

The Hepatokine TSK does not affect brown fat thermogenic capacity, body weight gain, and glucose homeostasis



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ABSTRACT

Objectives: Hepatokines are proteins secreted by the liver that impact the functions of the liver and various tissues through autocrine, paracrine, and endocrine signaling. Recently, Tsukushi (TSK) was identified as a new hepatokine that is induced by obesity and cold exposure. It was proposed that TSK controls sympathetic innervation and thermogenesis in brown adipose tissue (BAT) and that loss of TSK protects against diet-induced obesity and improves glucose homeostasis. Here we report the impact of deleting and/or overexpressing TSK on BAT thermogenic capacity, body weight regulation, and glucose homeostasis.

Methods: We measured the expression of thermogenic genes and markers of BAT innervation and activation in TSK-null and TSK-overexpressing mice. Body weight, body temperature, and parameters of glucose homeostasis were also assessed in the context of TSK loss and overexpression.

Results: The loss of TSK did not affect the thermogenic activation of BAT. We found that TSK-null mice were not protected against the development of obesity and did not show improvement in glucose tolerance. The overexpression of TSK also failed to modulate thermogenesis, body weight gain, and glucose homeostasis in mice.

Conclusions: TSK is not a significant regulator of BAT thermogenesis and is unlikely to represent an effective target to prevent obesity and improve glucose homeostasis.

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Keywords Tsukushi; Hepatokine; Brown adipose tissue; Thermogenesis; Glucose homeostasis; Obesity

1. INTRODUCTION

The liver plays a central role in regulating systemic metabolism. Alterations in liver functions linked to chronic overfeeding and obesity contribute to the development of several health issues including insulin resistance, type 2 diabetes, dyslipidemia, hypertension, and cardiovascular diseases [1,2]. Recent evidence indicates that the liver has an impact on metabolism through the secretion of specific proteins. These proteins, often referred to as *hepatokines*, are secreted by the liver and have an impact on the functions of not only the liver per se but also several extrahepatic tissues through autocrine, paracrine, and

endocrine mechanisms [3–5]. It is now well documented that the secretion of several hepatokines is altered in response to obesity and that some of these circulating proteins can directly contribute to the development of obesity-related metabolic disorders [3–5].

Tsukushi (TSK) is an atypical member of the small leucine-rich proteoglycan family that controls developmental processes in various organisms [6]. Recently, two groups, including ours, reported the identification of TSK as a new hepatokine [7–9]. These studies revealed that TSK is a liver-derived factor whose expression and circulating levels are strongly induced in response to obesity and cold exposure. Triglyceride deposition in the liver, a common characteristic

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associated with obesity and acute cold challenge, is strongly associated with *Tsk* expression and TSK release by the liver [7,9]. It was also shown that both inflammation and endoplasmic reticulum (ER) stress, two conditions closely linked to excessive lipid deposition in the liver, promote *Tsk* expression [7]. Collectively, these findings indicate that TSK is a new hepatokine whose circulating levels are linked to hepatic fat accumulation, inflammation, and ER stress.

The function of TSK in metabolism is becoming more clear. A recent study proposed a role for TSK in regulating the activation of brown adipose tissue (BAT), a key organ controlling thermogenesis, energy balance, and glucose metabolism [8]. Using TSK-null mice, Wang et al. reported that TSK loss increased sympathetic innervation and thermogenesis in BAT, protected mice against diet-induced obesity, and improved glucose homeostasis [8]. On the basis of these findings, they proposed that TSK could be part of a negative feedback mechanism emerging from the liver to repress thermogenesis in BAT and reduce energy expenditure. TSK was thus suggested as a potential target for therapeutic intervention in metabolic diseases. Here, we provide data showing that the loss of TSK does not affect BAT thermogenic capacity. We found that TSK-null mice are not protected against the development of obesity and do not show improvement in glucose metabolism. The overexpression of TSK also failed to modulate thermogenesis, body weight, and glucose tolerance. Thus, we conclude that TSK in not a significant regulator of BAT thermogenesis and that TSK is unlikely to represent an effective target for preventing obesity and improving glucose homeostasis.

2. MATERIALS AND METHODS

2.1. Animal care

All experimental protocols were approved by the Animal Ethics Committee of Université Laval (CPAUL) and were in accordance with the guidelines of the Canadian Council on Animal Care. All mice were on a C57BL/6J background and were purchased from the Jackson Laboratory (stock no. 000664). Obese mice (*ob/ob*; stock no. 000632) were also purchased from the Jackson Laboratory. The mice were maintained on a 12:12-h light-dark cycle (lights on 0600–1800) while individually housed in ventilated cages at an ambient temperature of 23 ± 1 °C. Unless stated, all mice were fed ad libitum chow. Low-fat diet (LFD; 10% kcal from fat, D12450B) and high-fat diet (HFD; 60% kcal from fat, D12492) were purchased from Research Diets.

2.2. Generation of *Tsk* knockout mice

Tsk knockout mice were obtained from Genentech/Lexicon and generated by homologous recombination, as described previously [10]. The insertion of the targeting vector was validated by polymerase chain reaction (PCR) and southern blotting. The *Tsk* wild-type allele was amplified using the following primers: forward 5'-GACATCAATCT-GAGCCATAAC-3', reverse 5'-ATGAAGGCATCTGGGTTGATG-3'. The integration of the Neo Cassette in the mutant *Tsk* allele was measured using the following primers: forward 5'-GTAGACTCTCCA-CAGGCATTGG-3', reverse 5'-GCAGCGCATCGCCTTCTATC-3'. The mice were back-crossed for 6 generations onto C57BL6/J before being used experimentally. All experiments were performed using age-matched littermates produced by breeding *Tsk* heterozygote mice.

2.3. Cold exposure

Age-matched littermates (male) were individually housed in clean cages and exposed to cold (10 ± 1 °C) for a period of 24 h. The mice had free access to chow and water throughout the experiment.

2.4. Overexpression of TSK in mice

Adeno-associated virus (AAV) vectors were packaged by the Canadian Neurophotonics Platform (Centre de recherche CERVO, QC, Canada). Briefly, viral particles were generated from a triple transfection of HEK 293T17 cells and collected from the culture media 5 days post-transfection. They were concentrated using a tangential flow filtration setup (Vivaflow 50R 100K MWCO, Sartorius) and then purified by iodixanol gradient and ultracentrifugation. The purified particles were collected in suspension buffer (phosphate-buffered saline [PBS] 320 mM NaCl, 5% D-sorbitol, and 0.001% Pluronic F-68) and titrated by quantitative PCR (qPCR; TaqMan) using an inverted terminal repeat-based probe and primers. Physical titer and purity were confirmed by separating the same volumes of AAV on 10% sodium dodecyl sulfate (SDS)—polyacrylamide gel electrophoresis (stain free; BioRad) in Tris-glycine-SDS buffer. Male C57BL6/J mice (10 weeks) were injected with 100 μ L of AAV8-GFP or AAV8-TSK through the tail vein (1×10^{11} plaque-forming units/mouse). The animals were sacrificed at the indicated time following the injection.

2.5. Quantitative real-time PCR (RT-qPCR)

Total mRNA was isolated from tissues using the RNeasy Lipid Tissue Mini Kit (Qiagen, 74104). The RNA concentrations were estimated by measuring the absorbance at 260 nm. cDNA synthesis was performed using the iScript™ Advanced cDNA Synthesis Kit for RT-qPCR (Bio-Rad) as described. mRNA extraction and cDNA synthesis were performed according to the manufacturer's instructions. cDNA was diluted in DNase-free water (1:15) before quantification by real-time PCR. mRNA transcript levels were measured in duplicate samples using CFX96 or CFX384 touch™ real-time PCR (Bio-Rad, Mississauga, ON, Canada). Chemical detection of the PCR products was achieved with SYBR Green (Bio-Rad, 172–5271). At the end of each run, melt curve analyses were performed, and representative samples of each experimental group were run on agarose gel to ensure the specificity of amplification. Gene expression was corrected for the expression level of the reference gene. The following primers were used.

Gene	Sense	Antisense
<i>Actb</i>	CTCTAGACTTCGAGCAGGAG	AGAGTACTTGCCTCAGGAG
<i>Arbp</i>	AGAAACTGCTGCCTCACATC	CATCACTCAGAATTTCAATGG
<i>Gapdh</i>	GGCAAATTCACGGCACAGT	CTCGTGGTTACACCCATCA
<i>Cidea</i>	AAAGGGACAGAAATGGACAC	CCTCAGCAGATTCTTAACAC
<i>Dio2</i>	CAGTGTGGTGCACGTCTCCAATC	TGAACCAAAGTTGACCACCAG
<i>Pgc1a</i>	AAGATCAAGGTCCCAGGCAGTAG	TGTCCCGTTGTGTGTCAGGTC
<i>Tsk</i>	TGCAGGGCATCCTCCATCTA	GCCTGAAAACACCTCAGCTC
<i>Ucp1</i>	GCAGTGTTCATTGGGCAGCC	GGACATCGCACAGCTTGGTAC

2.6. Glucose tolerance test (GTT) and insulin tolerance test (ITT)

For the glucose tolerance test (GTT), the mice were fasted for 6 h and injected intraperitoneally (ip) with 1 g/kg of D-glucose. For the insulin tolerance test (ITT), the mice were fasted for 6 h and injected ip with 0.75 U/kg of recombinant insulin (Humulin, Lilly, Canada). Blood samples were collected from the tail vein, and glucose was measured using a glucometer (Roche, Accu-Chek Performa).

2.7. Body composition analysis

Body composition was measured by dual-energy X-ray absorptiometry (DEXA) using the PIXIMUS mouse densitometry apparatus (Lunar Corporation, Madison, WI, USA) under isoflurane.

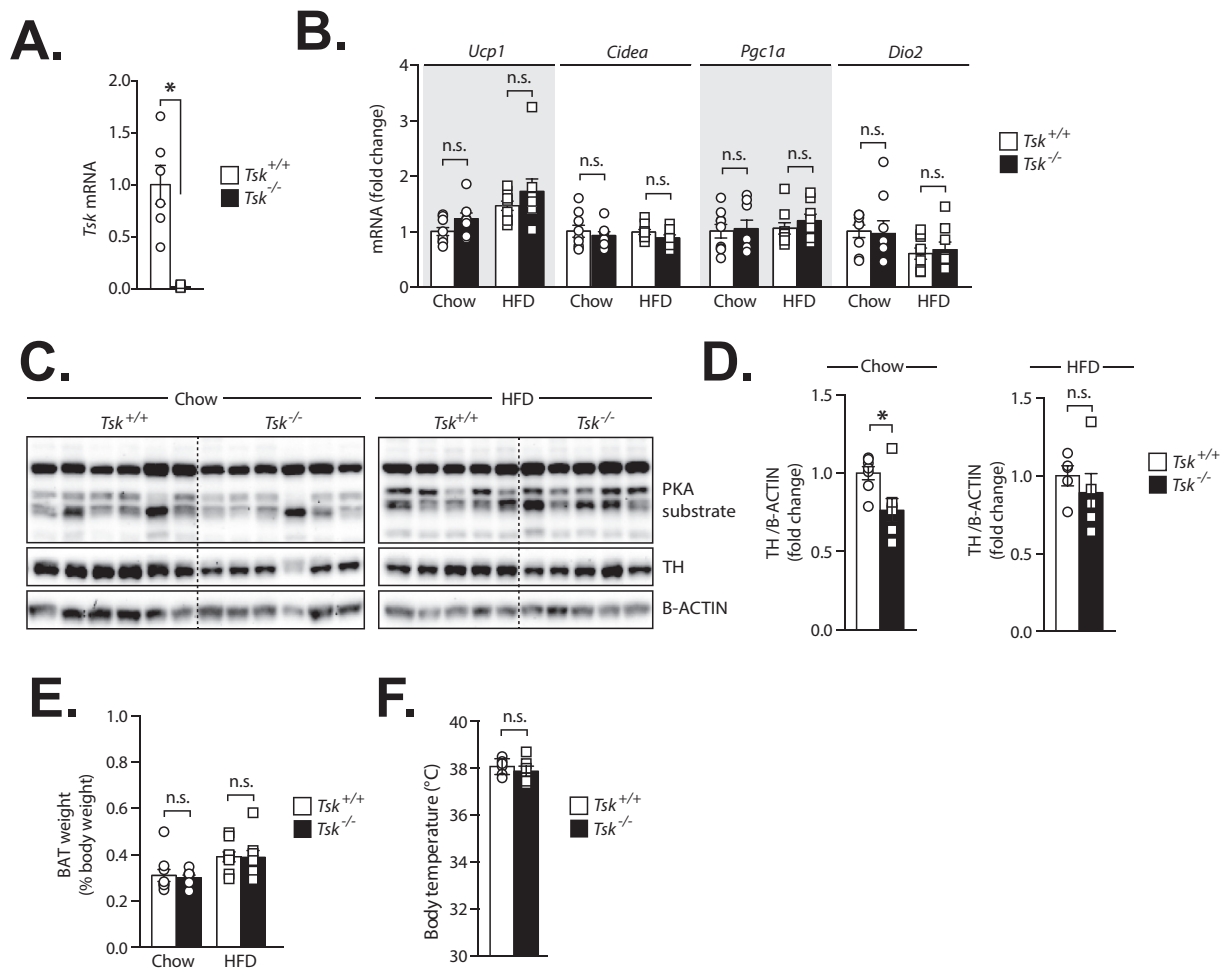


Figure 1: Loss of TSK does not affect BAT thermogenic capacity. (A) qPCR analysis of *Tsk* gene expression in the liver of wild-type (*Tsk*^{+/+}) and knockout (*Tsk*^{-/-}) mice (n = 6–7/group). (B) qPCR analysis of thermogenic gene expression in the BAT of *Tsk*^{+/+} and *Tsk*^{-/-} mice fed chow or HFD for 12 weeks (n = 8–9/group). (C) Western blot analyses performed on BAT protein lysates prepared from *Tsk*^{+/+} and *Tsk*^{-/-} mice fed chow or HFD for 12 weeks. Representative samples are shown. (D) Quantification of TH protein levels of samples described in panel C (n = 5–7/group). The results are normalized to β -actin. (E) BAT weight of *Tsk*^{+/+} and *Tsk*^{-/-} mice fed either chow or HFD diet for 12 weeks (n = 8–9/group). Results are presented as percentage of body weight. (F) Body temperature of *Tsk*^{+/+} and *Tsk*^{-/-} mice fed chow diet (n = 6/group). Data are presented as the mean \pm SEM. In panels A, D, and F, significance was determined by two-tailed, unpaired *t* test. **P* < 0.05 versus control. In panels B and E, two-way analysis of variance with Sidak’s multiple comparisons was performed. **P* < 0.05 versus wild-type. Nonsignificant differences (n.s.) are indicated.

2.8. Body temperature measurement

Rectal temperature was measured in mice using a digital thermometer with a precision of 0.1 °C (Mansfield, Montreal, QC, Canada).

2.9. Western blotting

Tissues were lysed with Triton-X 100-containing lysis buffer (50 mM HEPES, pH 7.4, 2 mM EDTA, 10 mM sodium pyrophosphate, 10 mM sodium glycerophosphate, 40 mM NaCl, 50 mM NaF, 2 mM sodium orthovanadate, 1% Triton-X 100, 0.1% sodium lauryl sulfate, and 1% sodium deoxycholate and one tablet of EDTA-free protease inhibitors per 25 ml). Tissues were rotated at 4 °C for 10 min, and then the soluble fractions of cell lysates were isolated by centrifugation for 10 min in a microcentrifuge. Protein levels were then quantified using Bradford reagent and analyzed by western blotting. Samples were loaded on 10% Tris-glycine precast gels (Life Technologies). Proteins were transferred to polyvinylidene difluoride membranes and then blocked in 5% milk diluted in PBS-Tween and incubated with their corresponding primary antibodies overnight at 4 °C. The following primary antibodies were used: Akt (pan) (4691S, Cell Signaling

Technology, dilution 1:1000), β -actin (4967, Cell Signaling Technology, dilution 1:1000), phospho-PKA substrate (RRXS*(T*)100G7E) (9624S), and tyrosine hydroxylase antibody (NB300-109, Novus, dilution 1:1000). Secondary antibodies were purchased from Cell Signaling Technology (7076S and 7074S) and diluted 1:5000. Amersham ECL Western Blotting Detection Reagent (RPN2106) was used to image the blots.

3. RESULTS

3.1. Loss of TSK does not affect BAT thermogenic capacity

TSK deficiency was recently shown to affect BAT function in mice [8]. An increase in thermogenic gene expression was reported in TSK-null animals, an effect that was linked to elevated sympathetic innervation and to a reduction in lipid deposition in BAT. To test the impact of TSK loss on BAT metabolism, we used the full-body TSK-null mouse model that was previously developed [10]. Confirming the validity of the model, *Tsk* expression was undetectable in the liver of TSK knockout mice (Figure 1A). We next measured the expression of classical

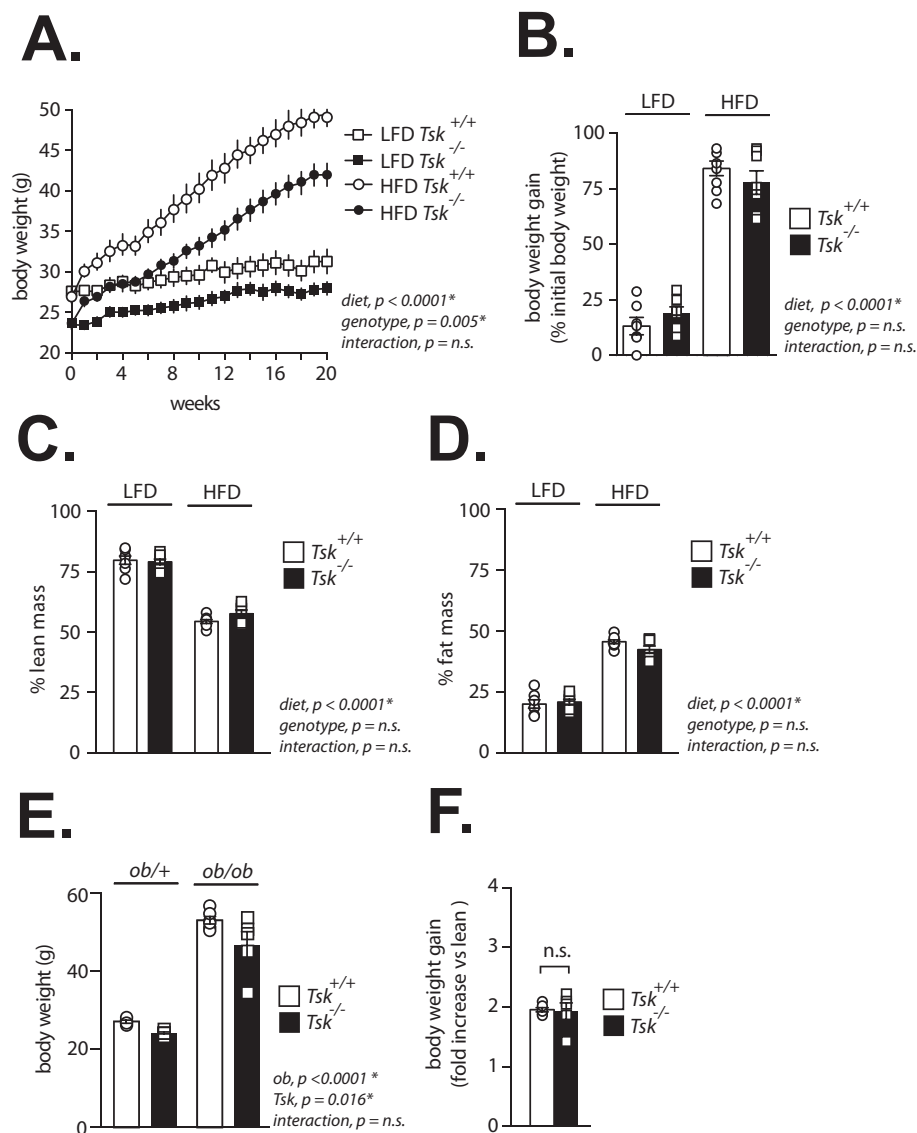


Figure 2: Loss of TSK does not protect mice against obesity development. (A) Body weight and (B) percentage of body weight gain of $Tsk^{+/+}$ and $Tsk^{-/-}$ mice fed either a LFD or a HFD diet for 19 weeks ($n = 6-8$ /group). (C) Percentage of lean and (D) fat mass measured by DEXA in mice described in A and B ($n = 6-8$ /group). (E) Body weight and (F) percentage of body weight gain measured in $ob/+$ $Tsk^{+/+}$, $ob/+$ $Tsk^{-/-}$, ob/ob $Tsk^{+/+}$, and ob/ob $Tsk^{-/-}$ mice ($n = 6-8$ /group). Data are presented as the mean \pm SEM. In panels A to D, two-way analysis of variance (ANOVA) with Sidak's multiple comparisons was performed. In panel A, body weight at week 20 was used in the analysis. Results from the ANOVA are presented on the side of each panel. In panel F, significance was determined by two-tailed, unpaired t test. * $P < 0.05$ versus wild-type mice. Nonsignificant differences (n.s.) are indicated.

thermogenic markers in BAT, including *Uncoupling protein 1 (Ucp1)*, *Cell death activator CIDE-A (Cidea)*, *Peroxisome proliferator-activated receptor gamma coactivator 1a (Pgc1a)*, and *Type II iodothyronine deiodinase (Dio2)*. As shown in Figure 1B, none of these genes were differentially expressed in the BAT of TSK-null mice as compared to the expression in littermate controls. This was observed in mice fed either chow or HFD. We next measured the phosphorylation of protein kinase A (PKA) substrates and tyrosine hydroxylase (TH) in BAT as markers of BAT activation and innervation. In contrast to the findings of Wang et al., we found no increase in the phosphorylation of PKA substrates and TH levels in TSK knockout mice (Figure 1C). In fact, we even observed a reduction in TH protein in the BAT of chow-fed mice (Figure 1C and 1D). No difference in BAT weight and body temperature between wild-type and knockout mice further confirmed the absence

of thermogenic induction in the BAT of TSK-null mice (Figure 1E and 1F). To test whether TSK might specifically have an impact on BAT activation in response to cold, wild-type and TSK-null mice were housed at 10 °C for 24 h. As shown in Figure S1, TSK loss did not increase the expression of the thermogenic gene, the phosphorylation of PKA substrates, or TH levels in BAT. The weight of BAT did not differ between wild-type and TSK null mice. Altogether, these results do not support a role for TSK in regulating BAT thermogenesis in mice.

3.2. Loss of TSK does not protect mice against the development of obesity

The elevation in BAT activity caused by TSK deletion was proposed to be sufficient to promote energy expenditure and prevent diet-induced obesity in mice [8]. To test the impact of TSK on body weight

regulation, wild-type and TSK knockout littermates were fed either a LFD or a HFD. At the beginning of the study, TSK-null animals were already smaller than wild-type littermates, a phenotype consistent with the established role of TSK in regulating development in mice (Figure 2A) [6]. In contrast to the results presented by Wang et al., TSK-null animals were not protected against diet-induced obesity. As shown in Figure 2B, TSK wild-type and TSK knockout mice gained the same amount of weight in response to LFD or HFD. Supporting these findings, DEXA analyses revealed no difference in lean and fat mass between the wild-type and TSK-null mice (Figure 2C and 2D). To test whether TSK loss could have an impact on body weight gain in severely obese mice, TSK wild-type and TSK knockout mice were established into the *ob/+* and *ob/ob* background, respectively. Although TSK-null mice were smaller than littermate controls (Figure 2E), the body weight gain measured in the *ob/ob* background was not affected by the loss of TSK (Figure 2F). These results further confirm that loss of TSK does not protect mice against the development of obesity.

3.3. Loss of TSK does not improve glucose homeostasis

The loss of TSK was previously reported to improve glucose tolerance in mice [8]. To define the impact of TSK loss on glucose metabolism, we next performed the GTT in TSK wild-type and knockout mice fed either a LFD or a HFD. We observed a mild deterioration in glucose tolerance in TSK knockout mice fed aLFD, but this effect was not statistically significant when the area under the curve was calculated (Figure 3A and B). More importantly, we did not find an impact of TSK loss on glucose intolerance induced by HFD feeding (Figure 3A and B). To test whether TSK loss could have an impact on glucose homeostasis in genetically obese mice, we performed GTT in TSK wild-type and TSK knockout mice bred into the *ob/ob* background. As presented in Figure 3C and D, we found no effect of TSK loss on glucose tolerance in this more severe model either. Altogether, these results

indicate that TSK does not improve glucose homeostasis in two well-established mouse models of obesity.

3.4. Overexpression of TSK does not affect BAT thermogenic capacity, body weight, and glucose homeostasis

To further examine the functions of TSK in regulating metabolism, complementary experiments were performed in TSK-overexpressing mice. Briefly, mice were infected with AAV serotype 8 (AAV8) coding for either a control green fluorescent protein (GFP) or a full-length TSK. As expected, we detected high levels of TSK in the plasma of mice injected with AAV8-TSK (Figure 4A). As observed in TSK-null mice, TSK overexpression did not affect the expression of thermogenic genes in BAT (Figure 4B). We did not observe any change in BAT weight or obvious differences in BAT morphology between AAV8-GFP and AAV8-TSK mice (Figure 4C and D). Moreover, we measured no difference in body temperature following TSK overexpression (Figure 4E). Consistent with these findings, we found no effect of TSK overexpression on body weight gain (Figure 4F). We next sought to define the impact of TSK overexpression on glucose homeostasis. As depicted in Figure 4G and H, TSK overexpression did not have any impact on glucose tolerance and insulin sensitivity. Overall, these results indicate that TSK overexpression does not affect BAT activation, body weight, and glucose homeostasis in mice.

4. DISCUSSION

Hepatokines are proteins released by the liver that have an impact on various organs to control systemic metabolism. The secretion of several hepatokines is altered in response to obesity, and growing evidence indicates that these changes can contribute to the development of glucose intolerance, insulin resistance, and cardiovascular diseases [3–5]. These observations suggest that hepatokines could represent new targets for the treatment of obesity-related metabolic diseases.

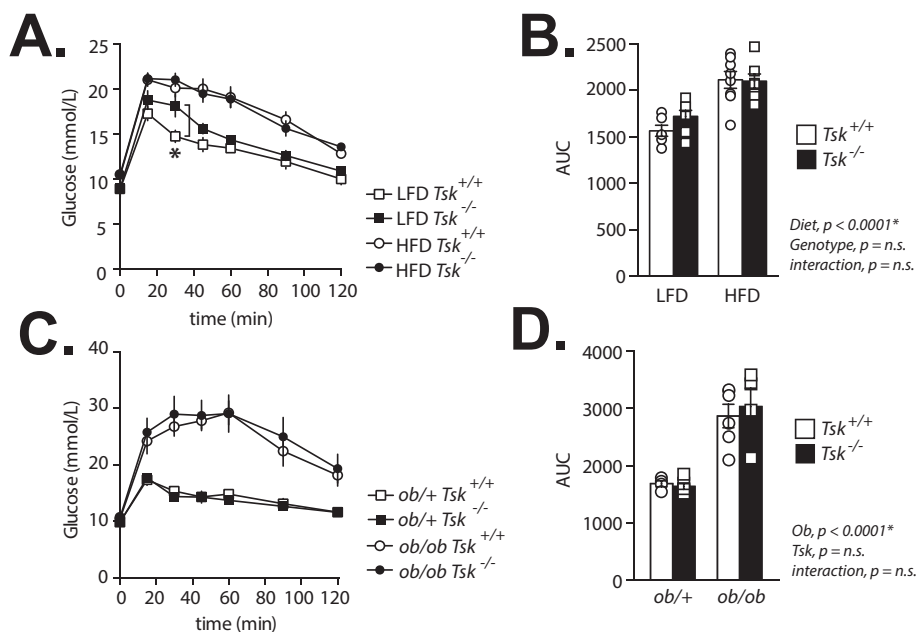


Figure 3: Loss of TSK does not improve glucose homeostasis. (A) Glucose tolerance test (GTT) performed in *Tsk*^{+/+} and *Tsk*^{-/-} mice fed either LFD or HFD (6–8/group). (B) Presentation of the area under the curve (AUC) calculated from the experiment described in panel A. (C) Glucose tolerance test (GTT) performed in *ob/+ Tsk*^{+/+}, *ob/+ Tsk*^{-/-}, *ob/ob Tsk*^{+/+}, and *ob/ob Tsk*^{-/-} mice (n = 6–8/group). (D) Presentation of the AUC calculated from the experiment described in panel C. Data are presented as the mean ± SEM. In panels A to D, two-way analysis of variance (ANOVA) with Sidak's multiple comparisons was performed. *P < 0.05 versus wild-type. In panels B and D, results from the ANOVA are presented on the side of the graph. Nonsignificant differences (n.s.) are indicated.

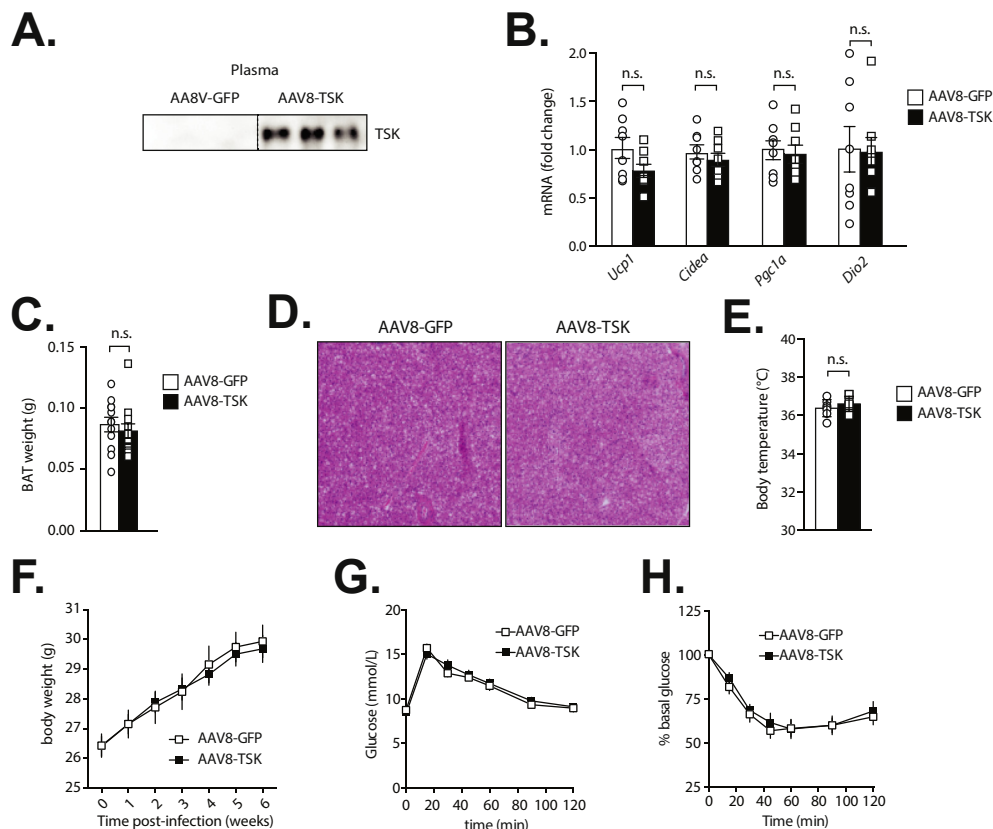


Figure 4: Overexpression of TSK does not affect BAT thermogenic capacity, body weight, and glucose homeostasis. (A) Western blot analysis of plasma collected from C57BL/6J mice injected with AAV8-GFP or AAV8-TSK. Plasma was collected 4 weeks following AAV8 injection. Representative samples are shown. (B) qPCR analyses of thermogenic gene expression in the BAT of AAV8-GFP and AAV8-TSK mice sacrificed 4 weeks post injection ($n = 8$ /group). (C) BAT weight of AAV8-GFP and AAV8-TSK mice ($n = 12$ /group). (D) Hematoxylin and eosin–stained sections of BAT samples collected from AAV8-GFP and AAV8-TSK mice. Representative samples are shown. (E) Body temperature of AAV8-GFP and AAV8-TSK mice 4 weeks post injection ($n = 7$ – 8 /group). (F) Body weight of AAV8-GFP and AAV8-TSK mice ($n = 12$ /group). (G) GTT and (H) ITT performed in AAV8-GFP and AAV8-TSK mice ($n = 12$ /group). Data are presented as the mean \pm SEM. In panels B, C, and E, significance was determined by two-tailed, unpaired t test. $*P < 0.05$ versus control. In panels F to H, two-way analysis of variance with Sidak's multiple comparisons was performed. $*P < 0.05$. The absence of an asterisk denotes no significant (n.s.) changes between the groups.

TSK is a newly identified hepatokine whose circulating levels are induced by obesity and cold exposure [7–9]. Liver steatosis, a common feature associated with obesity and acute cold challenge, is strongly associated with circulating TSK in several mouse models [7,9]. Interestingly, both inflammation and ER stress, two conditions linked to excessive lipid deposition in the liver, promote hepatic *Tsk* expression in mice [7]. Generally, these findings support the idea that TSK is produced when the liver faces various stressful conditions linked to excessive lipid flux in the tissue. Although both groups reporting the discovery of TSK generally agree on the conditions promoting its release by the liver, they also proposed different physiological roles for this hepatokine. Gain- and loss-of-function studies performed in our laboratory revealed that TSK has an impact on systemic cholesterol homeostasis [7]. In particular, we showed that TSK reduced circulating high-density lipoprotein cholesterol, lowered cholesterol efflux capacity, and decreased the conversion of cholesterol to bile acid in the liver. On the basis of these findings, we propose that TSK might be part of a biological response aimed at limiting reverse cholesterol transport to the liver when hepatocytes are exposed to elevated lipid flux, inflammation, and ER stress. Alternatively, because TSK is rapidly induced following cold exposure, a phenomenon that we found to be linked to the acute steatosis that develops upon sympathetic activation, a role for TSK in the regulation

of thermogenesis was investigated [8]. In this study, Wang et al. reported that TSK deletion increased sympathetic innervation and thermogenesis in BAT, protected mice against diet-induced obesity, and improved glucose homeostasis [8]. They proposed that TSK could be part of a negative feedback mechanism emerging from the liver to repress thermogenesis in BAT and reduce energy expenditure. With our past experience in the study of BAT and energy balance [11–17], we intuitively tested the effect of TSK on these parameters soon after the identification of TSK, with the objective of testing the possible role of this protein in the control of energy metabolism. Here, we provide compelling evidence showing that TSK does not impact the thermogenic activation of BAT under various experimental conditions. These results clearly show that neither the loss nor the overexpression of TSK has an effect on the expression of classical markers of thermogenesis in BAT. In these experiments, TSK did not affect body temperature and body weight gain in both lean and obese mice. Moreover, we did not observe any significant impact of TSK loss or overexpression on glucose homeostasis. Collectively, these observations raise questions about the importance of TSK in modulating BAT activation in mice, as recently proposed by Wang and colleagues [8]. The underlying reasons for these discrepant findings remain to be fully investigated, but it is worth pointing out some key experimental considerations that are likely contributing to the phenotypical discordance

between the studies. First, the TSK knockout models used by our respective teams were generated by two independent groups that used embryonic stem cells originating from different mouse strains (129S5/SvEvBrd vs. C57BL6 x CBA) [10,18]. Although both knockout mice were backcrossed to C57BL6J for six generations, differences in the genetic background may have persisted between the models, which could have been sufficient to alter the response to TSK loss. The impact of genetic background on thermogenesis has been well documented over the years [19,20]. Further supporting the differences between the two TSK knockout lines, a delay in hair cycle development was found in the TSK knockout mice used by Wang et al. [21], a phenotype that we did not observe in our model (Figure S2). Skin defects were also found in their TSK knockout mouse model [22]. As recently discussed by Nedergaard and Cannon, changes in the quality of skin, fur, and hair can reduce insulation and promote heat loss, which is often sufficient to activate the sympathetic nervous system and increase energy expenditure [23]. Taking this into account, it is thus possible that the link between TSK and BAT thermogenesis reported by Wang et al. may have been revealed secondary to a defect in heat conservation. The fact that their TSK knockout mice no longer show elevated BAT activity and protection against obesity when housed at thermoneutrality supports this hypothesis [8]. Lastly, we cannot exclude the possibility that variations in diet composition, housing conditions, or gut microbiota composition may have affected the biological outcomes of each study [24]. Although the experimental differences described above could explain part of the discordance between our studies with the TSK-null mouse models, they do not explain why TSK overexpression had no impact on BAT activation, body weight regulation, and glucose homeostasis. Collectively, the lack of effect of both TSK loss and overexpression on BAT activation and body weight regulation in our mouse models argues against a significant role of TSK in regulating thermogenesis and energy balance in mice.

5. CONCLUSION

Taken together, our data provide clear evidence that neither the loss nor the overexpression of TSK has any significant impact on BAT thermogenic capacity, body weight gain, and glucose homeostasis in mice. We thus feel that the conclusions drawn by Wang et al. should be interpreted with caution until additional studies from independent groups confirm or refute our respective findings.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molmet.2019.09.014>.

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