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Sphk2$^{-/-}$ mice are protected from obesity and insulin resistance

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ABSTRACT

Sphingosine kinases phosphorylate sphingosine to sphingosine 1-phosphate (S1P), which functions as a signaling molecule. We have previously shown that sphingosine kinase 2 (*Sphk2*) is important for insulin secretion. To obtain a better understanding of the role of Sphk2 in glucose and lipid metabolism, we have characterized 20- and 52-week old *Sphk2*$^{-/-}$ mice using glucose and insulin tolerance tests and by analyzing metabolic gene expression in adipose tissue. A detailed metabolic characterization of these mice revealed that aging *Sphk2*$^{-/-}$ mice are protected from metabolic decline and obesity compared to WT mice. Specifically, we found that 52-week old male *Sphk2*$^{-/-}$ mice had decreased weight and fat mass, and increased glucose tolerance and insulin sensitivity compared to control mice. Indirect calorimetry studies demonstrated an increased energy expenditure and food intake in 52-week old male *Sphk2*$^{-/-}$ versus control mice. Furthermore, expression of adiponectin gene in adipose tissue was increased and the plasma levels of adiponectin elevated in aged *Sphk2*$^{-/-}$ mice compared to WT. Analysis of lipid metabolic gene expression in adipose tissue showed increased expression of the *Atgl* gene, which was associated with increased Atgl protein levels. *Atgl* encodes for the adipocyte triglyceride lipase, which catalyzes the rate-limiting step of lipolysis. In summary, these data suggest that mice lacking the *Sphk2* gene are protected from obesity and insulin resistance during aging. The beneficial metabolic effects observed in aged *Sphk2*$^{-/-}$ mice may be in part due to enhanced lipolysis by Atgl and increased levels of adiponectin, which has lipid- and glucose-lowering effects.

1. Introduction

Sphingolipids are derived from ceramide, which is generated *de novo* from L-serine and palmitoyl-CoA catalyzed by serine palmitoyltransferases [1,2]. Ceramidases convert ceramide to sphingosine by hydrolyzing the fatty acids from ceramide. Sphingosine is then phosphorylated by two sphingosine kinases (*Sphk1/2*) to sphingosine 1-phosphate (S1P) [3]. *Sphk1* has pro-survival activity, while *Sphk2* is pro-apoptotic [3–5]. They differ in their activity, regulation, and localization [3,6]. *Sphk1* and *Sphk2* null mice are viable, but deletion of both genes results in embryonic lethality [7]. While the function of *Sphk1* has been studied in detail in various tissues, the exact function of *Sphk2* remains to be determined [8]. A recent study suggests that polymorphisms in the *Sphk2* gene may contribute to the genetic predisposition to type 1 diabetes [9].

Sphingolipids, including ceramide and S1P serve as signaling molecules [10,11]. S1P plays an important role in cell signaling, proliferation, cell survival and differentiation [11]. It has intracellular as well as extracellular signaling functions by serving as a ligand for five different G protein-coupled receptors ($S1P_1$-$S1P_5$) [11,12]. In mammals, there is a strong correlation between ceramide accumulation and age-related diseases, including type 2 diabetes, cardiovascular disease, cancer, and neurodegeneration [13–15]. While increased levels of ceramide are associated with insulin resistance in mice and humans, the role of S1P in regulation of glucose metabolism remains unclear [14,16–19]. However, recent data suggest that increased levels of S1P are also associated with obesity and insulin resistance [20]. Plasma S1P levels have been shown to be elevated in both obese humans and rodents [21]. Furthermore, palmitate-induced increases in S1P levels have been associated with insulin resistance in pancreatic beta cells as well as in hepatocytes via activation of the S1P receptor subtype 2 ($S1P_2$) [22,23]. Administration of JTE-013, an $S1P_2$ antagonist prevented the insulin resistance mediated by palmitate [20,22,23].

Adiponectin is mainly produced in adipocytes and has glucose- and

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lipid-lowering effects [24]. Recent studies suggest that the adiponectin receptors AdipoR1/R2 have ceramidase activity and are capable of converting ceramide to sphingosine [25]. Binding of adiponectin to AdipoR1/R2 receptors stimulates their ceramidase activity, thereby leading to lowering of ceramide levels. Ablation of the adiponectin receptors in mice results in accumulation of ceramide, which is associated with insulin resistance and glucose intolerance [26]. These findings suggest that the beneficial pleiotropic effects of adiponectin on glucose and lipid metabolism are due to its ability to lower ceramide levels.

We have previously shown that *Sphk2* is important for insulin secretion from pancreatic beta cells [27]. A detailed characterization of *Sphk2*$^{-/-}$ mice revealed that they are protected from age-related obesity and metabolic decline, which may be in part due to elevated adiponectin levels and enhanced lipolysis in adipose tissue of *Sphk2*$^{-/-}$ mice.

2. Materials and methods

2.1. Animals

All animals were housed in a specific pathogen-free animal facility at the University of Kentucky on a 14-h light and 10-h dark cycle and kept under standard humidity and temperature conditions with free access to water and rodent chow. All animal procedures were reviewed and approved by the University of Kentucky Institutional Animal Care and Use Committee (Protocol 2011-0806). *Sphk2*$^{-/-}$ mice on C57BL/6N background were obtained from the Jackson Laboratories (Stock No: 019140) and have been previously described in detail [7]. *Sphk2* heterozygous mating pairs were used to generate *Sphk2*$^{-/-}$ and wild type litter mates. Animals were euthanized with carbon dioxide followed by cervical dislocation.

2.2. Metabolic tests

All metabolic tests were carried out with male and female mice and followed the standard operating procedures as established previously [28]. The presented data were obtained with young mice (20–22 weeks of age) and old mice (50–54 weeks of age). For glucose tolerance tests (GTT), mice were fasted for 6 h and i.p. injected with glucose (2 g/kg). Mice were fasted for 4 h before insulin tolerance tests (ITT) were carried out by i.p. administration of insulin (0.75 units/kg). In all tests, blood glucose was measured from tail vein at the indicated time points using the Nova Max Plus glucometer. Plasma insulin levels were measured using an insulin ELISA kit (Crystal Chem). Circulating levels of adiponectin and leptin were quantified using ELISA kits from Chrystal Chem. Triglyceride levels were measured using colorimetric kits from Abcam. Homeostasis model assessment of insulin resistance (HOMA-IR) was determined using the following formula: fasting glucose (mg/dL) × fasting insulin (μU/ml)/405.

2.3. Indirect calorimetry and body composition measurements

Indirect calorimetry to monitor food consumption, physical activity, O_2 consumption, and CO_2 production was measured using the TSE LabMaster system by the COBRE Metabolic Core at the University of Kentucky. Fat and lean body mass was determined on conscious mice using an EchoMRI-5000 whole-body composition analyzer (Echo Medical System) that uses magnetic resonance relaxometry.

2.4. Preparation of protein extracts and Western blotting

Isolated tissues were rapidly frozen in liquid nitrogen and stored in −80 °C until extract preparation. Total cellular extracts were prepared in lysis buffer (50 mM Tris-HCl, pH 8.0, 20% glycerol, 140 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM DTT and protease/phosphatase inhibitors) using a tissue homogenizer. After incubation for 30 min at 4 °C, the lysates were centrifuged for 10 min at maximal speed and supernatants collected. Western blot analysis of whole cell lysates was conducted as previously described [29]. Sphk1 (sc-48825; rabbit polyclonal; dilution 1:1000) and Sphk2 (sc-22704; goat polyclonal; 1:1000) antibodies were from Santa Cruz Biotechnology, Inc. and β-actin antibodies (A2228) were obtained from Sigma-Aldrich. CD36 (ab133625) and Atgl (#2439) antibodies were from Abcam and Cell Signaling, respectively. Western blots were visualized using secondary antibodies conjugated to HRP in conjunction with ECL reagents (Thermo Scientific).

Table 1
Sequences of primers used for RT-PCR.

Gene	Forward primer	Reverse primer
Adipoq	GGAGAGAAAGGAGATGCAGGT	CTTTCCTGCCAGGGGTTC
Leptin	AAGACCATTGTCACCAGGATC	GAAGCCCAGGAATGAAGTCC
Acc1	GGCCAGTGCTATGCTGAGAT	CCAGGTCGTTTGACATAATGGATG
CD36	TGAGACTGGGACCATTGGTGAT	CCCAAGTAAGGCCATCTCTACCAT
Atgl	TGACCATCTGCCTTCCAGACT	TGTAGGTGGCGCAAGACAG
Cpt1	GCTGGGCTACTCAGAGGATG	CACTGTAGCCTGGTGGGTTT
Dgat2	AGGCCCTATTTGGCTACG TT	GATGCCTCCAGACATCAGGT
Sphk1	TCCTGGAGGAGGCAGAGATA	GCTACACAGGGGTTTCTGGA
ActB	CGTGGGCCGCCCTAG	TTGGCCTTAGGGTTCAGGGG

2.5. Realtime (RT) PCR analysis

Total RNA was isolated using Trizol (Life Technologies) and 1 μg of total RNA was used for cDNA synthesis with the qScript cDNA SuperMix kit (Quanta Biosciences). Quantitative RT-PCR (qRT-PCR) was performed on Mx3005P real-time PCR instrument (Stratagene) using SYBR Green qPCR Master Mix (Applied Biosystems) as previously described [30]. All qRT-PCR data were normalized to *β-actin* mRNA levels. The genes and primers used for qRT-PCR quantification are listed in Table 1.

2.6. Statistics

Results are expressed as mean ± SEM. For comparison between groups a two-tailed unpaired Student's *t*-test was used. A p value of < 0.05 was considered as statistically significant. Statistical analysis was performed using SPSS. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

3. Results

3.1. Sphk2$^{-/-}$ mice have reduced weight and fat mass and are protected from age-dependent insulin resistance

Aging is associated with weight gain and obesity. We measured the weight and fat mass of male *Sphk2*$^{-/-}$ and WT mice at 20 and 52 weeks of age (Fig. 1A). At 20 weeks of age, the weight of male *Sphk2*$^{-/-}$ mice was about 10% less than of that of WT mice; however at 52 weeks *Sphk2*$^{-/-}$ mice displayed a 35% decrease in body weight compared to WT (Fig. 1A). In fact, the weight of 52-week old male *Sphk2*$^{-/-}$ mice was similar to that of 20-week old mice. This suggests that while WT mice gained weight with age, the *Sphk2*$^{-/-}$ mice maintained a fairly constant body weight. The reduction in body weight in *Sphk2*$^{-/-}$ mice was first observed at 10 weeks of age (Fig. S1A) and persisted in 77-week old male mice (Fig. 1A). Echo-MRI analysis of lean and fat mass indicated a 36% decrease in fat and an 8% increase in lean mass (normalized to % BW) in male *Sphk2*$^{-/-}$ versus WT mice at 20 weeks of age (Fig. 1B & S1B). At 52 weeks of age, the difference in fat and lean mass in *Sphk2*$^{-/-}$ versus WT was more pronounced with 85% decrease in fat and 22% increase in lean mass in male *Sphk2*$^{-/-}$ compared to WT mice (Fig. 1B & S1B).

During a glucose tolerance test (GTT), 52-week old male WT mice

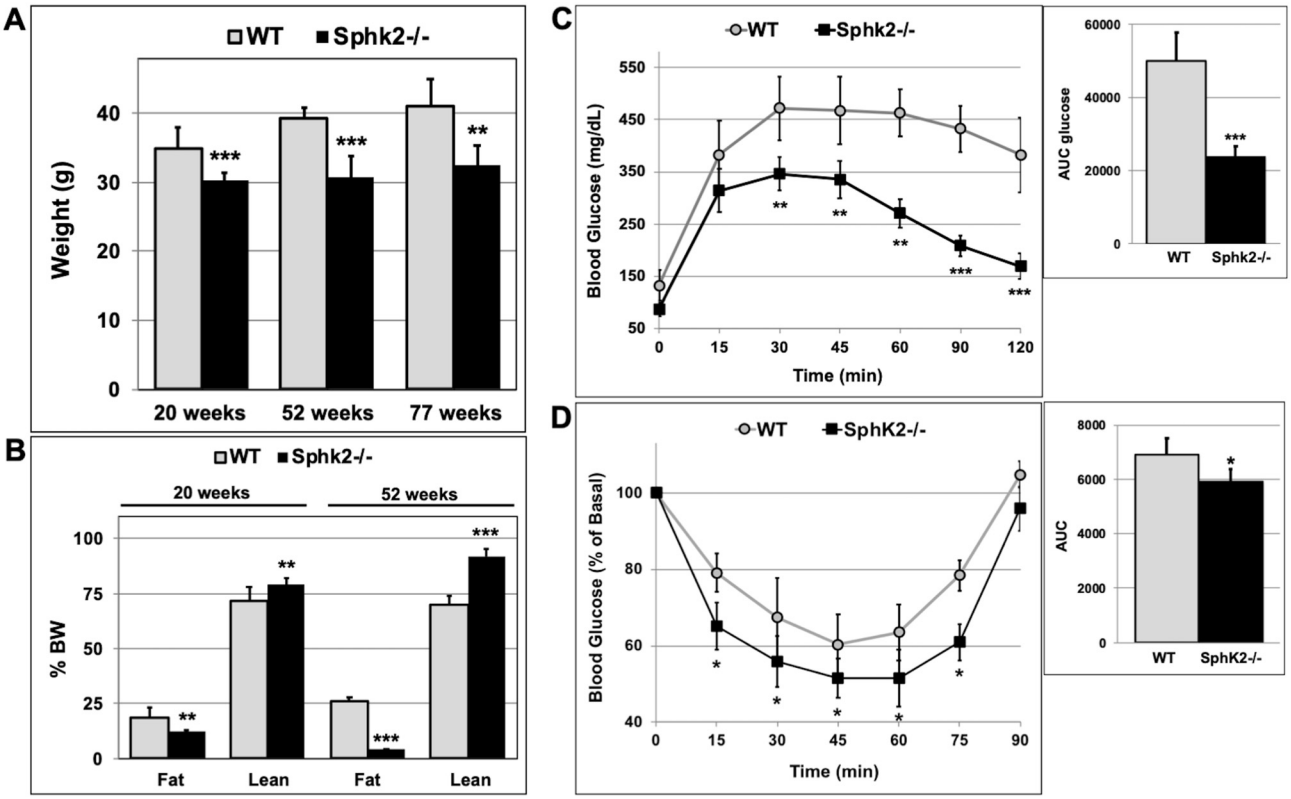

Fig. 1. Male *Sphk2*$^{-/-}$ mice have reduced weight and fat mass and increased glucose tolerance and insulin sensitivity during aging.
(A) Weight of male WT and *Sphk2*$^{-/-}$ mice was measured at 20, 52, and 77 weeks of age (n = 8–10). (B) Lean and fat mass in 20- and 52-week old male WT and *Sphk2*$^{-/-}$ mice was measured using Echo-MRI (n = 5–7). (C) GTT was performed in 52-week old male *Sphk2*$^{-/-}$ and WT mice after a 6-h fast; (n = 5). (D) ITT was carried out in 54-week old male *Sphk2*$^{-/-}$ and WT mice after a 4-h fast; (n = 5). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

were unable to normalize blood glucose levels after glucose administration within a 2-h time frame (Fig. 1C). In contrast, blood glucose levels in male *Sphk2*$^{-/-}$ mice were almost back to normal within 2 h. The area under the curve (AUC) for glucose was significantly lower for *Sphk2*$^{-/-}$ versus WT mice (Fig. 1C). Aged male *Sphk2*$^{-/-}$ mice were also more insulin sensitive compared to WT (Fig. 1D). Consistent with these findings, the HOMA-IR index for 52-week old male *Sphk2*$^{-/-}$ mice (2.83 ± 0.77) was significantly lower compared to WT mice (11.38 ± 1.37), confirming that *Sphk2*$^{-/-}$ mice are protected from insulin resistance during aging. There was no difference in glucose tolerance and insulin sensitivity in 20-week old male WT versus *Sphk2*$^{-/-}$ mice (Fig. S2A–B).

The decrease in weight gain was also observed in female *Sphk2*$^{-/-}$ mice at the age of 22, 50 and 76 weeks, although it was not as pronounced as in male *Sphk2*$^{-/-}$ mice (Fig. 2A). While there was no significant difference in fat and lean mass in 22-week old female *Sphk2*$^{-/-}$ mice, 50-week old female *Sphk2*$^{-/-}$ mice had a 30% decrease in fat and 20% increase in lean mass compared to WT, when normalized to % BW (Fig. 2B). 50-week old female *Sphk2*$^{-/-}$ mice displayed improved glucose tolerance compared to WT (Fig. 2C), however insulin sensitivity in female *Sphk2*$^{-/-}$ versus WT mice was not significantly different (Fig. 2D). These data suggest that ablation of *Sphk2* in mice prevents age-related weight gain and obesity.

3.2. Aged *Sphk2*$^{-/-}$ mice have lower fasting blood glucose levels and increased energy expenditure

While random blood glucose levels between *Sphk2*$^{-/-}$ and WT mice at 20 and 52 weeks of age were similar, fasting blood glucose levels in 52-week old male *Sphk2*$^{-/-}$ mice were significantly lower (35% decrease) than in WT mice (Fig. 3A). Moreover, 54-week old male WT mice showed signs of fatty liver compared to *Sphk2*$^{-/-}$ mice after H&E staining of paraffin-embedded liver sections (Fig. 3B). There was no change in fasting blood glucose levels between male *Sphk2*$^{-/-}$ and WT mice at 20 weeks of age (Fig. 3A). Fasting blood glucose levels in 50-week old female *Sphk2*$^{-/-}$ and WT were similar (Fig. S2C). These data suggest that male *Sphk2*$^{-/-}$ mice are protected from age-dependent glucose intolerance and hepatic insulin resistance.

Metabolic studies using indirect calorimetry with 22- or 54-week old male *Sphk2*$^{-/-}$ and WT mice suggested that food intake (corrected for body weight) was significantly increased during the dark cycle in 22- and 54-week old *Sphk2*$^{-/-}$ mice (Fig. 3C). The respiratory quotient was decreased during the light cycle in both 22- and 54-week old *Sphk2*$^{-/-}$ mice, while it was increased during the dark cycle in 54-week old *Sphk2*$^{-/-}$ mice (Fig. 3D). Although, total activity during the dark cycle was not significantly different between *Sphk2*$^{-/-}$ and WT mice, total activity during the light cycle was decreased in *Sphk2*$^{-/-}$ mice (Fig. 3E). Energy expenditure (corrected for body weight) was increased during the light and dark cycle in both 22- and 54-week old male *Sphk2*$^{-/-}$ versus WT mice (Fig. 3F). Energy expenditure corrected for lean body mass was also increased in *Sphk2*$^{-/-}$ male mice (Fig. S4A).

Female *Sphk2*$^{-/-}$ mice displayed lower food intake during the light cycle, but higher food intake during the dark cycle (Fig. S3A). Respiratory quotient in female *Sphk2*$^{-/-}$ mice was decreased during light cycle similar to male *Sphk2*$^{-/-}$ mice (Fig. S3B). In contrast to male mice, total activity in female *Sphk2*$^{-/-}$ mice was reduced by about 50% during the light cycle compared to WT (Fig. S3C). However, there was no significant difference in energy expenditure in female *Sphk2*$^{-/-}$ versus WT mice when normalized for body weight (Fig. S3D).

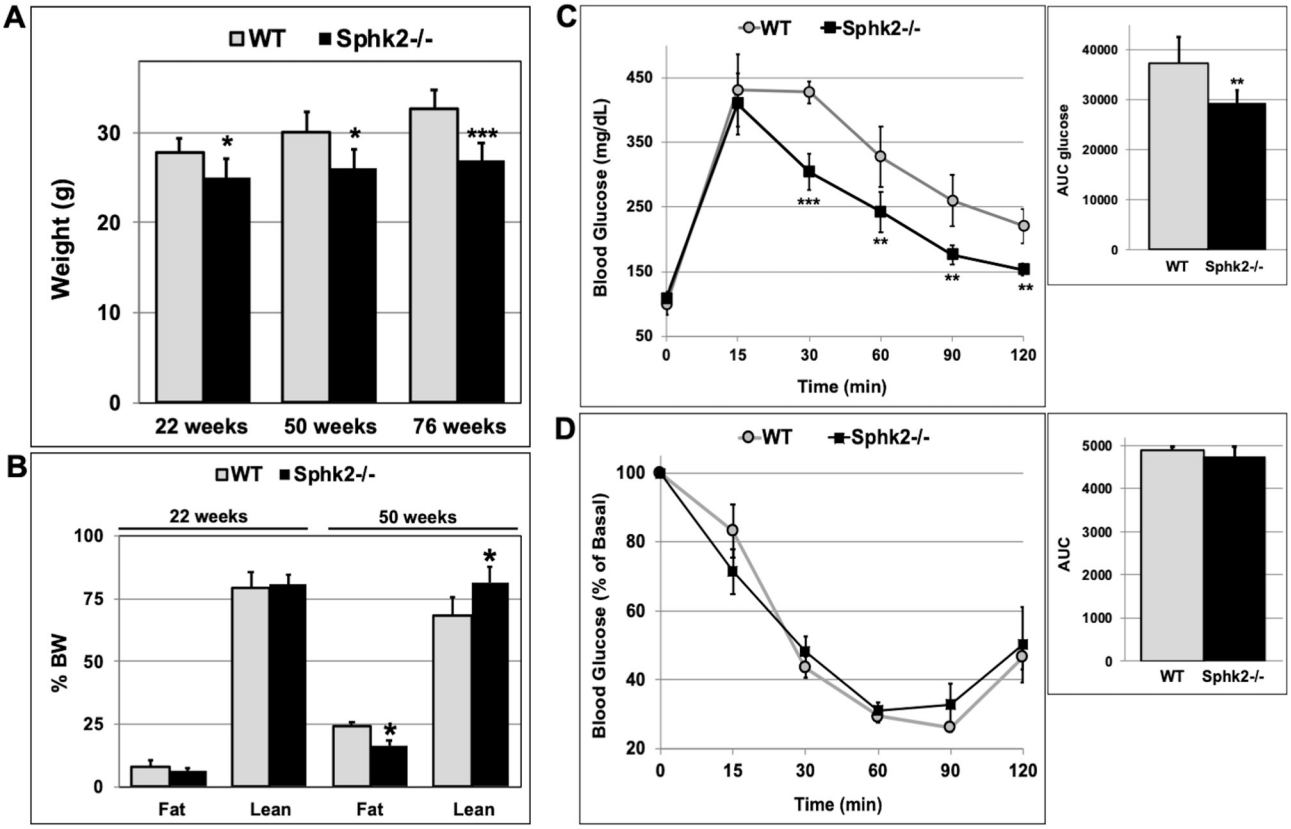

Fig. 2. Female *Sphk2*$^{-/-}$ mice have decreased weight and fat mass, and increased glucose tolerance with aging.
(A) Weight of female *Sphk2*$^{-/-}$ and WT mice was measured at 22, 50, and 76 weeks of age (n = 6–8). (B) Lean and fat mass in 22- and 50-week old female WT and *Sphk2*$^{-/-}$ mice was determined by Echo-MRI (n = 5). (C) GTT and (D) ITT was performed in 50-week and 52-week old female *Sphk2*$^{-/-}$ and WT mice, respectively (n = 5). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3.3. Sphk2$^{-/-}$ *mice have increased expression and circulating levels of adiponectin during aging*

Aging *Sphk2*$^{-/-}$ mice weigh less and have decreased fat mass compared to WT mice (Fig. 1), suggesting that they have altered lipid metabolism. Expression analysis of adipokines as well as lipid metabolic genes in gonadal white adipose tissue (WAT) indicated that the expression of adiponectin (2.6-fold), *CD36* (3.5-fold) and *Atgl* (4.7-fold) genes were significantly upregulated in 54-week old male *Sphk2*$^{-/-}$ versus WT mice (Fig. 4A). However, there were no significant changes in expression of these genes in WAT of 22-week old *Sphk2*$^{-/-}$ versus WT mice (Fig. S4B). Consistent with the gene expression data, the levels of CD36 and Atgl protein were about 2-fold increased in WAT of *Sphk2*$^{-/-}$ mice compared to WT (Fig. 4B–C). *Atgl* encodes for the adipocyte triglyceride lipase, which catalyzes the rate-limiting step in the hydrolysis of triglycerides [31,32]. Upregulation of Atgl suggests increased lipolysis in WAT of *Sphk2*$^{-/-}$ mice. Increased expression of adiponectin in WAT of *Sphk2*$^{-/-}$ mice correlated with increased plasma levels of adiponectin (Fig. 4D). Adiponectin displays glucose- and lipid-lowering effects [24]. Thus, it is possible that *Sphk2*$^{-/-}$ mice are protected from age-dependent obesity and insulin resistance in part due to increased levels of adiponectin. Plasma leptin, triglyceride and insulin levels were significantly lower in aged male *Sphk2*$^{-/-}$ versus WT mice (Fig. 4D).

4. Discussion

Sphingolipids have been implicated in many diseases, including obesity and diabetes. Two important sphingolipids that function as signaling molecules are ceramide and S1P. Increases in ceramide levels are associated with insulin resistance [14,33]. S1P has been shown to counteract ceramide function [11]. We report here that deletion of sphingosine kinase 2, which produces S1P, is beneficial and protects from metabolic decline during aging. Lack of *Sphk2* was associated with decreased weight and fat mass in both male and female mice, however the metabolic effects were more robust in male mice. Although aged female *Sphk2*$^{-/-}$ mice weighed less than WT, displayed decreased fat mass and had significantly improved glucose tolerance, there was no significant difference in fasting blood glucose levels, insulin sensitivity and energy expenditure between aged female *Sphk2*$^{-/-}$ and WT mice.

Aging is associated with weight gain and obesity. Age-related changes in adipose tissue composition and function lead to insulin resistance and metabolic dysfunction [34,35]. Male *Sphk2*$^{-/-}$ weighed about 35% less than WT mice and displayed an 85% reduction in fat mass at 52 weeks of age, suggesting that they are protected from age-related changes in adipose tissue function and composition. *Sphk2*$^{-/-}$ male mice are more glucose tolerant and have lower fasting blood glucose levels during aging compared to WT. One of the hallmarks of insulin resistance is hyperinsulinemia that serves as a compensation mechanism. While aging WT mice were insulin resistant and displayed hyperinsulinemia, *Sphk2*$^{-/-}$ mice had decreased plasma insulin levels and were protected from hyperinsulinemia. Interestingly, plasma insulin levels were reduced by about 40% even in 20-week old *Sphk2*$^{-/-}$ mice (Fig. S2D). This suggests that *Sphk2*$^{-/-}$ mice have chronically low levels of insulin, which is consistent with our previous observation that *Sphk2* is required for insulin secretion [27].

Caloric intake as well as energy expenditure was significantly increased in male *Sphk2*$^{-/-}$ versus WT mice at 22- and 54-weeks of age. Despite the increased caloric intake, male *Sphk2*$^{-/-}$ mice have decreased body weight and fat mass, which is likely to be attributed to

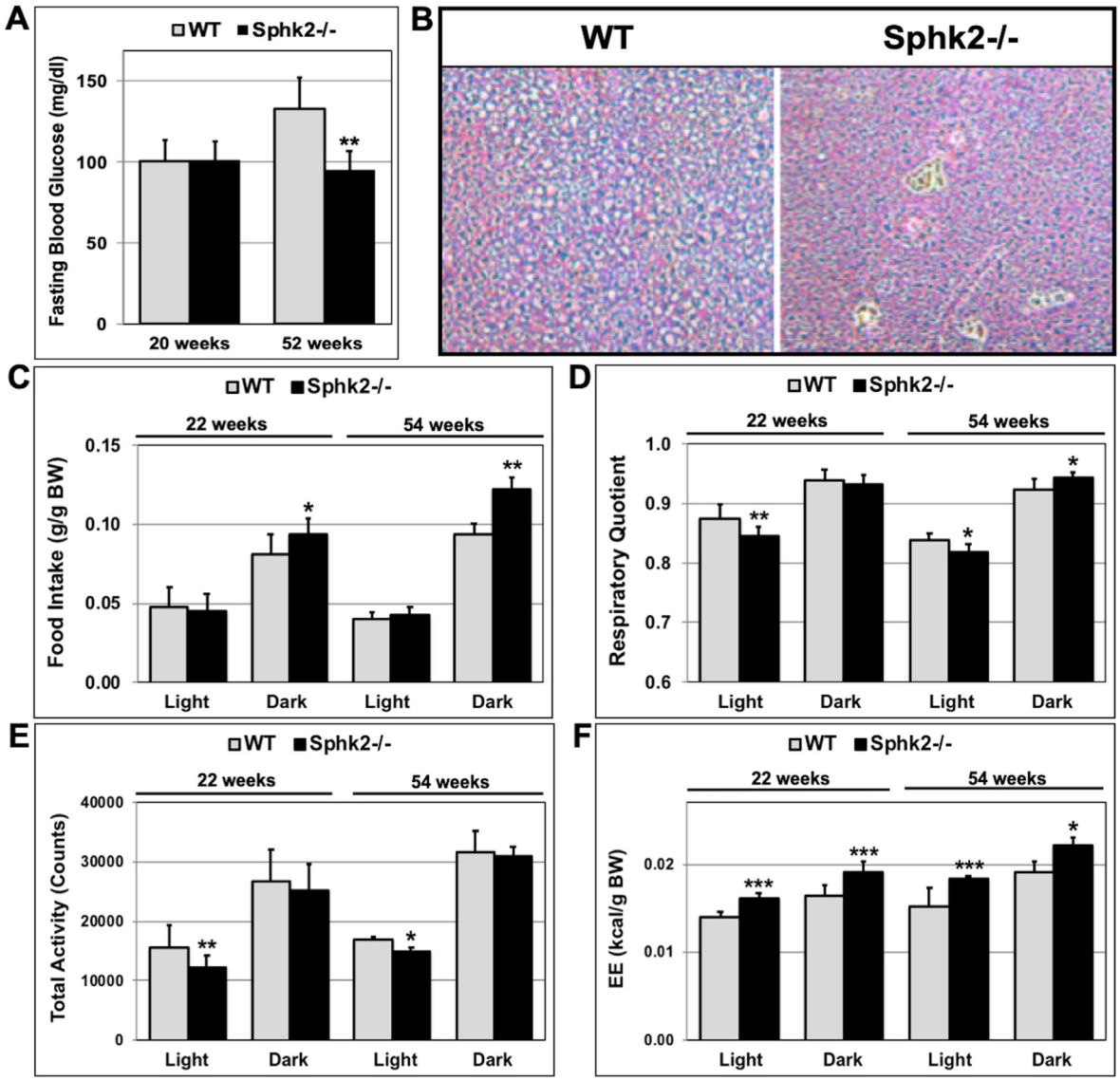

Fig. 3. Male *Sphk2*$^{-/-}$ mice have lower fasting blood glucose levels and increased energy expenditure. (A) Fasting blood glucose levels in 20- and 52-week old male *Sphk2*$^{-/-}$ and WT mice were measured after a 16 h-fast (n = 5). (B) Paraffin-embedded liver sections of 54-week old male *Sphk2*$^{-/-}$ and WT mice were stained with H & E. Food intake (C), respiratory quotient (D), total activity (E) and energy expenditure (F) were determined using indirect calorimetry with 22- and 54-week old male mice (n = 8–10). Food Intake and energy expenditure were normalized for body weight. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

increased energy expenditure. Interestingly, energy expenditure in 50-week old female *Sphk2*$^{-/-}$ mice was not different from that of WT mice, suggesting the idea that the weight loss observed in aging female *Sphk2*$^{-/-}$ mice is not due to increased energy expenditure. This indicates that *Sphk2* has different metabolic effects in male versus female mice.

Expression as well as circulating levels of adiponectin are increased *Sphk2*$^{-/-}$ mice compared to WT. Thus, it is possible that the improved metabolic effects seen in *Sphk2*$^{-/-}$ mice could be in part due to increased adiponectin levels. Adiponectin is produced from the white adipose tissue and its expression is decreased during obesity. Adiponectin has lipid- and glucose-lowering effects and improves glucose metabolism [24]. Interestingly, there is a strong connection between adiponectin and ceramide levels. Adiponectin receptors AdipoR1 and AdipoR2 have been previously shown to function as ceramidases [25]. Thus, binding of adiponectin to its receptors decreases ceramide levels, which may be responsible for the lipid- and glucose-lowering effects of adiponectin. However, it is possible that the increased adiponectin levels in *Sphk2*$^{-/-}$ mice may be due to decreased obesity in these mice. As reported previously, we confirmed that deletion of *Sphk2* leads to upregulation of plasma S1P by two-fold. Since S1P functions as a ligand for five different S1P receptors, increases in plasma S1P levels may lead to increased S1P signaling and thereby result in positive metabolic outcomes in *Sphk2*$^{-/-}$ mice.

Analysis of metabolic gene expression in WAT suggests that *Sphk2*$^{-/-}$ mice have increased expression of *Atgl* encoding for the adipose triglyceride lipase, which catalyzes the rate-limiting step of lipolysis [36]. This indicates that *Sphk2*$^{-/-}$ mice may be protected from obesity during aging in part due to increased lipolysis. The levels of the lipid transporter *CD36* were also significantly increased in WAT of *Sphk2*$^{-/-}$ mice [31,37], suggesting that lipid uptake into adipocytes of *Sphk2* null mice is increased. Since *Sphk2* is mainly localized to the nucleus, it is likely that is regulates the expression of lipid metabolic genes directly. Previous data suggest that Sphk2 and S1P interact with histone deacetylases HDAC1 and HDAC2 in the nucleus to inhibit their activity [38]. Consistent with the gene expression data, the levels of Atgl and CD36 protein were also increased about 2-fold in WAT of *Sphk2*$^{-/-}$ mice compared to WT. Since enzymes involved in the sphingolipid

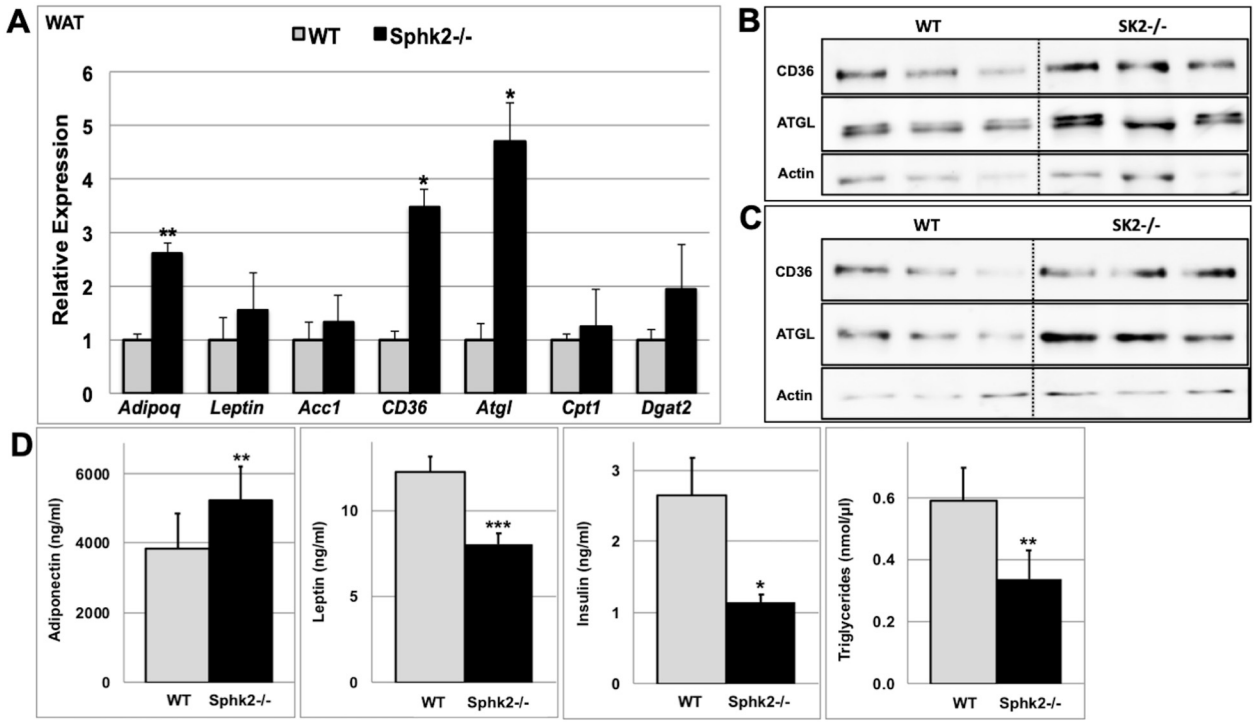

Fig. 4. Aged male *Sphk2*$^{-/-}$ mice display increased expression and plasma levels of adiponectin. The expression of various adipokines ((*Adipoq* (adiponectin) and leptin)), lipid metabolic genes (*Acc1* (acetyl CoA carboxylase), *CD36*, Atgl (adipocyte triglyceride lipase), *Cpt1* (carnitine palmitoyl transferase I), and *Dgat2* (diglyceride acyltransferase)) was quantified in WAT (A) of 54-week old *Sphk2*$^{-/-}$ and WT male mice by qRT-PCR and normalized to beta-actin levels (n = 4). CD36 and Atgl protein levels were measured in WAT of 54-week old *Sphk2*$^{-/-}$ and WT male mice under fed (B) or after 16 h fasting (C) conditions by immunoblotting with specific antibodies (n = 3). (C) Plasma adiponectin, leptin, insulin, and triglyceride levels were measured in 54-week old male *Sphk2*$^{-/-}$ and WT male mice using ELISA (n = 5). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

biosynthesis pathway have been shown to function as lipid sensors [39,40], it is possible that Sphk2 has a similar function. Sphk2 could sense the rate of sphingolipid biosynthesis by binding to sphingosine. During increased flux, Sphk2 could mediate the suppression of processes that increase intracellular fatty acid levels, such as lipolysis (Atgl) and lipid uptake (CD36) during obesity. Loss of *Sphk2* would lead to increased lipolysis and lipid uptake. Consistent with this idea, recent findings suggest that inhibition of fatty acid synthase (Fasn) and thereby downregulation of fatty acid synthesis decreases the flux through the sphingolipid biosynthesis pathway in colorectal cancer cells [41].

In agreement with our findings, recently published data suggest that Sphk2 and/or S1P are associated with negative health outcomes. Sphk2 has been shown to promote kidney fibrosis via phosphorylation of Fyn to activate STAT3 and AKT, which could not be rescued by addition of extracellular S1P [42]. Increased S1P levels have been recently associated with type 2 diabetes and obesity [21,22]. Palmitate-induced increases in S1P levels in pancreatic beta cells have been demonstrated to antagonize insulin-stimulated cell growth and survival via activation of the S1P receptor subtype 2 ($S1P_2$). Administration of JTE-013, an $S1P_2$ antagonist rescued the beta-cell damage attributed to the enhanced S1P- $S1P_2$ axis [22]. Similar data were also obtained in hepatocytes. An increase in hepatic S1P levels induced by palmitate caused insulin resistance via the S1P-$S1P_2$ axis, which could be reversed using the $S1P_2$ antagonist JTE-013 [23].

Sphk1 and Sphk2 both produce S1P, but they differ in their regulation, tissue-distribution, and localization. There were no changes in *Sphk1* expression and protein levels in livers of *Sphk2*$^{-/-}$ mice (Fig. S4C–D), suggesting that there is no compensation by overexpression of *Sphk1* in *Sphk2*$^{-/-}$ mice. This is in agreement with the original publication on *Sphk2*$^{-/-}$ mice, where it was reported that *Sphk1* expression was not altered in various tissue of *Sphk2*$^{-/-}$ mice [7]. However, several reports indicate that inhibition of Sphk2 results in upregulation of *Sphk1* expression. It is possible that Sphk2 inhibitors increase *Sphk1* levels by partially inhibiting Sphk1 activity. Although, there are no studies on Sphk1 function during aging, previously published data suggest that *Sphk1* null mice are protected from HFD-induced insulin resistance [43]. Interestingly, *Sphk1* null mice on a HFD gain as much weight as WT mice, but display improved glucose tolerance and insulin sensitivity [43]. Since *Sphk2*$^{-/-}$ mice do not gain weight during aging, this suggests that Sphk1 and Sphk2 have overlapping as well as unique functions with respect to obesity and insulin resistance.

In conclusion, mice lacking *Sphk2* are protected from obesity and insulin resistance during aging. This indicates that increased Sphk2 levels or activity, and/or increased S1P levels during aging may contribute to obesity and metabolic dysfunction. Therefore, Sphk2 may be a potential drug target for treatment of age-related obesity and insulin resistance.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbadis.2018.12.012.

Conflict of interest

The authors declare no conflict of interest.

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Contribution statement

SR contributed to the acquisition and analysis of data and the revision of the article. SÖ contributed to the conception and design of the study, the acquisition, analysis and interpretation of the data and drafting of the article. BSF and PAK contributed to the analysis and interpretation of the data and the revision of the article.

Transparency document

The Transparency document associated with this article can be found, in online version.

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