Glycerol kinase stimulates uncoupling protein 1 expression by regulating fatty acid metabolism in beige adipocytes

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Running title: Glycerol kinase regulates Ucp1 expression

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ABSTRACT

Browning, of adipose tissue is induced by specific stimuli such as cold exposure and consists of upregulation of thermogenesis in white adipose tissue. Recently, it has emerged as an attractive target for managing obesity in humans. Here, we performed a comprehensive analysis to identify genes associated with browning in murine adipose tissue. We focused on glycerol kinase (GYK) because its mRNA expression pattern is highly correlated with that of uncoupling protein 1 (UCP1), which regulates the thermogenic capacity of adipocytes. Cold exposure-induced Ucp1 upregulation in inguinal white adipose tissue (iWAT) was partially abolished by Gyk knockdown (KD) in vivo. Consistently, the Gyk KD inhibited Ucp1 expression induced by treatment with the β-adrenergic receptors (βAR) agonist isoproterenol (Iso) in vitro and resulted in impaired uncoupled respiration. Gyk KD also suppressed Iso- and adenylyl cyclase
activator-induced transcriptional activation and phosphorylation of the cAMP response element binding protein (CREB). However, we did not observe these effects with a cAMP analog. Therefore Gyk KD related to Iso-induced cAMP products. In Iso-treated Gyk KD adipocytes, stearoyl-CoA desaturase 1 (SCD1) was upregulated, and monounsaturated fatty acids such as palmitoleic acid (POA) accumulated. Moreover, a SCD1 inhibitor treatment recovered the Gyk KD-induced Ucp1 downregulation and POA treatment downregulated Iso-activated Ucp1. Our findings suggest that Gyk stimulates Ucp1 expression via a mechanism that partially depends on the βAR-cAMP-CREB pathway and on Gyk-mediated regulation of fatty acid metabolism.

INTRODUCTION

Adipose tissues are classified as white adipose tissue (WAT) and brown adipose tissue (BAT) according to their functions. WAT mainly consists of white adipocytes and stores extra energy in the form of triglycerides (TG) (1). BAT mostly comprises brown adipocytes and decomposes TG and dissipates thermal energy. It plays an important role in the regulation of thermogenic homeostasis (2). Nonshivering thermogenesis liberates heat via mitochondrial uncoupling protein 1 (UCP1) in the inner mitochondrial membrane. Mitochondrial adenosine triphosphate (ATP) synthesis is driven by a proton gradient produced by the respiratory electron transport chain. In contrast, UCP1 uses the proton gradient to generate heat rather than synthesize ATP (3).

Recent studies revealed that brown-like “beige” adipocytes appear in rodent WAT under cold exposure or the ingestion of certain dietary factors (4,5). Beige adipocytes expend energy via UCP1-mediated thermogenesis (6). Like other subcutaneous WAT, inguinal WAT (iWAT) browning is more readily induced than visceral WAT such as epididymal WAT (eWAT) (7). Several previous studies indicated that regulatory mechanisms of Ucp1 expression in each cell do not completely correspond (8). Moreover, the BAT in adult humans is mainly composed of beige adipocytes (9). Thus, elucidation of the browning mechanism in WAT may lead to effective new obesity prevention and treatment approaches.

Glycerol kinase (GYK) phosphorylates glycerol to glycerol 3-phosphate. This is an important step for the metabolism of glycerol that acts as the backbone of TG (10). Previous studies showed that deficiency of Gyk causes an X-linked recessive disease in humans, which shows hyperglycerolemia associated with congenital adrenal hypoplasia and developmental delay (11). In addition, Gyk KO mice show growth retardation, altered fat metabolism, and neonatal death (12). Although Gyk is more highly expressed in the liver and kidney than the adipose tissue (13), Gyk activity and expression in BAT has been reported to be upregulated in parallel by cold exposure or prolonged norepinephrine infusion (14,15). These reports indicated that Gyk might be related to thermogenic function in BAT. However the physiological functions of Gyk in WAT browning and their underlying mechanisms are unclear.

Here, we examined the effects of Gyk on Ucp1 expression during adipocyte browning in vitro and in vivo. Gyk positively regulated Ucp1 by controlling the βAR-cAMP-CREB signaling pathway via altering FA metabolism in
RESULTS

Ucp1 and Gyk had similar mRNA expression patterns in adipose tissues and adipocytes

First, we obtained comprehensive gene expression pattern in adipose tissue of mice during cold exposure (4 °C for 0 to 384 h) using microarray. Twenty nine genes which might be related to regulate thermogenesis in BAT were selected (Table 1). We then analyzed their expression pattern in iWAT via clustering analysis (see Methods). As shown in Fig. 1A, Gyk was located in the nearest cluster as Ucp1 and their expression levels were highly correlated (Pearson’s correlation coefficient = 0.951). Esrra located in a cluster next to the cluster of Ucp1 and Gyk is reported to be essential for high levels of mitochondrial biogenesis and oxidative capacity, characteristic of BAT, and thus for energy production in thermogenesis (16). On the other hand, the role of Gyk in BAT thermogenesis is unclear, and its function in iWAT has not been reported yet. We confirmed the gene expression levels of Ucp1 and Gyk in iWAT by real-time PCR. During cold exposure, mRNA expression levels of both Ucp1 and Gyk rapidly upregulated by 48 h (Fig. 1B). Therefore, we focused on the physiological role of Gyk in iWAT.

In iWAT, cold-induced Gyk and Ucp1 upregulation were observed in the adipocyte fraction but not the stromal vascular fraction (Fig. 1C). We used primary preadipocytes derived from iWAT to examine Gyk expression in this tissue. As shown in Fig. 1D, Ucp1 and Gyk were significantly upregulated after the induction of adipocyte differentiation. Furthermore, Ucp1 and Gyk were significantly upregulated in adipocytes treated with isoproterenol (Iso), a nonselective beta-adrenergic receptor (βAR) agonist (Fig. 1E).

Gyk knockdown suppressed βAR-induced Ucp1 upregulation in iWAT

To investigate the role of Gyk in adipocyte browning in vivo, we performed iWAT-targeting shRNA injection against Gyk using adeno-associated virus (AAV) as a vector. The shRNA against β-galactosidase (shLacZ) served as the control. Two weeks after AAV infection, the mice were individually housed either at room temperature (23 °C) or low temperature (10 °C) for 24 h. Cold stimulation reduced the weight of iWAT whereas Gyk KD did not cause significant variations in body or tissue weight (Table 2). Plasma TG levels were decreased both by cold exposure and Gyk KD. However, the effect of Gyk KD on plasma TG levels was abolished under low temperature (Table 2). These findings suggested that cold stress-activated βAR signaling may be involved in Gyk KD-regulated TG metabolism in vivo. As shown in Fig. 2A, fluorescence from ZsGreen encoded in the AAV vector was observed only in iWAT. The shRNA injection successfully downregulated Gyk in iWAT (Fig. 2B). No AAV infection-mediated Gyk downregulation was confirmed in the BAT, eWAT, or liver (Supplemental Fig. A), suggesting AAV infection-mediated Gyk downregulation occurred exclusively in iWAT. Browning marker genes such as Ucp1, Cidea, Pgc1a, and Pparg were also downregulated by Gyk KD in iWAT (Fig. 2B). In consistent with mRNA expression, UCP1 protein in iWAT was increased by cold exposure; however, this upregulation was
attenuated by Gyk KD (Fig. 2C). A histochemical analysis showed that cold exposure increased the number of small multilocular adipocytes in the iWAT of control mice whereas this response was relatively attenuated in the iWAT of Gyk KD mice (Fig. 2D). Low-temperature enhancement of the UCP1 immunohistochemical (IHC) staining intensity in iWAT was decreased by Gyk downregulation (Fig. 2D). On the other hand, Ucp1 gene expression and its protein expression levels in BAT were not affected by AAV infection (Supplemental Fig. B and C). H&E and IHC staining disclosed that AAV infection had no effect in BAT (Supplemental Fig. D). To examine the effect of iWAT-specific Gyk KD on cold-induced thermogenesis, we performed a cold tolerance test. Although there was no significant difference in rectal temperature changes during cold exposure, the rectal temperature in Gyk KD mice tended to be lower than that in control mice (Fig. 2E).

**Gyk knockdown suppressed βAR-induced Ucp1 upregulation in adipocytes**

Gyk was downregulated in immortalized primary iWAT cells infected with an adenovirus encoding Gyk-specific shRNA. Approximately 60% and 50% knockdown of Gyk mRNA expression occurred with and without Iso treatment, respectively (Fig. 3A). Ucp1 was upregulated by Iso in the control (shRNA for LacZ)-transfected cells. Gyk KD had no effect on Ucp1 expression in the absence of Iso treatment. Nevertheless, Ucp1 upregulation by Iso treatment was suppressed by Gyk KD (Fig. 3A). Cidea, a browning marker gene, was also upregulated by Iso treatment and this effect was suppressed by Gyk KD (Fig. 3A). Luciferase reporter assays revealed that Ucp1 promoter and cAMP-response element (CRE) transcriptional activities were upregulated by Iso treatment, and these upregulation were suppressed by Gyk KD (Fig. 3B). Next, phosphorylation of protein kinase A (PKA)-target protein were measured by western blot. In the presence of Iso, adipocytes with Gyk KD had lower hormone-sensitive lipase (HSL) and CRE binding protein (CREB) phosphorylation levels than shLacZ-induced adipocytes (Fig. 3C). A chromatin immunoprecipitation (ChIP) assay was performed to investigate whether CREB recruitment was reduced in the Ucp1 promoter region of Gyk KD adipocytes. Four CREs (CRE1-4) with the same core sequence were identified in the mouse Ucp1 5′ flanking region (17). However, CRE1 has no regulatory function and the impact of CRE3 is only marginally significant (18). Only CRE2 and CRE4 were reported to be necessary for Ucp1 regulation by CREB (18). Therefore, we investigated these two regions. In the presence of Iso, CREB recruitment increased in CRE2 and CRE4 and decreased in response to Gyk KD (Fig. 3D). These findings indicated that, Ucp1 was downregulated by Gyk KD via inhibition of the βAR signal. To investigate whether Gyk KD affect adipocytes energy metabolism, we performed a mitochondrial stress test. Gyk KD lowered the basal mitochondrial respiration rate. Proton leak also decreased without any apparent change in maximal respiration or ATP production, suggesting that Gyk KD decreased the uncoupling rate (Fig. 3E and 3F). These findings indicated that Gyk KD downregulated Ucp1 during Iso treatment in beige adipocytes by attenuating Iso-mediated βAR signaling.
activation.

**Gyk knockdown inhibited βAR-induced cAMP production in adipocytes**

To determine whether Gyk inhibition affects the βAR/ protein kinase A (PKA) pathway, we investigated the effects of Gyk KD on forskolin- and 8-Br-cAMP-induced Ucp1 expression. Forskolin activates adenylate cyclase (AC) and 8-Br-cAMP is a cell-permeable cAMP analog (Fig. 4A). Both forskolin and 8-Br-cAMP upregulated Ucp1 in a dose-dependent manner (Fig. 4B and 4C). However, Gyk KD inhibited Ucp1 expression induced by forskolin but not 8-Br-cAMP (Fig. 4B and 4C). Forskolin also enhanced CREB phosphorylation and Ucp1 promoter and CRE transcriptional activities but these were suppressed in Gyk KD cells (Fig. 4D and 4E). In contrast, in the case of 8-Br-cAMP, these Gyk KD-induced suppression were not observed (Fig. 4D and 4E). In control adipocytes, Iso elevated intracellular cAMP levels whereas Gyk KD attenuated them (Fig. 4F). cAMP, synthesized from ATP by AC, is catalytically degraded into AMP by phosphodiesterases (PDE). While Gyk KD showed no effect on AC protein levels, it significantly increased PDE activity (Fig. 4G and H), suggesting that the decrease in cAMP levels in Gyk KD adipocytes is mediated by increased PDE activity, at least partially. These results indicated that βAR-stimulated cAMP accumulation in adipocytes was downregulated by Gyk KD, leading to the suppression of Ucp1 upregulation.

**Gyk knockdown downregulated Ucp1 expression by altering fatty acid metabolism in adipocytes**

Gyk catalyzes the conversion of glycerol to glycerol-3-phosphate which is a substrate for TG synthesis. We hypothesized that glycerol and fatty acids (FAs) used for TG biosynthesis were accumulated in Gyk KD adipocytes. Glycerol and free FAs levels were significantly increased in the supernatant of Iso-treated Gyk KD adipocytes compared to Iso treated control adipocytes (Fig. 5A). Intracellular free FAs were detected by LC-MS to confirm the effects of Gyk on FA metabolism in Gyk KD cells. Iso treatment increased the monounsaturated FAs (palmitoleic acid (POA) and oleic acid (OA)), while these enhancements were further increased in Gyk KD cells (Fig. 5B). We hypothesized that monounsaturated FAs accumulation downregulates Ucp1 in Gyk KD cells. As shown in Fig. 5C, stearoyl-CoA desaturase (SCD) catalyzes the conversion of monounsaturated FAs, such as OA and POA, from saturated FAs, such as stearic acid (SA) and palmitic acid (PA). We measured the expression levels of four murine Scd isoforms in Gyk KD cells in response to Iso treatment. As shown in Fig. 5D, *Scd1* and *Scd2* were highly expressed in primary iWAT cells and *Scd1* was especially upregulated in Iso-treated Gyk KD cells. We investigated whether *Scd1* links Gyk-mediated regulation of Ucp1 expression. Under Iso treatment, Gyk KD suppressed Ucp1 expression but this inhibition was abolished by SCD1 inhibitor treatment (Fig. 5E). We evaluated SCD1 activity by calculating the ratio of saturated FA (PA+SA) to monounsaturated FA (POA+OA). It was markedly reduced in the Gyk KD group but the SCD1 inhibitor treatment rescued it (Fig. 5F). Thus, the influences of Gyk on Ucp1 expression may depend partially on SCD1-mediated intracellular monounsaturated...
FA accumulation.

**DGAT and ATGL inhibitors regulated Ucp1 expression**

To further investigate the relationship between intracellular FA accumulation and Ucp1 expression, we inhibited diglyceride acyltransferase (DGAT) and adipose triglyceride lipase (ATGL) which convert diacylglycerol to TG and regulate the first step in TG hydrolysis, respectively. No significant variations were detected in the absence of Iso. However, in the presence of Iso, DGAT inhibitor downregulated Ucp1 expression, while ATGL inhibitor upregulated it (Fig. 6A). With Iso addition, the Ucp1 promoter and CRE transcriptional activities were also decreased or increased by treating DGAT or ATGL inhibitor, respectively (Fig. 6B). As shown in Fig. 6C, PA, POA, and OA levels were dramatically augmented by DGAT inhibitor treatment but considerably decreased by ATGL inhibitor treatment. Thus, intracellular FAs may modulate βAR signaling-regulated Ucp1 mRNA expression.

**Palmitoleic acid treatment suppressed βAR agonist-induced Ucp1 expression**

As shown in Fig. 7A, Iso-induced Ucp1 upregulation was downregulated by only POA treatment. In the presence of Iso, POA reduced Ucp1 promoter and CRE transcriptional activities in a dose-dependent manner (Fig. 7B). Similar to the results of Gyk KD (Fig. 4B and 4C), POA treatment inhibited forskolin-induced Ucp1 promoter and CRE transcriptional activation but failed to inhibit 8-Br-cAMP-induced it (Fig. 7C). In addition, PDE activity was increased by POA treatment (Fig. 7D). To confirm whether POA treatment influenced FA accumulation, we measured the intracellular FAs. POA application induced intracellular POA accumulation (Fig. 7E). Thus, Gyk KD-inhibited Ucp1 upregulation is the result of βAR signaling attenuation by increased PDE activity via a mechanism partially dependent on enhanced POA accumulation.

**DISCUSSION**

Browning in white adipocytes is an inducible process which is a potential target for obesity treatment or prevention. Ucp1 is a classical thermogenic gene that has been widely used to evaluate adipocyte browning. Here, we searched for genes related to iWAT browning using transcriptome analysis and identified Gyk as a candidate gene. Gyk and Ucp1 upregulation were observed in adipocyte fraction in iWAT during cold exposure and Iso-treated primary iWAT cells (Fig.1C and D). Gyk KD suppressed cold exposure- or Iso-induced Ucp1 expression in vivo and in vitro (Fig. 2B and 3A) via βAR signaling, by suppressing the increase in cAMP production. Since Gyk catalyzed glycerol 3-phosphate formation which is a substrate of TG synthesis, we hypothesized that FAs metabolism might be related to Gyk KD-induced suppression of Ucp1 upregulation. Actually, the expression of Scd1 was enhanced in Iso-treated Gyk KD cells accompanied with an increase in monounsaturated FAs accumulation. Furthermore, POA addition suppressed Ucp1 upregulation via βAR signaling. As shown in Fig. 8, Gyk regulates Ucp1 expression, via POA accumulation-mediated suppression of the βAR-cAMP-CREB pathway.

Although Gyk has been reported to be highly expressed in the liver and kidney (13,19), recent study reported that Gyk expression levels
were induced by cold exposure in BAT (20,21). Lasar et al. reported that Gyk is partially related to peroxisome proliferator-activated receptor gamma function in BAT (22). These reports indicated that Gyk is implicated in BAT function but do not elucidate its detailed mechanism. Moreover, the physiological function of Gyk in iWAT browning remains unknown. To the best of our knowledge, this study is the first to indicate the role of Gyk in iWAT browning. The molecular signatures of adult human brown adipocytes resemble those of mouse beige adipocytes rather than brown ones (23). Therefore, functional analysis of mouse beige adipocytes would be more informative than that of brown adipocytes. Our findings could help uncover the role of Gyk in human BAT.

Thiazolidinediones administered for type 2 diabetes induce Gyk expression to promote TG synthesis and inhibit glycerol and FA secretion by adipocytes (24). Thus, Gyk upregulation increases TG synthesis and decreases intracellular glycerol and FAs. Intracellular FAs are thermogenesis substrates via UCP1. In Gyk KD adipocytes, intracellular FAs were increased, hence, Gyk KD had been considered acting positively for thermogenesis. Although the FAs content was enhanced, Gyk KD, DGAT inhibitor, and POA all downregulated Ucp1 expression. Previous study showed that lipogenesis is upregulated in BAT during cold exposure (25,26). In mice with BAT-specific hypothyroidism, cold-induced lipogenesis in BAT was inhibited, and this inhibition led to the reduction of thermogenic activity in BAT (26). These reports indicated that not only lipolysis but also lipogenesis in BAT during cold exposure are important for thermogenic activity in BAT. In this study, we showed in the presence of βAR stimuli, Gyk plays an important role to regulate intracellular FAs levels and composition. Therefore Gyk seems to be important regulator for the balance between lipolysis and lipogenesis in beige adipocytes.

The Gyk KD adipocytes that were not treated with Iso presented with comparatively lower fatty acid release levels (Fig. 5A). Concerning this point, we revealed that Gpat1 expression level was significantly higher in Gyk KD adipocytes than control adipocytes (data not shown). Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the conversion of glycerol-3-phosphate and long-chain acyl-CoA to lysophosphatidic acid. It was reported that GPAT is the rate-limiting enzyme in de novo glycerolipid biosynthesis (27). GPAT1 activity may be positively correlated with the rate of fatty acid storage in the form of triacylglycerol in human adipose tissue (28). Moreover, Gpat1 overexpression has been reported to increase cellular triacylglycerol accumulation (29). An increase in Gpat1 mRNA expression may not accurately reflect a corresponding increase in GPAT1 enzyme activity. However, the capacity for fatty acid acylation might be higher in Gyk KD adipocytes not treated with Iso than it is in control adipocytes.

Scd1 is expressed in various tissues including the liver and adipose tissue. Enser reported that obesity mouse models exhibiting high level of Scd1 expression present with liver steatosis and insulin resistance (30). In contrast, Scd1-deficient mice were resistant to diet-induced obesity possibly related to enhanced energy expenditure (31,32). In this study, we showed Scd1 upregulation-mediated
increase in POA levels suppressed Ucp1 upregulation in beige adipocytes, suggesting that Scd1 upregulation negatively regulates energy expenditure. This mechanism might contribute to anti-obese phenotype in Scd1-deficient mice.

Saturated FAs have been reported to enhance various cellular stresses, such as oxidative stress and endoplasmic reticulum stress and inflammation, in adipose tissue (33,34). We hypothesized that Gyk KD could reduce intracellular concentration of saturated FAs by upregulating Scd1 to attenuate saturated FAs-induced cellular stresses. However, as the results, accumulated POA may negatively regulate βAR stimulated Ucp1 upregulation.

Here, we proposed that POA functionally participates in Ucp1 regulation. POA may be an important signaling molecule produced mainly by WAT (35). POA functions as a WAT-derived lipokine implicated in insulin sensitivity in skeletal muscle, hepatic lipogenesis (36), and lipid metabolism in WAT (37). Although the effects of POA on the regulation of energy metabolism are largely unknown, Souza et al. reported that daily administration of 300 mg kg⁻¹ POA for 10 d successfully attenuated insulin resistance, liver inflammation, and damage caused by high-fat diets (38). Though several benefits of POA in mice were mentioned, POA may have several disadvantages in humans. Plasma POA levels were highly correlated with nonalcoholic fatty liver disease (39) and heart failure (40). As the data on the effects of POA on human health are inconsistent with those for animal and cell culture models, further human POA trials are warranted (41). Our results showed that the increase in intracellular POA levels is involved in the suppression of Ucp1 expression. Under Iso-treated conditions, POA cellular contents were increased to 4.3 fmol/cell (Fig. 5B), 6.6 fmol/cell (Fig. 6C) and 2.9 fmol/cell (Fig. 7D) by Gyk KD, DGAT inhibitor treatment, and POA treatment, respectively. Ucp1 suppression levels were 44 %, 53 % and only 16 %, respectively, suggesting that cellular POA contents are positively correlated with Ucp1 suppression levels. Although it is difficult to estimate accurate POA amounts required for Ucp1 suppression, approximately 2.9 fmol/cells or more POA seeds may be needed to suppress Ucp1 expression.

The present study disclosed that Gyk is an important gene in the white adipocyte browning process. We proposed that Scd1-regulated FA composition was in co-determine the role of Gyk in Ucp1 expression. The latter partially depends on the βAR-cAMP-CREB pathway in adipocytes (Fig. 8). Our findings provide insights into the potential mechanisms by which beige adipocyte thermogenesis is controlled via the regulation of Gyk expression. These modes of action could be exploited as a potential strategy to modulate Ucp1 expression and body temperature control.

**MATERIALS & METHODS**

**Materials**

All chemicals were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA), Nacalai Tesque (Kyoto, Japan), or Wako Pure Chemical Industries Ltd. (Osaka, Japan).

**Animal experiment**

Male C57BL/6J mice were purchased from Japan SLC (Shizuoka, Japan). They were kept in individual cages at 23 ± 1 °C and under a 12-h light/dark cycle. They had access to food...
and water ad libitum. They were fed a commercial chow diet (MF; Oriental Yeast Co. Ltd., Tokyo, Japan). The study was approved by the Animal Care Committee of Kyoto University.

**RNA sample preparation for microarray analysis**

Male 6-wk C57BL/6J mice were subjected to cold exposure (4 °C) for 0 h, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 48 h, 192 h, or 384 h. Then their iWAT was harvested and its RNA was extracted with the RNeasy lipid tissue mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The extracted total RNA was used to synthesize fluorescently labeled cDNA.

**Microarray analysis**

Microarray analysis was carried out using Agilent SurePrint G3 Mouse GE 8x60K microarrays (~60,000 probes including 39,430 Entrez Gene RNAs and 16,251 lincRNAs). All experiments were performed in triplicate (three microarray experiments or three different mouse RNA samples per time point). Microarray data quality control was performed by Agilent feature extraction (Agilent Technologies, Santa Clara, CA, USA) and GeneSpring software (Agilent Technologies, Santa Clara, CA, USA). The probes for each gene symbol were collapsed into one representative probe with maximum variance (24,163 genes). Raw signal intensities were normalized for each microarray by quantile normalization in the “limma” package (42). Expression values were then log-transformed (base 2). The batch effect was removed with the “Combat” function in the “sva” package (43).

**Clustering analysis**

A clustering analysis was run on the selected genes using the “pvclust” package (44). Pearson’s correlation coefficients were selected on the basis of similarity between gene expressions and the average linkage for the clustering method.

**Recombinant AAV vectors**

The DNA sequences corresponding to the short hairpin RNA (shRNA) sequences of *Gyk* were 5'-ACCCTCCATGCCTGAAACA-3' and 5'-ACCACTTTCTGGAGACTGAGTT-3'. They were annealed and ligated into the shRNA expression vector pAAV-U6-ZsGreen1 (TaKaRa Bio, Kusatsu, Shiga, Japan). Recombinant AAV6 expressing the *Gyk* shRNA were generated according to the manufacturer’s protocol. A recombinant AAV6 expressing a *LacZ* shRNA was generated as a negative control. The AAV particles were purified with the AAVpro purification kit (TaKaRa Bio, Kusatsu, Shiga, Japan). The virus titers (viral genomes [vg] mL⁻¹) were determined by qPCR.

**Administration of AAV vectors**

Male 10-wk C57BL/6J mice were anesthetized with isoflurane and injected into their bilateral iWAT with 40 μL AAV6 vector solutions (4 × 10¹² vg mL⁻¹) per pad. After 2 wks, the mice were subjected to cold treatment (10 °C) for 24 h and sampled for analysis.

**RNA preparation and quantification of gene expression**

Total RNA samples were isolated with a commercially available reagent (Sepasol Super-I, Nacalai Tesque, Kyoto, Japan). Aliquots of total RNA were reverse-transcribed with...
M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer’s instructions. To quantify mRNA expression, real-time RT-PCR was performed in a LightCycler system (Roche Diagnostics, Mannheim, Germany) with SYBR Green fluorescence signals as previously described (45). The oligonucleotide primers were designed in a PCR primer selection program available from the website of the GenBank database Virtual Genomic Center. They are shown in Supplemental Table All mRNA expression levels were normalized to those of 36B4 mRNA.

**Plasma characteristics**

Plasma glucose, TG, and free FA levels were enzymatically determined with glucose CII, triglyceride E, and NEFA C test kits (Wako Pure Chemicals Industries Ltd., Osaka, Japan), respectively.

**Histological analysis**

Tissues were excised from each mouse and fixed in 10% (v/v) paraformaldehyde/phosphate-buffered saline (PBS). After ethanol dehydration, the fixed samples were embedded in paraffin, cut into 5-μm sections with a microtome, and mounted on microscope slides (Matsunami Glass, Osaka, Japan). The sections were stained with modified Mayer’s hematoxylin (Merck, Darmstadt, Germany) and eosin Y (Wako Pure Chemical Industries Ltd., Osaka, Japan). For IHC analysis, the sections were incubated in 1% (v/v) hydrogen peroxide in methanol and then in 10% (v/v) normal goat serum, rabbit anti-UCP1 (U6382; 1:200; Sigma-Aldrich Corp., St. Louis, MO, USA), goat anti-rabbit IgG (Nichirei, Tokyo, Japan), and avidin-biotin-peroxidase complex (Nichirei, Tokyo, Japan) according to the standard avidin-biotin complex method (46).

**Cold tolerance test**

Two weeks after administration of AAV vectors, rectal temperature of mice was measured at 0, 1, 2, 4 hours after 10°C cold exposure using a thermometer probe (T&D Corp., Nagano, Japan). The area under the curve (AUC) was calculated using the trapezoidal rule.

**Cell culture**

Primary mouse iWAT preadipocytes were immortalized by transfection with Simian Virus 40 large T antigen (kind gift from Prof. S. Kajimura, University of California, San Francisco). Successfully transfected clones were screened for puromycin resistance. Mouse 10T1/2 preadipocytes were purchased from the American Type Culture Collection (Manassas, VA, USA). All cell lines were maintained in a humidified 5% CO₂ atmosphere at 37 °C in basic medium (Dulbecco’s modified Eagle medium-high glucose supplemented with 10% (v/v) fetal bovine serum (FBS), 10,000 units mL⁻¹ penicillin, and 10,000 μg mL⁻¹ streptomycin). The cell cultures were raised to 95–97% confluence. The iWAT preadipocytes cells were differentiated with basic medium plus 2 μg mL⁻¹ dexamethasone, 5 μg mL⁻¹ insulin, 0.5 mM 3-isobutyl-1-methylxanthine, 125 μM indomethacin, 1 nM T₃, and 0.5 μM rosiglitazone for 48 h. The media were replaced every 2d with growth medium (basic medium supplemented with 5 μg mL⁻¹ insulin and 1 nM T₃). To knock down Gyk, adenovirus (multiplicity of infection = 300) was inducted into cells at the 6-d differentiation stage (47). After 8 d differentiation, the cells were subjected
to 0.5 μM isoproterenol (Iso), 1–5 μM forskolin (FSK), and 100–500 μM 8-Br-cAMP for 4 h. The cells were pretreated for 1 h with 400 μM FAs, 100 μM SCD1 inhibitor (CAY 10566), 1 μM DGAT inhibitor (A922500), or 10 μM ATGL inhibitor (atglistatin) and co-treated with Iso for 4 h.

**Luciferase reporter assays**

Luciferase reporter assays were performed as previously reported (48). Briefly, pUCp1-pro-Luc or pCRE-Luc (kindly provided from Prof. Y. Kamei, Kyoto Kyoto Prefectural University) was transfected into 10T1/2 cells growing on 100-mm tissue culture dishes. Four hours after transfection, the cells were seeded in 96-well tissue plates and incubated with 0.5 μM Iso, 1-5 μM FSK, and 100-500 μM 8-Br-cAMP for 4 h. The cells were then lysed for the luciferase assay in a dual-luciferase reporter gene assay system (Promega, Madison, WI, USA) in accordance with the manufacturer’s protocol.

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and western blot**

Proteins from the cells and tissues were solubilized in lysis buffer (50 mM Tris·HCl, 150 mM NaCl, 1% (v/v) Triton X-100, 0.5% (w/v) deoxycholate, 0.1% (v/v) SDS, pH 7.4), and a protease/phosphatase inhibitor cocktail). The protein concentration was determined with a protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Protein samples were subjected to SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA, USA). The membranes were blocked with Blocking One–P and then incubated with anti-UCP1 (U6382; 1:1,000; Sigma-Aldrich Corp., St. Louis, MO, USA), anti-HSL (4107; 1:2,000), anti-phosphorylated HSL (4139; 1:2,000), anti-CREB (9197; 1:2,000), anti-phosphorylated CREB (9198; 1:2,000), anti-COX4 (4844; 1:1,000), anti-β-actin (4967; 1:1,000) (all from Cell Signaling Technology, Danvers, MA, USA) or anti adenylate cyclase 3 (NBP1-92683; 1:2,000; Novus Biologicals, Centennial, CO, USA) diluted with blocking buffer. Proteins were detected with an ECL western blot detection system (GE Healthcare, Chicago, IL, USA). For band quantification, AlphaEaseFC software (Alpha Innotec, Kasendorf, Germany) was used.

**Chromatin immunoprecipitation (ChIP) assay**

The ChIP assay was performed according to the manufacturer’s protocol (Upstate Cell Signaling Solutions, Lake Placid, NY, USA), with some modifications. The cells were fixed in 1% (v/v) formaldehyde, quenched with 125 mM glycine, collected, resuspended in 0.5% (w/v) SDS lysis buffer, and sonicated to shear their DNA into 100–1,000-bp fragments. The supernatant was collected and immunoprecipitated overnight with 5 μg CREB antibody (Cell Signaling Technology, Danvers, MA, USA) or with 1 μg rabbit IgG isotype (Novus Biological, Littleton, CO, USA) as a mock control along with Magna ChIP™ protein A+G magnetic beads (EMD Millipore, Burlington, MA, USA) at 4 °C in a rotary shaker followed by reverse cross-linkage and protease K digestion. The eluted DNA was purified with a MinElute PCR purification kit (Qiagen, Hilden, Germany) and analyzed by real-time PCR. Primer sequences are listed in Supplemental.
cAMP content quantification

The cAMP content was enzymatically determined with a cAMP-Glo™ max assay kit (Promega, Madison, WI, USA) according to the manufacturer’s instructions.

Phosphodiesterase (PDE) activity assay

The cells were lysed at 4 °C using the RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% (v/v) NP-40, 0.5% (w/v) deoxycholate, 1 mM EDTA, 0.1% (w/v) SDS, pH 7.4) mixed with protease and phosphatase inhibitors. The lysates were collected and centrifuged at 10,000 ×g at 4 °C for 5 min to remove cellular debris and the supernatant was used for the PDE activity assay using a colorimetric PDE Activity Assay Kit (Abcam, Cambridge, UK). Briefly, the supernatant was purified by gel filtration using desalting resin and desalting columns. The PDE activity was measured by the method based on the sequential hydrolysis of cyclic nucleotides by PDE and 5’ nucleosidase. The released phosphate by enzymatic cleavage is directly proportional to PDE activity and quantified using a modified Malachite Green reagent.

LC-MS for free FA quantitation

Free FA samples were collected in 99.5% (v/v) EtOH and centrifuged at 20,000 ×g and 4 °C for 10 min. The supernatant was filtered through a 0.2-μm PVDF membrane (Whatman, Brentford, UK) and the filtrate was used in the LC-MS which was performed as previously described (49). Briefly, a Waters Acquity UPLC system was coupled to a Xevo Quadrupole Time-of-Flight (QTOF)-MS system (Waters, Milford, MA) and a 3-mL FA sample aliquot was injected into an Acquity UPLC BEH-C18 reversed-phase column (2.1; 100 mm column size; 1.7 mm particle size). Mobile phase A consisted of 90% (v/v) acetonitrile, 10 mM ammonium formate, and 0.1% (v/v formic acid) and mobile phase B consisted of 98% (v/v) acetonitrile and 0.1% (v/v) formic acid. The buffer gradient was 0.1% B for 0–5 min, 0.1–99.9% B for 5–6 min, 99.9% B for 6–11 min, 99.9–0.1% B for 11–12 min, and 0.1% B for 3 min before the next injection. The flow rate was 400 mL min⁻¹. Data were acquired with MassLynx software (Waters, Milford, MA).

Oxygen consumption rate measurement

The oxygen consumption rate (OCR), reflects the mitochondrial respiration rate and was determined with the XF24 extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA, USA), according to the manufacturer’s instructions. Briefly, differentiated primary mouse iWAT adipocytes were seeded in 24-well plates (Seahorse Bioscience, North Billerica, MA, USA) and the OCRs were measured and recorded with a sensor cartridge and Seahorse XF-24 software (Seahorse Bioscience, North Billerica, MA, USA). Basal respiration was first measured and then the following were sequentially injected: 500 nM oligomycin (a mitochondrial ATP-synthase inhibitor), 1 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP; mitochondrial oxidative phosphorylation uncoupler), and a mixture of 15 μM antimycin A (a mitochondrial complex III inhibitor) and 15 μM rotenone (a mitochondrial complex I inhibitor). The OCR values related to basal respiration, ATP production, proton leak,
maximum respiration, and coupling and uncoupling rates were calculated as follows:

- Basal respiration = last point before oligomycin injection - minimum value after antimycin A and rotenone

- ATP production = last point before oligomycin injection - minimum value after oligomycin

- Proton leak = minimum value after oligomycin - minimum value after antimycin A and rotenone

- Maximum respiration = maximum value after FCCP - minimum value after antimycin A and rotenone

The coupling and uncoupling rates were calculated as follows:

- Coupling rate [%] = ATP production / basal respiration

- Uncoupling rate [%] = 100 - coupling rate

**Statistical analysis**

Data are presented as means ± SE. Student’s t-test or ANOVA followed by the Tukey-Kramer test were used to identify statistically significant differences among treatment means. Differences were considered significant at $P < 0.05$.

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**Conflict of interest statement**

The authors declare that they have no conflicts of interest with the contents of this article.

**Data availability**

All data for this publication are included in this published article and its supporting information.

**Author contributions**

M. I., S. S., Y.-S. Y., H. T., T. K., H. M., T. K., and T. G. designed the experiments and wrote the manuscript. M. I., S. T., T. M., and T. K. performed the experiments. M. I., S. T., S. S., Y.-S. Y., H. T., W. N., H.-F. J., T. K., N. O., H. M., T. K., and T. G. discussed and interpreted the data. S. M. and K. I. furnished technical assistance and analyzed the data. S. S., N. O., and H. M. conducted the transcriptomic analysis. T.G. supervised the project.
References

1. Zechner, R., Zimmermann, R., Eichmann, T. O., Kohlwein, S. D., Haemmerle, G., Lass, A., and Madeo, F. (2012) FAT SIGNALS—lipases and lipolysis in lipid metabolism and signaling. Cell metabolism 15, 279-291

2. Cannon, B., and Nedergaard, J. (2004) Brown adipose tissue: function and physiological significance. Physiological reviews 84, 277-359

3. Krauss, S., Zhang, C. Y., and Lowell, B. B. (2005) The mitochondrial uncoupling-protein homologues. Nature reviews. Molecular cell biology 6, 248-261

4. Goto, T., Naknukool, S., Yoshitake, R., Hanafusa, Y., Tokiwa, S., Li, Y., Sakamoto, T., Nitta, T., Kim, M., Takahashi, N., Yu, R., Daiyasu, H., Seno, S., Matsuda, H., and Kawada, T. (2016) Proinflammatory cytokine interleukin-1beta suppresses cold-induced thermogenesis in adipocytes. Cytokine 77, 107-114

5. Kim, M., Goto, T., Yu, R., Uchida, K., Tominaga, M., Kano, Y., Takahashi, N., and Kawada, T. (2015) Fish oil intake induces UCP1 upregulation in brown and white adipose tissue via the sympathetic nervous system. Scientific reports 5, 18013

6. Walden, T. B., Hansen, I. R., Timmons, J. A., Cannon, B., and Nedergaard, J. (2012) Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. American journal of physiology. Endocrinology and metabolism 302, E19-31

7. Sharp, L. Z., Shinoda, K., Ohno, H., Scheel, D. W., Tomoda, E., Ruiz, L., Hu, H., Wang, L., Pavlova, Z., Gilsanz, V., and Kajimura, S. (2012) Human BAT possesses molecular signatures that resemble beige/brite cells. PloS one 7, e49452

8. Garcia, R. A., Roemmich, J. N., and Claycombe, K. J. (2016) Evaluation of markers of beige adipocytes in white adipose tissue of the mouse. Nutrition & metabolism 13, 24

9. Wu, J., Bostrom, P., Sparks, L. M., Ye, L., Choi, J. H., Giang, A. H., Khandekar, M., Virtanen, K. A., Nuutila, P., Schaart, G., Huang, K., Tu, H., van Marken Lichtenbelt, W. D., Hoeks, J., Enerback, S., Schrauwen, P., and Spiegelman, B. M. (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366-376

10. Beale, E. G., Hammer, R. E., Antoine, B., and Forest, C. (2002) Glyceroneogenesis comes of age. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 16, 1695-1696

11. Lewis, B., Harbord, M., Keenan, R., Carey, W., Harrison, R., and Robertson, E. (1994) Isolated glycerol kinase deficiency in a neonate. Journal of child neurology 9, 70-73

12. Huq, A. H., Lovell, R. S., Ou, C. N., Beaudet, A. L., and Craigien, W. J. (1997) X-linked glycerol kinase deficiency in the mouse leads to growth retardation, altered fat metabolism, autonomous glucocorticoid secretion and neonatal death. Human molecular genetics 6, 1803-1809

13. Yue, F., Cheng, Y., Breschi, A., Vierstra, J., Wu, W., Ryba, T., Sandstrom, R., Ma, Z., Davis, C., Pope, B. D., Shen, Y., Pervouchine, D. D., Djebali, S., Thurman, R. E., Kaul, R., Rynes,
E., Kirilusha, A., Marinov, G. K., Williams, B. A., Trout, D., Amrhein, H., Fisher-Aylor, K., Antoschechkin, I., DeSalvo, G., See, L. H., Fastuca, M., Drenkow, J., Zaleski, C., Dobin, A., Prieto, P., Lagarde, J., Bussotti, G., Tanzer, A., Denas, O., Li, K., Bender, M. A., Zhang, M., Byron, R., Groudine, M. T., McCleary, D., Pham, L., Ye, Z., Kuan, S., Edsall, L., Wu, Y. C., Rasmussen, M. D., Bansal, M. S., Kellis, M., Keller, C. A., Morrissey, C. S., Mishra, T., Jain, D., Dogan, N., Harris, R. S., Cayting, P., Kawli, T., Boyle, A. P., Euskirchen, G., Kundaje, A., Lin, S., Lin, Y., Jansen, C., Malladi, V. S., Cline, M. S., Erickson, D. T., Kirkup, V. M., Learned, K., Sloan, C. A., Rosenbloom, K. R., Lacerda de Sousa, B., Beal, K., Pignatelli, M., Flicek, P., Lian, J., Kahveci, T., Lee, D., Kent, W. J., Ramalho Santos, M., Herrero, J., Notredame, C., Johnson, A., Vong, S., Lee, K., Bates, D., Neri, F., Diegel, M., Canfield, T., Sabo, P. J., Wilken, M. S., Reh, T. A., Giste, E., Shafer, A., Kuttyavin, T., Haugen, E., Dunn, D., Reynolds, A. P., Nep, S., Humbert, R., Hansen, R. S., De Bruijn, M., Selleri, L., Rudensky, A., Josefowicz, S., Samstein, R., Eichler, E. E., Orkin, S. H., Levasseur, D., Papayannopoulou, T., Chang, K. H., Skoultchi, A., Gosh, S., Distech, C., Treuting, P., Wang, Y., Weiss, M. J., Blobel, G. A., Cao, X., Zhong, S., Wang, T., Good, P. J., Lowdon, R. F., Adams, L. B., Zhou, X. Q., Pazin, M. J., Feingold, E. A., Wold, B., Taylor, J., Mortazavi, A., Weissman, S. M., Stamatoyannopoulos, J. A., Snyder, M. P., Guigo, R., Gingeras, T. R., Gilbert, D. M., Hardison, R. C., Beer, M. A., Ren, B., and Mouse, E. C. (2014) A comparative encyclopedia of DNA elements in the mouse genome. *Nature* 515, 355-364

14. Festuccia, W. T., Guerra-Sa, R., Kawashita, N. H., Garofalo, M. A., Evangelista, E. A., Rodrigues, V., Kettelhut, I. C., and Migliorini, R. H. (2003) Expression of glycerokinase in brown adipose tissue is stimulated by the sympathetic nervous system. *American journal of physiology. Regulatory, integrative and comparative physiology* 284, R1536-1541

15. Kawashita, N. H., Festuccia, W. T., Brito, M. N., Moura, M. A., Brito, S. R., Garofalo, M. A., Kettelhut, I. C., and Migliorini, R. H. (2002) Glycerokinase activity in brown adipose tissue: a sympathetic regulation? *American journal of physiology. Regulatory, integrative and comparative physiology* 282, R1185-1190

16. Villena, J. A., Hock, M. B., Chang, W. Y., Barcas, J. E., Giugere, V., and Kralli, A. (2007) Orphan nuclear receptor estrogen-related receptor alpha is essential for adaptive thermogenesis. *Proceedings of the National Academy of Sciences of the United States of America* 104, 1418-1423

17. Quinn, P. G., Wong, T. W., Magnuson, M. A., Shabb, J. B., and Granner, D. K. (1988) Identification of basal and cyclic AMP regulatory elements in the promoter of the phosphoenolpyruvate carboxykinase gene. *Molecular and cellular biology* 8, 3467-3475

18. Kozak, U. C., Kopecky, J., Teisinger, J., Enerback, S., Boyer, B., and Kozak, L. P. (1994) An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene. *Molecular and cellular biology* 14, 59-67

19. Miao, L., Yang, Y., Liu, Y., Lai, L., Wang, L., Zhan, Y., Yin, R., Yu, M., Li, C., Yang, X., and
Ge, C. (2019) Glycerol kinase interacts with nuclear receptor NR4A1 and regulates glucose metabolism in the liver. FASEB journal: official publication of the Federation of American Societies for Experimental Biology 33, 6736-6747

20. Rahib, L., MacLennan, N. K., Horvath, S., Liao, J. C., and Dipple, K. M. (2007) Glycerol kinase deficiency alters expression of genes involved in lipid metabolism, carbohydrate metabolism, and insulin signaling. European journal of human genetics: EJHG 15, 646-657

21. Festuccia, W. T., Blanchard, P. G., Richard, D., and Deshaies, Y. (2010) Basal adrenergic tone is required for maximal stimulation of rat brown adipose tissue UCP1 expression by chronic PPAR-gamma activation. American journal of physiology: Regulatory, integrative and comparative physiology 299, R159-167

22. Lasar, D., Rosenwald, M., Kiehlmann, E., Balaz, M., Tall, B., Opitz, L., Lidell, M. E., Zamboni, N., Krznan, P., Sun, W., Varga, L., Stefanicka, P., Uкроpec, J., Nuutila, P., Virtanen, K., Amri, E. Z., Enerback, S., Wahlil, W., and Wolfram, C. (2018) Peroxisome Proliferator Activated Receptor Gamma Controls Mature Brown Adipocyte Inducibility through Glycerol Kinase. Cell reports 22, 760-773

23. Shinoda, K., Luijten, I. H., Hasegawa, Y., Hong, H., Sonne, S. B., Kim, M., Xue, R., Chondronikola, M., Cypess, A. M., Tseng, Y. H., Nedergaard, J., Sidossis, L. S., and Kajimura, S. (2015) Genetic and functional characterization of clonally derived adult human brown adipocytes. Nature medicine 21, 389-394

24. Guan, H. P., Li, Y., Jensen, M. V., Newgard, C. B., Steppan, C. M., and Lazar, M. A. (2002) A futile metabolic cycle activated in adipocytes by antidiabetic agents. Nature medicine 8, 1122-1128

25. McCormack, J. G. (1982) The regulation of fatty acid synthesis in brown adipose tissue by insulin. Progress in lipid research 21, 195-223

26. Christofolette, M. A., Linardi, C. C., de Jesus, L., Ebina, K. N., Carvalho, S. D., Ribeiro, M. O., Rabelo, R., Curcio, C., Martins, L., Kimura, E. T., and Bianco, A. C. (2004) Mice with targeted disruption of the Dio2 gene have cold-induced overexpression of the uncoupling protein 1 gene but fail to increase brown adipose tissue lipogenesis and adaptive thermogenesis. Diabetes 53, 577-584

27. Yu, J., Loh, K., Song, Z. Y., Yang, H. Q., Zhang, Y., and Lin, S. (2018) Update on glycerol-3-phosphate acyltransferases: the roles in the development of insulin resistance. Nutrition & diabetes 8, 34

28. Morgan-Bathke, M., Chen, L., Oberschneider, E., Harteneck, D., and Jensen, M. D. (2016) More insights into a human adipose tissue GPAT activity assay. Adipocyte 5, 93-96

29. Linden, D., William-Olsson, L., Ahnmark, A., Ekroos, K., Hallberg, C., Sjogren, H. P., Becker, B., Svensson, L., Clapham, J. C., Oscarsson, J., and Schreyer, S. (2006) Liver-directed overexpression of mitochondrial glycerol-3-phosphate acyltransferase
results in hepatic steatosis, increased triacylglycerol secretion and reduced fatty acid oxidation. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 434-443

30. Enser, M. (1975) Desaturation of stearic acid by liver and adipose tissue from obese-hyperglycaemic mice (ob/ob). *The Biochemical journal* **148**, 551-555

31. Miyazaki, M., Sampath, H., Liu, X., Flowers, M. T., Chu, K., Dobrzyn, A., and Ntambi, J. M. (2009) Stearoyl-CoA desaturase-1 deficiency attenuates obesity and insulin resistance in leptin-resistant obese mice. *Biochemical and biophysical research communications* **380**, 818-822

32. Jiang, G., Li, Z., Liu, F., Ellsworth, K., Dallas-Yang, Q., Wu, M., Ronan, J., Esau, C., Murphy, C., Szalkowski, D., Bergeron, R., Doebber, T., and Zhang, B. B. (2005) Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *The Journal of clinical investigation* **115**, 1030-1038

33. Hunnicutt, J. W., Hardy, R. W., Williford, J., and McDonald, J. M. (1994) Saturated fatty acid-induced insulin resistance in rat adipocytes. *Diabetes* **43**, 540-545

34. Kennedy, A., Martinez, K., Chuang, C. C., LaPoint, K., and McIntosh, M. (2009) Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. *The Journal of nutrition* **139**, 1-4

35. Frigolet, M. E., and Gutierrez-Aguilar, R. (2017) The Role of the Novel Lipokine Palmitoleic Acid in Health and Disease. *Advances in nutrition* **8**, 173S-181S

36. Cao, H., Gerhold, K., Mayers, J. R., Wiest, M. M., Watkins, S. M., and Hotamisligil, G. S. (2008) Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* **134**, 933-944

37. Bolsoni-Lopes, A., Festuccia, W. T., Farias, T. S., Chimim, P., Torres-Leal, F. L., Derogis, P. B., de Andrade, P. B., Miyamoto, S., Lima, F. B., Curi, R., and Alonso-Vale, M. I. (2013) Palmitoleic acid (n-7) increases white adipocyte lipolysis and lipase content in a PPARalpha-dependent manner. *American journal of physiology. Endocrinology and metabolism* **305**, E1093-1102

38. Souza, C. O., Teixeira, A. A., Lima, E. A., Batatinha, H. A., Gomes, L. M., Carvalho-Silva, M., Mota, I. T., Streck, E. L., Hirabara, S. M., and Rosa Neto, J. C. (2014) Palmitoleic acid (n-7) attenuates the immunometabolic disturbances caused by a high-fat diet independently of PPARalpha. *Mediators of inflammation* **2014**, 582197

39. Merino, J., Sala-Vila, A., Plana, N., Girona, J., Vallve, J. C., Ibarretxe, D., Ros, E., Ferre, R., Heras, M., and Masana, L. (2016) Serum palmitoleate acts as a lipokine in subjects at high cardiometabolic risk. *Nutrition, metabolism, and cardiovascular diseases : NMCD* **26**, 261-267

40. Djousse, L., Weir, N. L., Hanson, N. Q., Tsai, M. Y., and Gaziano, J. M. (2012) Plasma phospholipid concentration of cis-palmitoleic acid and risk of heart failure. *Circulation.*
41. de Souza, C. O., Vannice, G. K., Rosa Neto, J. C., and Calder, P. C. (2018) Is Palmitoleic Acid a Plausible Nonpharmacological Strategy to Prevent or Control Chronic Metabolic and Inflammatory Disorders? *Molecular nutrition & food research* **62**

42. Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., and Smyth, G. K. (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic acids research* **43**, e47

43. Leek, J. T., Johnson, W. E., Parker, H. S., Jaffe, A. E., and Storey, J. D. (2012) The sva package for removing batch effects and other unwanted variation in high-throughput experiments. *Bioinformatics* **28**, 882-883

44. Suzuki, R., and Shimodaira, H. (2006) Pclust: an R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics* **22**, 1540-1542

45. Yeh, Y. S., Jheng, H. F., Iwase, M., Kim, M., Mohri, S., Kwon, J., Kawarasaki, S., Li, Y., Takahashi, H., Ara, T., Nomura, W., Kawada, T., and Goto, T. (2018) The Mevalonate Pathway Is Indispensable for Adipocyte Survival. *iScience* **9**, 175-191

46. Goto, T., Hirata, M., Aoki, Y., Iwase, M., Takahashi, H., Kim, M., Li, Y., Jheng, H. F., Nomura, W., Takahashi, N., Kim, C. S., Yu, R., Seno, S., Matsuda, H., Aizawa-Abe, M., Ebihara, K., Itoh, N., and Kawada, T. (2017) The hepatokine FGF21 is crucial for peroxisome proliferator-activated receptor-alpha agonist-induced amelioration of metabolic disorders in obese mice. *J Biol Chem* **292**, 9175-9190

47. Orlicky, D. J., and Schaack, J. (2001) Adenovirus transduction of 3T3-L1 cells. *Journal of lipid research* **42**, 460-466

48. Sakamoto, T., Takahashi, N., Sawaragi, Y., Naknukool, S., Yu, R., Goto, T., and Kawada, T. (2013) Inflammation induced by RAW macrophages suppresses UCP1 mRNA induction via ERK activation in 10T1/2 adipocytes. *American journal of physiology: Cell physiology* **304**, C729-738

49. Takahashi, H., Suzuki, H., Suda, K., Yamazaki, Y., Takino, A., Kim, Y. I., Goto, T., Iijima, Y., Aoki, K., Shibata, D., Takahashi, N., and Kawada, T. (2013) Long-chain free fatty acid profiling analysis by liquid chromatography-mass spectrometry in mouse treated with peroxisome proliferator-activated receptor alpha agonist. *Bioscience, biotechnology, and biochemistry* **77**, 2288-2293

**FOOTNOTES**

**Abbreviations**: AAV, adeno associated virus; AC, adenylate cyclase; ATGL, adipose triglyceride lipase; ATP, adenosine triphosphate; βAR, beta adrenergic receptor; BAT, brown adipose tissue; ChIP, chromatin immunoprecipitation; CRE, cAMP response element; CREB, cAMP response element binding protein; DGAT, diacylglycerol acyltransferase; eWAT, epididymal white adipose tissue; FA, fatty acid; FSK, forskolin; GPAT, Glycerol-3-phosphate acyltransferase; Gyk, glycerol kinase; HE,
hematoxylin and eosin; HSL, hormone sensitive lipase; Iso, isoproterenol; iWAT, inguinal white adipose tissue; KD, knockdown; OA, oleic acid; PA, palmitic acid; PDE, Phosphodiesterases; POA, palmitoleic acid; PKA, protein kinase A; SA, stearic acid; SCD, stearoyl CoA desaturase; shRNA, small hairpin RNA; SVF, stromal vascular fraction; TG, triglyceride; UCP1, uncoupling protein 1; WAT, white adipose tissue
Table 1. Thermogenesis related gene in BAT

| Gene  | Bmp7 | Bmp8b | Cebpb | Cebpd | Cidea |
|-------|------|-------|-------|-------|-------|
| Adrb3 |      |       |       |       |       |
| Ebf2  |      |       |       |       |       |
| Fgf21 |      |       |       |       |       |
| P2rx5 |      |       |       |       |       |
| Prdm16|      |       |       |       |       |

Table 2. Body and tissues weight, and plasma characteristics in Gyk knockdown mice

|                          | 23 °C          | 10 °C          |
|--------------------------|----------------|----------------|
|                          | shLacZ | shGyk | shLacZ | shGyk |
| Weight                   |         |       |        |       |
| Body weight (g)          | 26.8 ± 0.4 | 26.31 ± 0.5 | 26.29 ± 0.5 | 25.8 ± 0.55 |
| BAT (mg)                 | 47.5 ± 3.2 | 43.0 ± 2.0 | 46.1 ± 1.4 | 42.7 ± 1.7 |
| iWAT (mg)                | 216.6 ± 10.0 | 209.4 ± 12.3 | 176.4 ± 14.3 ** | 155.2 ± 12.8 ** |
| eWAT (mg)                | 302.8 ± 20.3 | 289.1 ± 18.8 | 263.2 ± 29.4 | 233.2 ± 17.9 |
| Liver (mg)               | 1312 ± 82   | 1226 ± 57   | 1217 ± 20   | 1143 ± 45   |
| Plasma characteristic    |         |       |        |       |
| TG (mg/dL)               | 115.1 ± 11.8 | 79.9 ± 8.9 * | 45.4 ± 2.9 ** | 50.0 ± 2.8 ** |
| Free FA (mEq/L)          | 0.34 ± 0.03 | 0.42 ± 0.04 | 0.16 ± 0.01 | 0.25 ± 0.05 |
| Glucose (mg/dL)          | 185.3 ± 13.7 | 197.8 ± 15.1 | 162.9 ± 6.3 | 165.4 ± 8.5 |

BAT, brown adipose tissue; iWAT, inguinal white adipose tissue; eWAT, epididymal white adipose tissue; TG, triglyceride; FA, fatty acid

#, P < 0.05 vs. shLacZ RT. *, P < 0.05; **, P < 0.01 RT vs CE.
**FIGURE LEGENDS**

**Fig. 1.** *Ucp1* and *Gyk* showed similar mRNA expression in adipose tissues and adipocyte cells

Mice were housed at 4 °C for 0–384 h. The iWAT was harvested for total RNA extraction. Heatmap- and clustering analyses of the selected genes (see Methods). Heatmap colors were normalized by Z-score based on their time-course gene expression profiles. The time points in each profile were measured in triplicate (n = 3) (A). The expression levels of *Ucp1* (left panel) and *Gyk* (right panel) in iWAT were analyzed by real-time PCR (B). Mice were housed at room temperature (23 °C) or at low temperature (4 °C) for 24 h. Expression levels of *Ucp1* (left panel) and *Gyk* (right panel) in the adipocyte fraction (AT) and the stromal vascular fraction (SVF) of the iWAT (C). D and E, Expression levels of *Ucp1* (left panel) and *Gyk* (right panel) in primary iWAT adipocytes at various stages of differentiation (D) or after 0.5 μM Iso treatment (E). Data are means ± SE (n = 3–5). *, P < 0.05; **, P < 0.01 vs. AT, 4 °C, 0 d, or 0 h.

**Fig. 2.** *Gyk* knockdown downregulated *Ucp1* expression in mice

Non-targeting (shLacZ) or Gyk-targeting (shGyk) shRNA were transfected to mouse iWAT. Mice with iWAT-specific knockdown were prepared with AAV. Mice were housed at room temperature (23 °C) or at low temperature (10 °C) for 24 h. Zsgreen protein fluorescence in the AAV vector. Left image was from eWAT and right image was from iWAT, respectively. Scale bars in panels represent 1 cm (A). *Gyk* and thermogenesis-related genes (*Ucp1*, *Cidea*, *Pgc1a*, and *Pparg*) expression levels were measured and normalized to 36B4 (B). UCP1 and COX4 protein expression levels were evaluated by western blotting. Cold-stimulated BAT samples were used for positive control (P.C.) (C). Histochemical analyses of iWAT were performed by hematoxylin and eosin (HE) or immunohistochemical (IHC) staining using anti-UCP1 antibody. Scale bars in panels represent 200 μm (D). Changes of rectal temperature and the area under the curve (AUC) calculated from rectal temperature curve in the cold tolerance test were shown (E). Data are means ± SE. (n = 8–12). *, P < 0.05; **P < 0.01 vs. shLacZ.

**Fig. 3.** *Gyk* knockdown downregulated *Ucp1* expression in primary iWAT adipocytes

Non-targeting (shLacZ) or Gyk-targeting (shGyk) shRNA were transfected to primary iWAT adipocytes. After 8 d differentiation, the cells were subjected to 0.5 μM isoproterenol (Iso) for 4 h. *Gyk*, *Ucp1*, and *Cidea* expression levels were measured and normalized to 36B4 (A). *Ucp1* promoter and cAMP-response element (CRE) activities were measured by luminometer (B). Phospho-HSL, HSL, phospho-CREB, and CREB expression levels were analyzed by western blot (C). CRE binding sites were located from -2514 to -2496 (CRE2) and from -140 to -122 (CRE4). CREs-related *Ucp1* promoter was analyzed by chromatin immunoprecipitation (ChIP) assay using CRE2 and CRE4 primers (D). E and F, Differentiated primary iWAT adipocytes were sequentially injected 500 nM oligomycin, 1 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), and a mixture of 15 μM antimycin A and 15 μM rotenone. Oxygen consumption rate (OCR) change determined by XF24 extracellular flux analyzer (E) and OCR related to basal respiration, proton leak, maximum respiration,
and ATP production were calculated (F). Data are means ± SE. (n = 4–6). *, P < 0.05; **, P < 0.01 vs. shLacZ Iso-. #, P < 0.05; ##, P < 0.01 vs. shLacZ Iso+.

**Fig. 4. Effects of Gyk knockdown were ameliorated by forskolin but not cAMP analog treatment**

Schematic illustration of β-adrenergic receptor (βAR) signaling linkage to Ucp1 (A). B and C, Gyk knockdown cells treated with 1-5 μM forskolin (FSK) (B) or 100-500 μM 8-Br-cAMP (C) for 4 h and Ucp1 expression levels were measured. D and E, Ucp1 promoter and CRE activities and phospho-CREB and total CREB protein expression levels in response to 1-5 μM FSK (D) or 100-500 μM 8-Br-cAMP (E). Effect of 0.5 μM Iso-induced cAMP levels were quantified (F). Effect of 0.5μM Iso-induced PDE activity was quantified (G). AC and β-actin expression levels were analyzed by western blot (H). AC, adenylate cyclase; PDE, phosphodiesterase; PKA, protein kinase A. Data are means ± SE. (n = 4–6). *, P < 0.05; **, P < 0.01 vs. shLacZ Iso-.

**Fig. 5. Monounsaturated fatty acids were increased by Gyk knockdown and attenuated isoproterenol-induced Ucp1 expression**

Glycerol (left panel) and free fatty acid (right panel) in supernatant were measured for cells with or without Gyk knockdown or 0.5 μM isoproterenol (Iso) treatment (A). Intracellular free fatty acids were measured by LC-MS (B). Data was indicated percent of the shLacZ Iso- group. Actual fatty acids concentrations are MA: 0.81, PA: 10, POA: 1.0, SA: 11, OA: 2.6, LA: 0.31 fmol/cell. Schematic illustration of biosynthesis pathway of unsaturated FAs (C). Scd1, Scd2, Scd3, and Scd4 expression levels were measured and shown as % relative to Scd1 of shLacZ Iso- group (D). Gyk knockdown cells were treated with 100 μM stearoyl CoA desaturase 1 (SCD1) inhibitor 1 h before 0.5 μM Iso treatment. Ucp1 expression levels were detected (E). Desaturation ratios (saturated FA [PA+SA] / monounsaturated FA [POA+OA]) were used to estimate SCD1 activity with 0.5 μM Iso treatment (F). Data are means ± SE. (n = 3–5). n.d, not detected; MA, myristic acid; PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid. *, P < 0.05; **, P < 0.01 vs. shLacZ Iso- vs. shGyk Iso- or shLacZ Iso+ vs. shGyk Iso+.  

**Fig. 6. DGAT and ATGL inhibitors downregulated Ucp1 expression**

Gyk knockdown cells were treated with 10 μM diacylglycerol acyltransferase (DGAT) inhibitor or 10 μM adipose triglyceride lipase (ATGL) inhibitor for 1 h before 0.5 μM isoproterenol (Iso) treatment. Ucp1 expression levels were measured (A). Ucp1 promoter (left panel) and CRE (right panel) activities were measured (B). Intracellular free fatty acids were quantified by LC-MS (C). Data was indicated percent of the Control group. Actual fatty acids concentrations are MA: 0.62, PA: 5.8, POA: 2.8, SA: 4.4, OA: 4.4, LA: 0.13 fmol/cell. MA, myristic acid; PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid. Data are means ± SE. (n = 3–5). *, P < 0.05; **, P < 0.01 vs. Cont.

**Fig. 7 Fatty acid treatment affected Ucp1 expression**
Primary iWAT adipocytes were treated independently with various fatty acids (400 μM) for 1 h before 0.5 μM isoproterenol (Iso) treatment. *Ucp1* expression levels were measured (A). *Ucp1* promoter and CRE activities were measured in response to dose dependent (50-200 μM) POA treatment (B). Effects of 400 μM POA on *Ucp1* expression were quantified in cells with or without 5 μM forskolin (FSK) (left panel) or 500 μM 8-Br-cAMP (right panel) treatment (C). Effect of 400 μM POA on PDE activity was quantified in cells with or without 0.5 μM Iso (D). Effects of 400 μM POA on intracellular free fatty acids were measured (E). Data was indicated percent of the Cont group. Actual fatty acids concentrations are MA: 0.87, PA: 4.5, POA: 1.7, SA: 0.59, OA: 1.2, LA: 0.052 fmol/cell. PDE, phosphodiesterase; MA, myristic acid; PA, palmitic acid; POA, palmitoleic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid. Data are means ± SE. (n = 3–5). *, P < 0.05; **, P < 0.01 vs. Cont or Cont Iso+.

**Fig. 8. Gyk regulates Ucp1 expression via the βAR-cAMP-CREB pathway**

In this study demonstrates that *Gyk* is an important gene for white adipocytes browning. *Gyk* KD upregulated *Scd1* expression and induced desaturation of saturated FAs by SCD1. Monounsaturated FAs, especially POA, accumulated in adipocytes downregulate *Ucp1* expression via attenuate βAR-cAMP-CREB pathway by increased PDE activity. AC, adenylate cyclase; βAR, beta adrenergic receptor; CREB, cAMP response element binding protein; Gyk, glycerol kinase; PA, palmitic acid; POA, palmitoleic acid; PDE, phosphodiesterase; PKA, protein kinase A; SCD, stearoyl CoA desaturase; UCP1, uncoupling protein 1.
Fig. 1

(A) Heatmap showing the expression levels of various genes over time. The color key indicates the range of expression levels. Gene names include: Ppargc1b, Stat5a, Bmp7, Ebf2, Cebpd, Pdgfra, Lep, Ehmt1, Sirt1, Prdm16, Adrb3, Hoxc8, Cebpb, Hoxc9, Pparg, Bmp8b, Esrrb, Fgf21, Esrrg, Slc27a1, Ebf2, Fabp4, Cebpb, Hoxc9, Ppargc1a, Ucp1, Ppara, Fndc5, Cidea, P2rx5.

(B) Graphs showing the relative expression of Ucp1 and Gyk over time. The x-axis represents time in hours, and the y-axis represents relative expression as a percentage of the initial expression.

(C) Bar graphs comparing the relative expression of Ucp1 and Gyk in adipocyte and SVF under different conditions: 23°C and 4°C. The black bars indicate the relative expression compared to the initial expression.

(D) Bar graphs showing the relative expression of Ucp1 and Gyk in adipocyte and SVF under different conditions: AF and SVF. The black bars indicate the relative expression compared to the initial expression.

(E) Graphs showing the relative expression of Ucp1 and Gyk over different time points. The x-axis represents time in days or hours, and the y-axis represents relative expression as a percentage of the initial expression.
Fig. 2

| Gene   | shLacZ 23°C | shGyk 23°C | shLacZ 10°C | shGyk 10°C |
|--------|-------------|------------|-------------|------------|
| Gyk    | ![](shLacZ_23°C) | ![](shGyk_23°C) | ![](shLacZ_10°C) | ![](shGyk_10°C) |
| Ucp1   | ![](shLacZ_23°C) | ![](shGyk_23°C) | ![](shLacZ_10°C) | ![](shGyk_10°C) |
| Cidea  | ![](shLacZ_23°C) | ![](shGyk_23°C) | ![](shLacZ_10°C) | ![](shGyk_10°C) |
| Pgc1a  | ![](shLacZ_23°C) | ![](shGyk_23°C) | ![](shLacZ_10°C) | ![](shGyk_10°C) |
| Pparg  | ![](shLacZ_23°C) | ![](shGyk_23°C) | ![](shLacZ_10°C) | ![](shGyk_10°C) |

**D**

* iWAT HE staining
  - 23°C
  - 10°C

* iWAT IHC (anti UCP1)
  - 23°C
  - 10°C

**E**

Rectal temperature change (hour)

- shLacZ
- shGyk

AUC (% of shLacZ)

* P = 0.06
Fig. 3
**Fig. 4**

A. βAR \( \rightarrow \) Isoproterenol

\[ \downarrow \]

AC \( \rightarrow \) Forskolin

\[ \downarrow \]

PKA \( \rightarrow \) 8Br-cAMP

\[ \downarrow \]

UCP1

B. Relative Ucp1 expression (% of shLacZ 0 µM)

| FSK (µM) | shLacZ | shGyk |
|----------|--------|--------|
| 0        |       | **     |
| 1        |       | **     |
| 5        |       |        |

C. Relative Ucp1 expression (% of shLacZ 0 µM)

| 0       | shLacZ | shGyk |
|---------|--------|--------|
| 0       | **     | **     |
| 100     |       |        |
| 500     |       |        |

D. Relative luciferase activity (% of shLacZ 0 µM)

| FSK (µM) | shLacZ | shGyk |
|----------|--------|--------|
| 0        | 100    | **     |
| 1        | 150    | **     |
| 5        | 200    |        |

| 8-Br-cAMP (µM) | shLacZ | shGyk |
|---------------|--------|--------|
| 0             | 50     | **     |
| 100           | 150    |        |
| 500           | 200    |        |

E. Relative luciferase activity (% of shLacZ 0 µM)

| Ucp1 promoter | CRE |
|---------------|-----|
| 0             | 100 |
| 1             | 150 |
| 5             | 200 |

| 8-Br-cAMP (µM) | Ucp1 promoter | CRE |
|---------------|---------------|-----|
| 0             | 50            | ** |
| 100           | 150           |    |
| 500           | 200           |    |

F. cAMP activity (% of shLacZ Iso-)

| shLacZ | shGyk |
|--------|--------|
| 0      | 150    |
| 100    | 200    |
| 500    | 250    |

G. PDE activity (% of shLacZ Iso-)

| shLacZ | shGyk |
|--------|--------|
| 0      | 150    |
| 100    | 200    |
| 500    | 250    |

H. βAR activity

| βAR | FSK - | FSK + | 8-Br-cAMP - | 8-Br-cAMP + |
|-----|-------|-------|-------------|-------------|
| 40 kDa | shLacZ | shGyk | shLacZ | shGyk |
| 50 kDa | shLacZ | shGyk | shLacZ | shGyk |
**Fig. 5**

**A**

Glycerol (mg/dL) and free fatty acid (mEq/L) levels with and without SCD1 inhibitor.

**B**

Relative fatty acid content (% of shLacZ).

**C**

Pathway of fatty acid metabolism:
- Myristic acid (C14:0) → Palmitic acid (C16:0) → Palmitoleic acid (C16:1) → Stearic acid (C18:0) → Oleic acid (C18:1) → SCD.

**D**

Relative gene expression (% of Scd1 shLacZ). SCD1 inhibitor levels.

**E**

Relative Ucp1 expression (% of shLacZ inhibitor). SCD1 inhibitor levels.

**F**

SFA/MUFA (precursor/product) ratio with and without SCD1 inhibitor.
Fig. 6
Fig. 7
Control adipocytes

Gyk knockdown adipocytes

noradrenaline

β-AR

AC

cAMP

PKA

Glycerol-3-P

Glycerol

CREB

CREB

UCP1 promoter

UCP1

CREB

CREB

UCP1

PA

POA

SCD1

Glycerol-3-P

Glycerol

AMP

PDE

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