Loss of the ciliary gene Bbs4 results in defective thermogenesis due to metabolic inefficiency and impaired lipid metabolism

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Abstract

Adipose tissue is central to the regulation of energy balance. While white adipose tissue (WAT) is responsible for triglyceride storage, brown adipose tissue specializes in energy expenditure. Deterioration of brown adipocyte function contributes to the development of metabolic complications like obesity and diabetes. These disorders are also leading symptoms of the Bardet–Biedl syndrome (BBS), a hereditary disorder in humans which is caused by dysfunctions of the primary cilium and which therefore belongs to the group of ciliopathies. The cilium is a hair-like organelle involved in cellular signal transduction. The BBSome, a supercomplex of several Bbs gene products, localizes to the basal body of cilia and is thought to be involved in protein sorting to and from the ciliary membrane. The effects of a functional BBSome on energy metabolism and lipid mobilization in brown and white adipocytes were tested in whole-body Bbs4 knockout mice that were subjected to metabolic challenges. Chronic cold exposure reveals cold-intolerance of knockout mice but also ameliorates the markers of metabolic pathology detected in knockouts prior to cold. Hepatic triglyceride content is markedly reduced in knockout mice while circulating lipids are elevated, altogether suggesting that defective lipid metabolism in adipose tissue creates increased demand for systemic lipid mobilization to meet energetic demands of reduced body temperatures. These findings taken together suggest that Bbs4 is essential for the regulation of adipose tissue lipid metabolism, representing a potential target to treat metabolic disorders.

Abbreviations: Adipoq, adiponectin; ALT, alanine aminotransferase; Angptl4, angiopoietin like 4; Angptl8, angiopoietin like 8; Apoa1, apolipoprotein A1; Apoe, apolipoprotein E; AST, aspartate aminotransferase; ATGL, adipose triglyceride lipase; BAT, brown adipose tissue; BBS, Bardet–Biedl syndrome; Cds6, cluster of differentiation 36; Chol, cholesterol; Cyp51, cytochrome P450 family 51; eWAT, epididymal white adipose tissue; Fatp1, long-chain fatty acid transporter 1; FFA, free fatty acids; Gly, glycerol; HDL, high-density lipoprotein; Hmgr, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HSL, hormone sensitive lipase; iWAT, inguinal white adipose tissue; Ldr, low density lipoprotein receptor; Lep, leptin; Lpc, hepatic lipase; Lpl, lipoprotein lipase; Lrp1, lipoprotein receptor-related protein 1; Plin1, perilipin 1; Pparg, peroxisome proliferating factor gamma; SVF, stromal vascular fraction; TG, triglycerides; TP, total protein stain; Ucp1, uncoupling protein 1; Vldlr, very low density lipoprotein receptor.
1 | INTRODUCTION

The metabolic syndrome, which is accompanied by dyslipidemia and insulin resistance, is a primary risk factor for the development of cardiovascular disease and type 2 diabetes mellitus. As obesity and adipose tissue dysfunction are key aspects of this pathology, the analysis of the metabolic functions of brown and white adipose tissue (WAT) is important to understand the contributing mechanisms. While WAT is responsible for storage of triacylglycerides in large unilocular adipocytes, brown adipose tissue (BAT) dissipates energy in form of heat in a process known as non-shivering thermogenesis. Brown adipocytes are multilocular cells rich in mitochondria and uniquely express the Uncoupling protein 1 (UCP1) which uncouples the mitochondrial proton gradient from ATP synthesis, generating heat instead. Thermogenesis is activated upon cold exposure in brown adipocytes. Prolonged cold exposure or other mechanisms of sustained adrenergic activation lead to browning of white fat, i.e., the formation of brown-like adipocytes that express Ucp1. In humans, metabolically active brown adipocytes have recently received renewed scientific attention and have been proposed as a target for the treatment of metabolic diseases. Interestingly, adiposity is a leading symptom of the so-called ciliopathies, like the Bardet–Biedl Syndrome (BBS) and the Alström Syndrome. BBS is a pleiotropic autosomal recessive disorder caused by genetically heterogeneous mutations known to target at least 12 Bbs genes, therefore showing a wide range of clinical symptoms. The primary cilium is a non-motile, hair-like organelle that consists of a microtubular structure called axoneme with a sensory function to detect extracellular signals and which is involved in directed protein transport processes known as intraflagellar transport. The axoneme emanates from the basal body, which is thought to regulate protein sorting to and from the ciliary structure through the transition zone (TZ). The TZ serves as diffusion barrier, that maintains the concentration of ciliary membrane proteins and prevents diffusion of proteins from the surrounding (apical) plasma membrane. The BBSome is a multiprotein complex that is localized at the basal body. It is composed of distinct proteins encoded by highly conserved Bbs genes. Among these, the gene Bbs4 is thought play a role in microtubule anchoring and cell cycle progression, but most cellular functions of Bbs4 have not been studied in great detail and with respect to functions in different cell types. In addition to regulation of cilia development and function, it was reported that basal body proteins fulfill cilia-independent functions, and have been reported to be involved in the regulation of signaling pathways such as Notch signaling through endosomal trafficking and Wnt signaling by selective proteolysis.

Like in many other BBS-ciliopathies, the development of morbid obesity is a key symptom of patients carrying mutations of Bbs4. Studies using Bbs4 knockout mice (Bbs4-KO) have revealed an association of obesity with hyperleptinemia, suggesting that loss of responsiveness to leptin might be due to attenuated leptin receptor signaling. Gerdes et al previously reported that loss of Bbs4 contributes to impaired glucose metabolism due to an insulin secretion defect in pancreatic beta cells and perturbations of overall islet architecture in diabetic rats. Insulin-dependent regulation of Bbs4 was further linked to regulation of adipocyte differentiation, as it was shown that insulin inhibits Bbs expression during early adipogenesis and that silencing of Bbs4 impaired glucose uptake into adipocytes. Furthermore, absence of functional Bbs4 led to accelerated preadipocyte proliferation, resulting in abnormal adipocyte differentiation by suppression of adipogenic marker expression. To this end, aberrant function of the BBS4 protein may result in aberrant triglyceride accumulation in mature adipocytes, where lipids are stored in smaller lipid droplets, but in larger number.

Taken together these findings suggest that ciliary dysfunctions caused by mutations of Bbs4 might affect adipose tissue function, but the molecular processes that depend on Bbs4 in the regulation of energy expenditure and adipose tissue remodeling remain unknown. Here we show that loss of Bbs4 negatively affects thermogenic potential and leads to cold intolerance potentially due to an impairment of lipid mobilization in adipose tissues and the liver and defective fatty acid metabolism.

2 | MATERIAL AND METHODS

2.1 | Adipogenic progenitor (APC) isolation and differentiation

Adipose tissue derived progenitor cells were isolated from the BAT and inguinal white adipose tissue (iWAT) of mice according to published procedures. Primary adipocytes and stromal vascular fractions (SVF) were resuspended in QIAzol lysis reagent (Qiagen, Hilden, Germany) directly after isolation for gene expression analysis.
Primary APCs were isolated by fluorescence-activated cell sorting, cultivated and differentiated as described previously.\textsuperscript{25}

### 2.2 Animal housing and experiments

All procedures were approved by the ethics committee for animal welfare of the State Office of Environment, Health, and Consumer Protection (State of Brandenburg, Germany). Bbs4-KO mice (German Center for Diabetes Research (DZD), München-Neuherberg, Germany) and male C57Bl6J mice (Charles River Laboratories, Sulzfeld, Germany) were housed in a controlled environment (22 ± 2°C, 12/12 h light/dark cycle), maintained on a standard diet (Ssniff, Soest, Germany). For metabolic analysis of mice in the cold, whole body composition was analyzed by nuclear magnetic resonance (NMR) technology (EchoMRI\textsuperscript{TM}-100H, EchoMRI LLC, Houston, TX, USA) directly before housing mice in a climate chamber at 4°C for ten days and was repeated after 5 days of cold exposure. Blood was sampled at room temperature directly before decreasing ambient temperature from the vena facialis.

Body weight and temperature were controlled daily using a rectal probe thermometer (Bioseb, FL, USA). For acute β3-adrenergic stimulation, mice were fasted for 2 h followed by intraperitoneal injection of 1 mg/kg body weight CL316,243 (Sigma-Aldrich, Taufkirchen, Germany) disolved in PBS. Plasma was sampled before CL-injection and after 90 and 180 min and immediately frozen in liquid nitrogen. Mice were sacrificed subsequently by cervical dislocation and organs were flash-frozen in liquid nitrogen for RNA and protein analysis. The tissues were ground in liquid nitrogen and the powder was aliquoted for the different analyses. A power analysis was performed with $\alpha = .05$, $\beta = .2$ (Power 80%) and with an effect size of $\delta = 1.2$ to determine maximum group sizes. However, final group sizes during experiments were adjusted depending on data significance levels during intermediate analyses in line with the 3R principles of animal experimentation, i.e., reduction, which requires minimization of animal numbers used during research.

### 2.3 Plasma collection and analysis

Blood was sampled by punctation of the vena facialis and collected in EDTA-coated tubes to prevent coagulation. Samples were kept on ice and centrifuged at 8000 rpm for 10 min. Clear plasma-supernatants were collected, and blood parameters were measured on a Cobas Mira analyzer (Roche Diagnostics, Mannheim, Germany) by using commercially available assay kits for free fatty acids (FFA) (Wako chemicals, Neuss, Germany), glycerol (Randox, Crumlin, UK), triglycerides, cholesterol, high-density lipoprotein (HDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glucose (Axonlab, Stuttgart, Germany).

### 2.4 Histological analysis

Tissues were overnight-fixed directly after dissection in 4% formalin at room temperature, subsequently dehydrated and embedded in paraffin. Paraffin sections (4 µm thickness) were stained with hematoxylin and eosin (H&E). All sections were photographed at 200×. Additionally, liver sections were captured at 400× magnification. For measurements of lipid droplet size, IWAT images were analyzed at a magnification of 200× using ImageJ (NIH, Bethesda, MA, USA) as previously described.\textsuperscript{26} A total of three non-overlapping histological images from each section were averaged for each animal.

### 2.5 Triglyceride analysis in livers

Ground tissue was homogenized in a buffer containing 10 mM sodium dihydrogen phosphate, 1 mM EDTA, and 1% polyoxyethylene (10) tridecyl ether at pH 7.4. Samples were then incubated for 5 min at 70°C, were cooled on ice to 4°C, and subsequently centrifugated at 13 000 rpm and 4°C for 10 min. Triglycerides were determined in clear supernatants using commercially available assay kit (Axonlab, Stuttgart, Germany) on a Cobas Mira instrument according to kit manufacturer’s instructions (Roche Diagnostics, Mannheim, Germany).

### 2.6 Quantitative real-time PCR

For primary adipocytes, RNA was extracted with trizol and purified by using a column-based extraction kit (Zymo Research, Freiburg, Germany). From 50 mg of ground tissue, total RNA was extracted with 500 µl trizol and homogenized in the bullet blender for 5 min at 4°C followed by centrifugation for 10 min at 4°C and 12 000 g to separate the lipid layer. Phase separation was initiated by adding 100 µl chloroform. After 15-min centrifugation at 12 000 g and 4°C, RNA was precipitated from the upper clear phase with 250 µl of isopropanol. Lastly, RNA was purified with 500 µl of 75% ethanol, dried and solubilized in 30 µl DEPC water. Purified RNA was reversely transcribed into cDNA using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, Dreieich, Germany). Quantitative real-time PCR was performed using Maxima SYBR Green/ROX qPCR Master Mix.
(Thermo Fisher Scientific, Dreieich Germany) on a CFX384 Touch instrument (Bio-Rad, München, Germany). Primers were designed as intron spanning sequences to specifically amplify cDNA (Table S1).

2.7 Immunoblotting

Samples were homogenized in RIPA buffer with addition of 0.5% Triton X 114 (Sigma-Aldrich, Taukirchen, Germany). Protein concentrations were determined using a BCA protein assay kit (Thermo Fisher Scientific, Dreieich, Germany). Proteins were separated on a 12% SDS-polyacrylamide gel electrophoresis and transferred on PVDF membranes for detection using the following antibodies: anti total HSL (Cell signaling Technology, Danvers, MA, USA), anti-phospho-HSL (Cell signaling Technology, Danvers, MA, USA), anti ATGL (Abcam, Cambridge, United Kingdom) followed by incubation with a species-specific horseradish peroxidase-conjugated secondary antibody. As loading controls, total protein was stained using a commercially available kit (Thermo Fisher Scientific, Dreieich, Germany) were used.

2.8 Statistical analyses

Significance levels of differences between groups were evaluated using either parametric unpaired two-tailed Student’s t-test with Welch correction or nonparametric Mann–Whitney test using the GraphPad Prism software (Version 8.1.2).

3 | RESULTS

3.1 Expression of Bbs4 is enriched in mature white adipocytes

To determine the distribution of Bbs4 gene expression in adipose tissue, we compared Bbs4 mRNA levels in the mature adipocyte and the SVFs freshly isolated from brown and iWAT of young healthy C75B6/J mice. While Bbs4 mRNA was significantly lower in the adipocyte fraction of BAT compared to its SVF, we measured a significant increase in its expression in white adipocytes compared to the SVF of iWAT (Figure 1A). To verify whether Bbs4 was indeed expressed in mature adipocytes, we isolated brown and white APCs of wildtype mice by flow cytometry and analyzed Bbs4 gene expression throughout a time course of adipogenic differentiation. Under these conditions, only a trend toward increased expression was detected in BAT-derived APCs during differentiation (Figure 1B). Conversely, Bbs4 expression increased significantly from the fourth day of differentiation onward in APCs isolated from iWAT (Figure 1C).

3.2 Loss of Bbs4 in mice results in adiposity and cold-intolerance

To assess metabolic effects of Bbs4-deficiency on adipose tissue function under thermal stress, we exposed mice with a whole-body ablation of the Bbs4 gene (Bbs4-KO) and wildtype littermates (WT) to a temperature of 4°C for ten days. Under these conditions of chronic cold-exposure,
thermogenesis in brown adipocytes is activated and WAT depots undergo morphological adaptations known as browning to ensure efficient thermoregulation. Body weight and body composition were determined immediately before cold exposure and after five and ten days of cold exposure. Bbs4-KO mice were generally lighter compared to wild-type littermate controls which was mainly due to significantly reduced lean mass in knockout animals (Figure 2A,B). Moreover, body weight was significantly reduced after five days of cold exposure in Bbs4-KO, but not in WT mice and did not change further significantly between five and ten days of cold exposure (Figure 2A). Accordingly, and confirming the previously described obesity phenotype, total fat content and body weight-normalized fat mass were significantly increased in Bbs4-KO before cold, which was completely ameliorated after five days in the cold and no significant differences were measured after ten days of cold exposure between WT and KO mice (Figure 2C,D).

In addition to body weight monitoring, we performed daily measurements of body core temperature which revealed a decreased body temperature in Bbs4-KO compared to WT during the whole period of housing mice in the cold, altogether suggesting that Bbs4-KO are unable to maintain normal thermogenesis under these conditions (Figure 2E). The analysis of plasma metabolites before cold exposure showed significantly higher levels of triglycerides, and HDL and a trend for elevated cholesterol in knockout compared to wildtype mice (Figure 2F, d0), while no differences were detected for plasma levels of glycerol, FFA and glucose. We also measured an increase in the liver enzymes ALT and AST in plasma of Bbs4-KO mice housed at room temperature (Figure 2G, d0). Sustained cold exposure led to an overall normalization of elevated TG, cholesterol, HDL, ALT and AST levels in Bbs4-KO mice (Figure 2F,G, d10). In summary, while loss of Bbs4 leads to impaired hepatic metabolism and adiposity, cold exposure ameliorates this metabolic phenotype despite uncovering defective thermogenesis in mice lacking Bbs4.

### 3.3 | Bbs4 knockout attenuates browning capacity of inguinal WAT

To examine functional consequences of the Bbs4 knockout in adipose tissue, we further analyzed BAT and iWAT morphology. While no effect was visible in BAT (Figure 3A), the occurrence of brown-like adipocytes was less pronounced in iWAT of cold-exposed Bbs4-KO compared to WT mice (Figure 3B). This observation was corroborated by quantification of lipid droplet size in iWAT, showing higher abundance of larger lipid droplet areas in iWAT of Bbs4-KO mice (Figure 3C). To further clarify the thermogenesis defect, we measured different marker genes. No significant differences in the expression profiles of adipogenic genes were found in BAT of WT and Bbs4-KO besides a mild reduction of the mitochondrial genes Cox7a and Cpt1b and reduced expression of perilipin 1 (Plin1) and fatty acid transport protein 1 (Fatp1; Figures 3D and S1A). Conversely, we observed a significant reduction of thermogenic and mitochondrial marker genes in iWAT of Bbs4-KO mice after cold exposure, while more general adipogenic genes as well as genes regulating fatty acid transport and lipoprotein metabolism remained generally unaffected, confirming the observations of the histological analysis regarding the impaired formation of brown-like adipocytes (Figures 3E and S1B).

To further assess the fat mass loss and defective thermoregulation of Bbs4-KO during cold exposure, we measured markers of lipolysis, as this process is central to the activation of BAT thermogenesis by providing the necessary nutrients to fuel heat generation. Under these conditions, we observed a reduction of adipose triacylglyceride lipase (ATGL) protein in BAT of Bbs4-KO mice (Figure 3F), whereas no significant effect was detected in iWAT (Figure 3G). Additionally, no significant changes of the phosphorylation of hormone sensitive lipase were found in BAT and iWAT (HSL, Figure S2A,B).

As Bbs4-KO mice were reported to suffer from mild liver damage when housed at room temperature and showed abnormal plasma liver enzyme profiles when housed at room temperature (Figure 2G), we analyzed liver morphology in the cold exposed animals. Here, we found decreased amounts of ectopic fat in livers of KO mice compared to WT littermates and concomitantly a significantly reduced concentration of triglycerides in liver homogenates was measured (Figure 4A,B). Although mRNA expression of genes regulating lipoprotein and cholesterol metabolism were generally not changed in Bbs4-KO livers, we detected significantly higher expression of fatty acid transporter cluster of differentiation 36 (Cd36) and simultaneous reduction of lipase C (Lipc), which codes for the enzyme catalyzing the initial step of hepatic lipolysis (Figure 4C). Phosphorylation of HSL was reduced in livers of knockout mice compared to wildtype mice (Figures 4D and S2C). It should be noted that HSL expression in liver is considered to be very low, but our analysis shows that it is expressed at detectable levels in liver after chronic cold exposure.

### 3.4 | Systemic enhancement of lipid mobilization may compensate for dysfunctional WAT in Bbs4-KO mice

Since chronic cold exposure affected lipid mobilization and metabolism in adipose tissue and the liver, we next sought to assess the effects of Bbs4 gene deletion on
acute alterations of lipid metabolism following adrenergic stimulation. To this end, WT and Bbs4-KO mice were injected with a β3-adrenergic receptor (ADRB3) agonist, CL316,243 (CL), which mimics the cold stimulus by directly binding to ADRB3, which is primarily expressed on adipocytes.2 This short-term treatment was not sufficient to influence body or adipose tissue weight of the two genotypes (Figure 5A,B). Changes to the plasma lipids displayed a typical pattern in response to adrenergic stimulation in both genotypes, which show increases in FFA and TG over time following CL treatment. However, and paralleling the observations in mice prior to the chronic cold challenge, TG and cholesterol levels, and to a milder extent also levels of HDL, remained elevated in KO mice compared to controls for the duration of the acute stimulation (Figure 5C, panels 1–3). As without stimulation, no differences were detected between genotypes for plasma levels of glycerol, FFAs, and glucose (Figure 5C, panels 4–6). Thus, while chronic cold was able to ameliorate the detrimental effects of Bbs4 gene deletion on plasma lipids, this was apparently not a consequence of acute, adrenergic stimulation-induced intake of lipids into adipose tissue.
3.5 | Loss of Bbs4 leads to impaired lipid metabolism in adipose tissue and liver

Similar to the observations after chronic cold exposure, we detected no significant effects of acute adrenergic stimulation on mRNA expression of marker genes of mitochondrial function, (brown) adipogenesis or lipid metabolism in BAT (Figures 5D and S3A). Conversely, the expression of marker genes of browning and mitochondrial function was broadly attenuated in iWAT of Bbs4-KO mice, indicating a dysfunctional response was already evident under these acute conditions of adrenergic stimulation (Figure 5E). Similar to cold exposure, no differences were found in iWAT for general adipogenic marker genes (Figure S3B).

To test whether acute adrenergic stimulation also affects lipid mobilization, we assessed ATGL protein expression in adipose tissues and while there was a trend toward increased levels in BAT, no difference was observed in iWAT (Figures 6A,B and S4A,B). Since the time course of acute stimulation may not have been sufficient to affect protein expression, we also assessed
HSL activation by phosphorylation. Indeed, phosphorylated HSL levels were markedly increased in BAT, iWAT, and liver, albeit not always reaching statistical significance (Figures 6C–E and S4C–E). Unexpectedly, total HSL levels were strongly induced in iWAT, but not BAT or liver, which resulted in a decreased phospho-HSL/HSL ratio in iWAT, while the ratio tended to be increased in BAT and liver (Figures 6C–E and S4C–E). Unlike after chronic cold-exposure, hepatic triglyceride levels were unchanged or even showed an upward trend in Bbs4-KO livers which could also reflect the elevated liver enzymes observed in the first cohort prior to cold exposure (Figure 6F). In summary, loss of Bbs4 results in defective systemic lipid homeostasis, with adipose tissue and the liver showing a dysregulated lipid metabolism response following acute adrenergic stimulation. However, increased mobilization and availability of lipids does not result in an adequate thermogenic response.

4 | DISCUSSION

The Bardet–Biedl syndrome is characterized by dysfunction of the primary cilium due to mutations in individual members of the Bbs gene family. Among other symptoms, patients develop morbid obesity and metabolic complications with elevated prevalence of type 2 diabetes.30 Here we show that loss of Bbs4 results in defective thermogenesis, which is mainly due to a defective lipid metabolism response in WAT and subsequent depletion of lipid stores in liver under conditions of sustained cold stress, suggesting that obesity is due to attenuated adipose tissue lipid metabolism in this model of BBS.

Central to adipose tissue integrity is the activation of preadipocytes upon metabolic demand, which go on to differentiate into mature adipocytes, a process that is regulated by multiple factors. One of the relevant characteristics is the transient ciliation of differentiating adipocytes. Hence, the expression of several Bbs genes...
increases after adipogenic induction and was reported to decrease in the late phase, as adipocytes are thought to lose their primary cilia upon reaching full maturity.\textsuperscript{31,32} This would indicate a functional relevance of the cilium in adipocyte maturation, and accordingly it was reported that loss of various \textit{Bbs} gene products leads to augmented accumulation of triglycerides, alterations of fatty acid profiles and morphological changes of white adipocytes.\textsuperscript{22,33} While adipose tissue dysfunction due to loss of \textit{Bbs} genes is well-established, very little is known about the metabolic consequences and the distinct implications for white versus brown adipocytes. As we observed higher expression of one of the \textit{Bbs} genes, \textit{Bbs4}, in the adipocyte fraction of iWAT and induction of its expression during differentiation of iWAT-derived preadipocytes, we conclude a higher relevance of \textit{Bbs4}-dependent processes in white adipocyte maturation compared to formation of brown adipocytes. This is further supported by the physiological characterization of \textit{Bbs4} deficient mice housed at room temperature, which manifest an obesogenic phenotype and abnormal plasma lipid profiles, indicated by increased circulating triglycerides, cholesterol and HDL concentrations.\textsuperscript{18,29} More importantly, the ability to maintain normal body temperature through thermogenesis appears to be impaired in Bbs4-KO mice, which could be indicative of overall reduced energy metabolism to
Exacerbate obesity. While lipid metabolism and mobilization in white fat and liver of Bbs4-KO animals is clearly impaired, brown fat appears to be phenotypically unremarkable aside from reduced ATGL expression. Secondly, ectopic fat storage occurred in livers of KOs, which may also explain the previously described impairment of liver function. Given that Bbs4-KO mice appear underweight and runted compared to WT littersmates before obesity develops after 12 weeks of age, our observations suggest that a mild metabolic inefficiency, combined with an inability for adequate lipid handling in adipocytes, is present in these animals.

Adrenergic stimulation due to cold exposure acutely leads to activation of thermogenesis in brown adipocytes and lipid mobilization in liver and white fat, whereas chronic stimulation results in additional remodeling of iWAT in a process known as browning. Heat is generated by adaptive thermogenesis through mitochondrial oxidation of nutrients to maintain body temperature. Accelerated lipid uptake into BAT is thought to be a main fuel contributor for thermogenesis, as BAT was described as key organ for correction of hyperlipidemia following adrenergic stimulation.

Consequently, our data suggest that Bbs4 ablation does not affect adrenergic activation of BAT thermogenesis per se, but that the decreased thermogenic activity under chronic cold exposure occurs as a result of reduced substrate availability in adipocytes of Bbs4 deficient mice. Therefore, elevated levels of triglycerides in Bbs4-KO mice may indicate an inability of brown (and white) adipocytes to take up and metabolize lipids from the circulation. This may be linked to attenuated lipid uptake mechanisms, such as reduced expression of lipid transporters, including Fatp1, which mediates membrane crossing of long chain fatty acids. This is particularly evident when intracellular lipid storage is depleted and thermogenic activity depends on the direct import of lipids into activated brown adipocytes. Accordingly, Fatp1 knockout mice show increased efflux of mobilized lipids upon acute adrenergic stimulation, but depletion of lipid storages occurs under

![Graph showing activation of liver lipid metabolism upon acute adrenergic stimulation.](image-url)
chronic challenges due to defective replenishment of lipid stores in adipocytes. Accordingly, we find that livers of cold-exposed Bbs4-KO mice feature significantly reduced triglyceride storage which could be interpreted as a consequence of increased energy demand from the ineffective adipose tissues.

An acute adrenergic stimulation activates breakdown of triglycerides in adipose tissue and liver by increasing lipolytic activity, leading to elevated mobilization of lipids to compensate for defective import of lipids to brown adipocytes. It could be assumed that intracellular lipid stores of Bbs4-KO mice, which feature increased fuel demand due to inefficient thermogenesis. In agreement with this, Bbs4-KO mice showed a marked loss of body fat content after chronic cold exposure and the systemic markers of metabolic dysfunction, such as elevated liver enzymes and circulating triglycerides, were ameliorated. Therefore, cold acclimatization is sufficient to normalize the metabolic defects of our animal model, presumably by reducing the detrimental effects of systemic ectopic lipid accumulation. The role of bona fide browning in this process remains to be elucidated. While attenuation of iWAT browning was observed under acute and chronic stimulation, the differences in gene expression between Bbs4-KO mice and WT are relatively mild compared to the absolute changes that are routinely observed in response to adrenergic stimulation, which can easily reach several orders of magnitude. It therefore stands to reason that defective processing steps of lipids for metabolism, and not browning of iWAT per se, are the main drivers of hyperlipidemia and late-onset adiposity and that cold exposure is sufficient to counteract these effects.

A remaining question is the direct involvement of a functional cilium in adipocyte metabolic inefficiency. Adipocytes are formed within adipose tissue by de novo adipocyte development from pre-adipocytes. This process requires morphological rearrangements that may be regulated by a transient presence of a cilium on differentiating adipocytes. However, since mature adipocytes are most likely not ciliated, it is unclear how this translates into a progressive metabolic inefficiency effect that is mainly prevalent in adult mice. Bbs4 silencing decreases differentiation capacity, giving rise to cells with smaller lipid droplets that are morphologically more reminiscent of preadipocytes. Therefore, it could be hypothesized that the maturation of adipocytes is generally impaired due to loss of a functional cilium during their development and that the aging organism becomes less able to cope with this defect, ultimately resulting in hepatic dysfunction, hyperlipidemia and obesity. Another potential explanation may be that the BBSome and some BBS proteins may possess extra-ciliary function, for instance in intracellular cargo trafficking. Such mechanisms have been proposed but it remains to be determined whether this also pertains to the mature adipocyte.

5 CONCLUSION

In summary, our data indicate that cilium-dependent and -independent mechanisms may be involved in the regulation of mature adipocyte function. Bbs4 ablation directly affects thermogenic potential by targeting the ability of adipocytes to efficiently take up and metabolize lipids. Chronic cold exposure, despite leading to collateral cold intolerance, is sufficient to overcome the metabolic inefficiency. By increasing systemic lipid mobilization, the metabolic activation improves hyperlipidemia and body fat accumulation, suggesting that the primary consequences of adrenergic stimulation may not be directly linked to effective thermogenesis but rather to breakdown of superfluous lipids. Understanding regulatory mechanisms of lipid homeostasis as regulators of metabolic efficiency, even independent of brown adipocyte function, could provide potential targets to treat metabolic disorders, such as ciliopathies and other metabolic disorders.

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DISCLOSURES

The authors declare no conflict of interest in relation to this study.

AUTHOR CONTRIBUTIONS

T.J.S. and S.G. conceived experiments, analyzed the majority of the data, and wrote the manuscript. S.G. performed the majority of experiments. C.M. and F.G.-C. Performed gene expression analyses and analyzed data.
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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of the article at the publisher’s website.

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