeared almost exclusively to affect BAT depots and liver, which in the cold acts as an important supplier of de novo synthesized lipids for oxidation by BAT.

**L-PGDS ablation increases glucose tolerance.** Given that our data had indicated that loss of L-PGDS in cold-acclimated animals appeared to increase glucose utilization to meet energy demands, we next investigated whether a lack of L-PGDS could affect lipid and carbohydrate metabolism under standard laboratory conditions (24°C housing). In animals fed a high-fat diet, lack of L-PGDS caused a significant improvement in glucose tolerance (Fig. 5A). Surprisingly, this effect was observed despite apparently similar degrees of insulin resistance induced by high-fat diet in WT and L-PGDS KO mice, as indicated by markers of insulin sensitivity including serum insulin levels during the glucose tolerance test (Fig. 5B and Supplementary Fig. 4G); adiposity (Fig. 5C); adipocyte size (Supplementary Fig. 4A); markers of adipogenesis in BAT,
subcutaneous WAT, and epididymal WAT (Supplementary Fig. 4B–D), tissue weights (Supplementary Fig. 4E–F); and fasted insulin, adiponectin, and leptin levels (Fig. 5E). Tissue markers of insulin sensitivity were also unchanged between wild-type and L-PGDS KO mice (Supplementary Fig. 5). Consistent with increased glucose utilization, mice lacking L-PGDS had lower fasting glucose levels (Fig. 5D). Because the changes in glucose tolerance and fasting glucose did not seem to be associated with markers of improved insulin sensitivity, we investigated whether there were any changes in other processes that mediate glucose uptake. In BAT norepinephrine has been shown to promote glucose uptake independently of insulin (33). Consistent with increased sympathetic tone or sensitivity to sympathetic tone, mice lacking L-PGDS had elevated thermogenic markers in BAT, and the constitutive glucose transporter gene Glut1 was also upregulated (Fig. 5F). To test whether response to sympathetic tone in terms of plasma glucose levels could be modulated by L-PGDS, we injected wild-type and L-PGDS KO mice with norepinephrine (1 mg/kg). As expected norepinephrine increased serum glucose levels as a result of its effects on hepatic glucose production (34,35); however, in L-PGDS KO mice the increase in plasma glucose levels was blunted relative to that in wild-type animals (Fig. 5G). To determine whether this was due to changes in glucose uptake as opposed to hepatic glucose uptake, we subsequently included 2[14C]-DG as a tracer to measure tissue-specific glucose uptake. We detected elevated uptake of glucose into BAT in response to norepinephrine, with no changes in muscle and a tendency (P = 0.059) toward increased glucose disposal into WAT. Although hepatic glucose production could not be directly assessed in the same assay, the elevated accumulation of 2-deoxy-D-glucose in liver in L-PGDS KO mice was suggestive of decreased glucose output, because 2-deoxy-D-glucose accumulates in liver by mass action (through Glut2) but can be actively exported.

**Loss of L-PGDS does not affect prostaglandin D<sub>2</sub> levels in BAT.** Finally, to try to identify a mechanism for L-PGDS in the regulation of fuel utilization, we measured prostaglandin D<sub>2</sub> and E<sub>2</sub> in BAT from cold acclimated wild-type and L-PGDS KO mice (Fig. 5I). We detected no differences in prostaglandin levels, suggesting that L-PGDS may act as a lipocalin.

**DISCUSSION**

In this study, we demonstrated that L-PGDS contributes to the balance between carbohydrate and lipid utilization in vivo. We also demonstrated that the physiological levels of L-PGDS mRNA in BAT are strongly and positively correlated with activation of BAT metabolism. In vivo characterization of cold-exposed mice lacking L-PGDS demonstrated that lack of L-PGDS resulted in a modest impairment in metabolic rate but a substantial increase in RER. These changes in metabolic rate and RER suggested that the principal role of L-PGDS was to control the type of fuel utilized by BAT, rather than the maximal thermogenic capacity.

The first evidence for a role for L-PGDS in BAT metabolism came from its dynamic regulation in response to both physiological and genetic manipulations which affect BAT function. L-PGDS mRNA was upregulated in states of elevated BAT function, including cold acclimation and high-fat feeding, as well as in the Rip140 KO mouse model, which has substantially increased metabolic rate with elevated markers of brown adipocyte genes within WAT (32). Conversely, L-PGDS mRNA was downregulated by thermoneutral housing and in mouse models with reduced BAT function, including PGC1α and PGC1β KO mice. Notably, both Rip140 KO and PGC1β KO mice have RER values that inversely correlate with their L-PGDS levels (20,36), consistent with the elevated RER seen in L-PGDS KO mice.

Although differences in metabolic rate observed between L-PGDS KO mice and wild-type controls were modest, there were much more substantial changes in RER. An elevated RER would usually be considered a marker of increased carbohydrate utilization and decreased lipid utilization by an organism; however, conversion of carbohydrate to lipid and its subsequent oxidation has the same RER as oxidizing carbohydrate itself, suggesting that either increased direct metabolism of carbohydrate or increased de novo lipogenesis followed by fatty acid oxidation could explain the differences in RER between wild-type and KO mice. In agreement with this, molecular analysis of BAT from L-PGDS KO mice demonstrated an increase in the proportion of de novo synthesized lipid located within BAT, as well as increased expression of glycolytic and de novo lipogenic enzymes, when compared with wild-type controls. Under conditions of cold acclimation, the principal sites of de novo lipogenesis are BAT and liver (14). Similarly to BAT, the livers of L-PGDS KO mice also exhibited a gene expression profile consistent with converting carbohydrate to lipid but did not exhibit any increase in hepatic TG levels, suggesting that any increased TG synthesis was potentially being exported. The molecular prolipogenic changes in liver and BAT were supported by lower serum glucose levels. Overall, these data suggest that under conditions of 4°C housing, L-PGDS is required for appropriate utilization of dietary lipids. In the absence of L-PGDS, mice were still able to meet the thermogenic demands of cold exposure; however, this appeared to be at the expense of a substantial increase in carbohydrate utilization.

In addition to cold exposure, high-fat feeding has been shown to promote BAT activation. After feeding with a very high-fat diet (60% calories from fat), L-PGDS KO mice had improved glucose tolerance relative to wild-type mice. Although euglycemic-hyperinsulinemic clamp data would be needed to formally confirm an absence of alterations in insulin sensitivity, there were no molecular markers of insulin resistance that differed between WT and L-PGDS KO mice, suggesting that the improved glucose tolerance of the L-PGDS KO mice could be driven by mechanisms not insulin dependent. Consistent with this concept, the thermogenic program in BAT and the

### TABLE I

Serum biochemistry of WT and L-PGDS KO mice after cold acclimation

|            | Wild-type | L-PGDS KO | P value |
|------------|-----------|-----------|---------|
| Insulin (µg/L) | 1.69 ± 0.36 | 1.51 ± 0.32 | 0.736   |
| Glucose (mmol/L) | 16.94 ± 1.67 | 13.21 ± 0.45 | **0.045** |
| FFA (µmol/L) | 555 ± 109 | 572.6 ± 31.4 | 0.876   |
| TG (mmol/L) | 1.54 ± 0.27 | 1.7 ± 0.128 | 0.593   |
| Cholesterol (mg/dL) | 3.14 ± 0.39 | 2.866 ± 0.088 | 0.481   |
| HDL (mg/dL) | 1.92 ± 0.15 | 1.818 ± 0.053 | 0.518   |
| Adiponectin (ng/mL) | 18,889 ± 1911 | 17,885 ± 646 | 0.611   |
| Leptin (pg/mL) | 6,642 ± 1933 | 5,191 ± 711 | 0.473   |

Data are mean ± SEM for male mice, n > 5 per group, C57/Bl6 background, 7 months old. Bold type indicates significant differences between groups. FFA, free fatty acid.
FIG. 5. A: Glucose levels during an intraperitoneal glucose tolerance test in wild-type control (WT) mice fed chow (black circles), L-PGDS KO mice fed chow (white circles), WT mice fed a high-fat diet (black squares), and L-PGDS KO mice fed a high-fat diet. B–I: Area under the curve for insulin levels during glucose tolerance test (B); body weight (BW), fat mass (FM), and lean mass (LM) (C); fasting blood glucose levels (D); fasting serum hormone levels (E); gene expressions in intrascapular BAT (F); glucose levels in response to subcutaneous injection of norepinephrine (1 mg/kg) (G); tissue-specific glucose uptake in response to subcutaneous injection of norepinephrine (1 mg/kg) (H); and prostaglandin levels from BAT of cold-acclimated WT and L-PGDS KO mice (I). All animals were 7-month-old C57Bl/6 males, fed either chow or 60% calories-from-fat diet (HFD), n = 8 per group, fasted animals (except in H and I), n = 7 per group. a, P < 0.05 for diet; b, P < 0.05 for genotype; c, P < 0.05 for genotype and diet interaction. *P < 0.05.
constitutive glucose transporter Glut1 were upregulated in mice lacking L-PGDS. To test the concept that the sympathetic nervous system could regulate glucose levels in L-PGDS KO mice, we injected mice lacking L-PGDS with norepinephrine in combination with 2[1-13C]DG. Mice lacking L-PGDS exhibited a 60% increase in glucose disposal to BAT relative to wild-type controls, supporting the concept of sympathetically mediated glucose uptake into BAT being elevated in the absence of L-PGDS. BAT exhibits very high rates of glucose uptake in response to either norepinephrine or insulin, and although the exact contribution of glucose as a substrate for thermogenesis remains debatable, it seems likely that it does contribute significantly (37). Several publications have demonstrated sympathetic control of glucose uptake into BAT (38–42) and that this uptake is dependent on UCPI (39). Insulin stimulated uptake of glucose to BAT is not dependent on it being thermogenically functional (40); however, cold exposure does increase the rate of glucose disposal into BAT in response to insulin (42). Overall, these results suggest that BAT can have a major impact on systemic glucose levels and, importantly, that it can do so independent of the actions of insulin.

The finding that L-PGDS can mediate fuel provision to BAT is a potentially important one given the renewed excitement in the potential of BAT to treat metabolic disease after its rediscovery in adult humans (8,12,43). Although much research into BAT has focused on its recruitment and molecular activation, relatively less research has gone into the supply of nutrients to BAT. Recent articles regarding angiogenesis in BAT (44) and the control of lipid uptake into BAT (45) have helped to further our understanding of fuel supply to BAT; however, if BAT is ever to be used to treat human obesity, then it must be both fully activated and appropriately supplied with nutrients. The ability of L-PGDS to control in part the balance of lipid and carbohydrate utilization by BAT highlights another potential layer of regulation of BAT activity.

To try to address in part how L-PGDS may affect BAT function on a molecular level, we measured prostaglandin levels in cold-acclimated BAT and detected no differences. Because L-PGDS is a bifunctional molecule capable of acting both as a carrier of lipophilic molecules (46) and as a prostaglandin synthase (15), the absence of changes in prostaglandins suggested that L-PGDS may act primarily as a lipocalin in terms of its regulatory role in BAT. What L-PGDS binds to and how that molecule may regulate BAT function will be an area for future study.

In summary, L-PGDS appears to regulate the balance between carbohydrate and lipid metabolism in BAT. The association between metabolic health and an ability to switch between carbohydrate and lipid metabolism is already established; however, the ability of L-PGDS to control the balance between lipid and carbohydrate utilization, with only minor effects on metabolic rate, highlights a new type of control mechanism for whole organism substrate handling that could potentially be used to bypass states of nutritionally induced or genetic insulin resistance.

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S.V., H.F., M.Ch., M.Ca., M.D., C.L., M.M., P.V., and K.B. performed experiments. S.V., C.Y.T., M.M., J.L.G., H.F., and B.C. analyzed data. S.V., M.P., P.V., J.K.S., J.L.G., B.C., and A.V.-P. designed experiments. S.V. and A.V.-P. wrote the manuscript. Y.U. and N.E. provided essential reagents. S.V. and A.V.-P. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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