# Central Administration of Sargahydroquinoic Acid Elevates Peripheral Thermogenic Signaling and Ameliorates High Fat Diet-Induced Obesity

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**Central administration of sargahydroquinoic acid elevates peripheral thermogenic signaling and ameliorates high fat diet-induced obesity**

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**Abstract**

*Sargassum serratifolium* (C.Agardh) C.Agardh, a marine brown alga, has been consumed as food and traditional medicine in Asia. A previous study showed that the meroterpenoid-rich fraction of an ethanolic extract of *Sargassum serratifolium* (MES) induced adipose tissue browning and suppressed diet-induced obesity and metabolic syndrome. Sargahydroquinoic acid (SHQA) is a major component in MES. However, it is unclear whether SHQA can regulate energy homeostasis through the central nervous system. To figure it out, SHQA was administrated through the third ventricle of the hypothalamus in high-fat diet-fed C57BL/6 mice and investigated its effects on energy homeostasis. Bath application of SHQA increases the intrinsic neuronal excitability of hypothalamic ARC neurons in acutely prepared brain slices. Thus, we further tested its effect on energy balance. Chronic administration of SHQA into the brain reduced body-weight without a change in food intake and improved metabolic syndrome-related phenotypes. Cold experiments and biochemical analyses indicate that SHQA elevates thermogenic signaling pathways evidenced by an increase in body temperature and UCP1 signaling in white and brown adipose tissues. As partial mechanisms, SHQA significantly elevated mRNA levels of genes associated with sympathetic outflow and GABA signaling pathways. Our data indicate that hypothalamic injection of SHQA elevates peripheral thermogenic signaling and ameliorates diet-induced obesity.

**Keywords:**sargahydroquinoic acid, hypothalamus, GABAergic neurons, thermogenesis

Obesity is a pandemic worldwide and arises from an extreme imbalance between energy intake and expenditure. Because obesity is generally related to a variety of metabolic disorders such as cardiovascular diseases, diabetes, hypertension, and fatty liver, countless attempts have been made to fight obesity [1]. Although various options can be chosen for anti-obese effects, pharmacotherapy is one of the popular ways to ameliorate severe obesity [2]. Orlistat is probably the only anti-obese drug approved for long-term use but the effect is mild and unfavorable side effects have been reported [3]. Also, many drugs for anti-obesity have been withdrawn from the market because of serious side effects [2]. Therefore, natural products and their-derived compounds have attracted attention to developing safer therapeutics for combating obesity.

Brown adipose tissue (BAT) is the main organ for maintaining body temperature by dissipating energy as heat. Similarly, conversion of white adipocytes to beige adipocytes, called the browning of white adipose tissue (WAT), has also been highlighted as a strategy to reduce obesity through elevating thermogenesis. BAT activation and adipocyte browning are induced by the direct effects of peripheral factors on adipocytes, such as thyroid prohormone thyroxine (T4), retinoblastoma interacting zinc finger protein homology domain containing 16 (PRDM16), chronic cold exposure, exercise, peroxisome proliferator-activated receptor γ (PPARγ) agonists, β3-adrenergic receptors, irisin, and norepinephrine [4,5]. Thermogenesis can also be regulated by the central nervous system (CNS). The hypothalamus can regulate the outflow signaling that drives the sympathetic nerve activity to white WAT and BAT, regulating energy homeostasis and heat generation [6]. Various signals associated with energy homeostasis can be sent to the hypothalamus and integrated for giving a response expressing orexigenic or anoresigenic peptides controlling the feeding behavior. Besides, the hypothalamus can regulate glucose and lipid metabolism in peripheral tissues via the autonomic nervous system, containing WAT browning and BAT activation [6-8]. Growing evidence supports that WAT

browning and BAT activation are attractive pharmaceutical mechanisms underlying the effects of anti-obesity and anti-metabolic syndrome associated with elevating energy expenditure [5,9,10].

*Sargassum serratifolium* (C.Agardh) C.Agardh, a marine brown alga belonging to the Sargassaceae family and mainly inhabits the Korean and Japanese coastal areas. Previous studies showed that *S. serratifolium* included a high level of meroterpenoid, including sargahydroquinoic acid (SHQA), sargachromenol, and sargaquinoic acid, of which SHQA is the most abundant compound [11-14]. A previous study reported that the meroterpenoid-rich fraction of an ethanolic extract of *S. serratifolium* (MES) inhibited diet-induced obesity and related metabolic syndrome [14]. MES supplementation notably decreased body weight and fatty liver and improved blood lipid profile without altering food intake in HFD-fed mice. Especially in adipose tissue of mice with MES supplementation, uncoupling protein 1 (UCP1)-positive cells, and related signaling were significantly elevated and macrophage infiltration reduced[14]. Another study examined the direct effect of SHQA, the most abundant compound in MES on adipocyte metabolism [15]. SHQA appears to activate lipid catabolic and adipocyte browning pathways presumably through activating PPARγ, PPARα, and AMPKα signaling [15]. Although MES supplementation was effective for weight loss in mice and direct treatment of SHQA on adipocyte elevated signaling pathways related to lipid catabolism [14,15], it is unclear whether SHQA can regulate energy homeostasis through CNS. To figure it out, we administrated SHQA through cannula into the third ventricle (3V) of the brain and investigated its effect on energy homeostasis in HFD-fed mice.

## Results

### Acute treatment of SHQA increases the neuronal excitability of hypothalamic ARC neurons.

We determined whether the acute treatment of SHQA affects the intrinsic neuronal excitability of hypothalamic ARC neurons. Toward this aim, ARC neurons on acutely prepared hypothalamic brain slices were whole-cell patched under a current-clamp configuration, and a change in the firing frequency was monitored. We found that ARC neurons continuously fired action potentials at high frequency even in the absence of any external stimulation (2.55± 0.42 Hz), and the treatment with DMSO vehicle did not affect the frequency of spontaneous firing (2.47 ± 0.45 Hz). However, when the hypothalamic slices were acutely treated with SHQA (4.3 ng/μL), the frequency of spontaneous firing was increased to 3.12 ± 0.36 Hz (Fig. 1), indicating that SHQA increases the intrinsic neuronal excitability of hypothalamic ARC neurons.

### Administration of SHQA into the 3V decreased HFD-induced obesity

SHQA is a major component in the meroterpenoid-rich fraction of an ethanolic extract of *Sargassum serratifolium* and the structure of isolated compound was idnetifed in figure 2A. Based on the beneficial effects of SHQA on neuronal excitability, we further tested whether hypothalamic SHQA injection regulates energy balance. To examine the central administration of SHQA is effective on the regulation of energy balance, C57BL/6 mice were fed anHFD (60% of calorie as fat) with or without the central injection of SHQA at low and high dose (20ng/μl and 100ng/μl, respectively), and body weight and food intake were monitored. As expected, HFD notably increased body weight but SHQA administration especially at a high dose significantly inhibited body weight gain (Fig.2B). Consistently, SHQA decreased the weights of fat depots at different sites (Fig.2E), indicating that the central administration of SHQA is

effective to reduce HFD-induced weight gain. To test whether the SHQA-mediated change in the energy balance is due to an alteration in feeding behavior, we measured food intake at various time points in normal diet condition (ND diet fed). Although SHQA at a high dose slightly reduced food intake at 2 h and 4 h, no apparent changes were observed during 24 h (Fig. 2C). Consistently, food intake (7 d) was unaltered among groups at 1 m and 2 m of age (Fig. 2D), suggesting that SHQA uncontrol food intake signaling in CNS.

**Administration of SHQA into the 3V control energy expenditure**

Because the SHQA decreased body weight without altering food intake, we hypothesized that hypothalamic SHQA may elevate energy expenditure. To determine this, indirect calorimetry was performed during the light and dark cycles using mice fed a chow diet or HFD with or without SHQA injection. The data showed that the SHQA injection at a high concentration elevated metabolic rate during the light cycle (Fig. 3A and B). To further test whether the increase in metabolic rate by the SHQA-injected group is associated with the elevation of thermogenic energy expenditure, a cold challenge test was performed. The data exhibited that the rectal body temperature was higher in the mice injected with SHQA at a high concentration than HFD-fed mice in a cold chamber (4℃) (Fig. 3C), indicating that the hypothalamic SHQA-mediated thermogenesis may contribute to the increase in energy expenditure.

**Hypothalamic administration of SHQA ameliorates blood metabolic parameters**

We tested whether the SHQA-induced bodyweight reduction is related to the improvement of metabolic parameters by measuring blood glucose and lipid profile. The glucose tolerance test showed that the HFD-mediated increase in the level of blood glucose was significantly decreased by SHQA injection at a high dose (Fig.4A). Although there are no clear effects of

SHQA on free fatty acid (FFA) levels in the blood, possibly due to no dramatic increase of FFA by HFD feeding, the levels of triglycerides and total cholesterols were reduced by the SHQA injection (Fig. 4B–D). These data indicate that the body weight-lowering effect of SHQA is related to the amelioration of some metabolic parameters.

**Hypothalamic administration of SHQA elevates thermogenesis signals**

Oral MES supplementation improved diet-induced obesity and metabolic syndrome partially by elevating signals associated with lipid catabolism including lipolysis, mitochondria biogenesis, and UCP1-positive cells in adipose tissues [14]. We investigated using qPCR whether the hypothalamic injection of SHQA can control genes related to lipid catabolism including thermogenic signaling pathways in WAT and BAT.In BAT of chow and HFD-fed mice, no significant changes were observed in the mRNA levels of genes associated with BAT activation and functions such as UCP1, UCP2, peroxisome proliferator-activated receptor gamma co-activator 1 (PGC1α), and PR-domain containing 16 (PRDM16), lipid catabolism such as carnitine palmitoyltransferase I (CPT1), mitochondrial transcription factor A (TFAM), acetyl-CoA carboxylase (ACC), and lipid metabolism-related transcription factor including peroxisome proliferator-activated receptorα (PPARα) and PPARγ (Fig.5). Although SHQA injection at the low concentration did not show significant effects, the high concentration significantly upregulated the mRNA levels of UCP1, PGC1α, and PPARγ in BAT (Fig. 5A). Consistently, SHQA at the high dose also elevated mRNA levels of these genes in WAT (Fig. 5B), suggesting that the hypothalamic injection of SHQA stimulates thermogenic signaling cascade in adipose tissues.

**SHQA broadly alters the mRNA expression levels of genes related to sympathetic outflow**

**and GABA signaling pathways**

The hypothalamus is the primary central place integrating the outflow signals that induce the sympathetic nerve activity to BAT and WAT, stimulating thermogenesis, and regulating energy homeostasis. To investigate potential mechanisms underlying the hypothalamic SHQA injection-mediated stimulation of thermogenesis, we measured mRNA expression levels of several genes including CART, LepRb, LepRb2, PGC1α, CRHR1, THR, HCRTR1, HCRT2, PTPN2, BDNF, GABRA1, GABRA2, GABRB1, GABRB2, GABRG1, GABRG2, and VGAT associated with sympathetic nerve activity and thermogenesis in the hypothalamus. Of these genes, mRNA levels of CRHR1 and THR were most notably increased by a hypothalamic SHQA injection at a high concentration (Fig. 6A), indicating that SHQA may stimulate neural signaling pathways involved in energy homeostasis mediated by corticotropin releasing factor and thyroid hormone. SHQA also significantly up-regulated mRNA levels of BDNF, GABRA2, GABRB1, and GABRG2 in the hypothalamus (Fig. 6B). Because BDNF has been shown to boost sympathetic outflow and GABAergic signaling acting on the paraventricular nucleus of the hypothalamus can induce thermogenesis, SHQA-stimulated BDNF up-regulation and GABA-related signaling may contribute to the elevation of peripheral thermogenic signaling.

## Discussion

Our previous study showed that MES induced thermogenic signaling in adipose tissues and improved diet-induced obesity and metabolic syndrome when supplemented with an HF diet [14]. The chemical composition analysis suggests that SHQA is a primary compound in MES [14]. Further *in vitro* study indicated that SHQA treatment elevated mitochondria numbers and thermogenic signaling pathways in adipocytes. However, it was questionable whether SHQA works on the central nervous system to regulate energy balance. Based on the current study, it is quite clear that the hypothalamic injection (3V) of SHQA significantly improved HF-diet induced body weight gain and metabolic profile partly due to the increase in thermogenic signaling in adipose tissues. The underlying mechanisms include but not limited to the broad up-regulation of genes associated with sympathetic outflow and GABA-related signaling pathways.

Boosting sympathetic outflow is important to integrate signaling from the central nervous system and adipose thermogenesis [16]. Several factors in the hypothalamus have been reported to stimulate the activation of BAT and WAT browning. It has been reported that BDNF neurons in the medial and posterior paraventricular nucleus of the hypothalamus stimulate thermogenesis through the release of BDNF into the spinal cord to elevate sympathetic outflow [17]. Also, corticotropin-releasing factors that act at hypothalamic corticotropin-releasing factor receptors are related to regulating energy balance.

The central injection of corticotropin-releasing factor or corticotropin-releasing factor microinjection into the preoptic area or the dorsomedial hypothalamus stimulated the sympathetic nerve activity and temperature of BAT [18]. Thyroid hormones have also been shown to boost the sympathetic nervous system and stimulate thermogenesis in BAT and WAT browning partly by suppressing AMP-activated protein kinase (AMPK) in the ventromedial

nucleus of the hypothalamus [6]. Our study showed that SHQA significantly up-regulated hypothalamic CRHR1, THR, and BDNF. Although thermogenic mechanisms mediated by each gene may be different, these genes are closely associated with the stimulation of sympathetic outflow. We assumed that SHQA may stimulate sympathetic nerves in the hypothalamus for BAT activation and WAT browning although more detailed experiments are necessary.

Hypothalamic GABA signaling is important for thermogenesis and energy expenditure [19]. Hypothalamic injections (the preoptic area) of GABA or a GABA receptor agonist elevated the rate of energy expenditure and body core temperature of urethane-chloralose-anesthetized, artificially ventilated rats [19]. In the same study, the author proved that the GABA-mediated thermogenic energy expenditure was accompanied by a tachycardic response and electromyographic activity recorded from the femoral or neck muscles [19]. It appears that GABA signaling in the preoptic area of the hypothalamus mediates cold-induced thermogenesis. Hypothalamic injection of SHQA significantly up-regulated mRNA levels of GABRA2, GABRB1, and GABRG2 in the hypothalamus. We speculate that SHQA may also regulate hypothalamic GABA signaling for activating peripheral thermogenesis. It is necessary to further study whether SHQA controls a tachycardic response and electromyographic activity related to GABA signaling pathways in the hypothalamus.

The roles of AMPK in thermogenic energy expenditure are different between the hypothalamus and adipose tissues. A study using adipose tissue-specific AMPK knockout mice showed that AMPK is necessary for WAT browning and thermogenic energy expenditure [20], whereas the activation of AMPK in the hypothalamus elevates food intake and reduce adaptive thermogenesis in adipose tissues to decrease energy expenditure in response to food deprivation [21]. Our previous study showed that SHQA treatment activates AMPK and lipid catabolic pathways including thermogenic signaling in 3T3-L1 adipocytes [15]. In the current

study, the hypothalamic injection of SHQA in HFD-fed mice elevated peripheral thermogenic signaling and body temperature, thereby preventing diet-induced obesity. Although not directly tested here, we assume that the effects of SHQA on hypothalamic AMPK may not be strong to reduce thermogenesis unlike its effects on adipose tissues or that other thermogenic signaling pathways in the hypothalamus induced by SHQA may be stronger than its effect on AMPK.

In conclusion, our data support that SHQA works on the hypothalamus to induce peripheral thermogenesis and prevent HFD-induced obesity. The underlying mechanisms include but are not limited to SHQA-mediated alteration in the mRNA expression of genes associated with the sympathetic outflow and GABA signaling pathways. Thus, SHQA may be applied to a pharmaceutical compound that prevents obesity and obesity-related metabolic disorders.

## Methods

### Animals

Wild-type C57BL/6 male mice (4 weeks old, n=27) were obtained from DBL Inc. through the Saeron Bio Inc. (Uiwang, Republic of Korea). The animals were housed in a room with temperature of 23 ± 1 ℃, a humidity of 50 ± 10%, a 12 h light/dark cycle, and freely accessed to food and water. Following arrival, mice were acclimatized to the KIST animal room for one week. After one week for adaptation, mice were randomly divided into four groups (n=6~7); (1) Control group: mice for normal diet (ND) feeding and PBS treatments (injected with a guided cannular into third-ventricle), (2) HFD + Vehicle group: mice for 60% high fat-diet (HFD) and PBS treatments (injected with a guided cannular into third-ventricle), (3) HFD + SHQA-low group: mice for 60% high fat-diet (HFD) and 20 ng/μl SHQA (low) treatments (injected with a guided cannular into third-ventricle) and (4) HFD + SHQA-high group: mice for 60% high fat-diet (HFD) and 100 ng/μl SHQA (high) treatments (injected with a guided cannular into third-ventricle). The Institutional Animal Care and Use Committee (IACUC) and the Institutional Biosafety Committee (IBC) at the Korea Institute of Science and Technology (KIST) approved all the procedures (Approval number, KIST-2019-048). All experiments were performed in accordance with relevant guidelines and regulation of the IACUC and the IBC in KIST. A statement confirming the study was carried out in compliance with the ARRIVE guidelines.

### Cannulation of and chronic injection into the hypothalamic third ventricle

The hypothalamic third ventricle was cannulated as described [22,23]. The ultraprecise small animal stereotactic apparatus (Kopf Instruments) is used to implant a 26-gauge guide cannula (Plastics One) at the midline coordinates of 2.0 mm posterior to the bregma and 5.0 mm below the bregma. PBS, as a vehicle, and SHQA (20 or 100 ng/μl, three times a week) were injected

via a cannula into the third-ventricle of the brain for three month.

**Metabolic and energy expenditure analyses**

To measure metabolic rates, metabolic parameters in the treated mice were analyzed with indirect calorimetry [24,25] using Comprehensive Laboratory Animal Monitoring System (CLAMS) system (Columbus Instruments, OH, USA) at normal temperature (22℃) with 12 hours light/dark cycles. The mice were individually housed 2~3 days prior to the experiment for habituation and then fed the same diet and water provided *ad libitum* in clear respiratory chambers (20.5 × × 10.5 × 12.5 cm). The experimental animals of $O_2$ consumption, $CO_2$ production, energy expenditure, heat and activities were measured for 24 hours. These data were collected to Oxymax for windows (version 5.40.14, Columbus Instruments, OH, USA), and analyzed with CLAX software (version 2.2.15, Columbus Instruments, OH, USA). Based on collected data, respiratory quotient or exchange ratio (CO2/O2) and delta-heat values were calculated by CLAX software.

**Body temperature measurements**

Using rectal thermometry is a common method for measuring body temperature in mice [24,26]. After central treatments of Veh, SHQA/L and SHQA/H for 3 months, we put the mice in a 4℃ cold room for two days. Decrease of mice's body temperatures in a cold room were measured every 0, 0.5, 1, 2, 3, 5, 7, 9, 26, 28, 30, 32 and 34 hours by inserting a small-diameter temperature probe the anus (Thermometer DT-610B, CEM). The insertion depth of > 2cm was conducted into mice in a cold room with 12 hr light/dark cycle, and free access to food and water.

**Electrophysiology**

C57BL/6 mice (male, 4-weeks-old) were anesthetized, and the brain was rapidly dissected out. Acute coronal brain slices were prepared in an ice-cold cutting buffer (in mM: 234 sucrose, 2.5 KCl, 1.25 $NaH_2PO_4$, 24 $NaHCO_3$, 11 glucose, 0.5 $CaCl_2$, 10 $MgSO_4$, saturated with 95% $O_2$ and 5% $CO_2$) with the 300-μm thickness on a vibratome (Leica, VT1000S). Subsequently, the brain slices were recovered in a recovery artificial cerebrospinal fluid (aCSF) containing (in mM) 124 NaCl, 3 KCl, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 10 glucose, 6.5 $MgSO_4$, 1 $CaCl_2$, saturated with 95% $O_2$ and 5% $CO_2$ at 35 °C, and maintained at an ambient temperature thereafter. The brain slices containing the hyphothalamic region were used for electrophysiology experiments in a recording aCSF solution containing (in mM) 124 NaCl, 3 KCl, 1.25 $NaH_2PO_4$, 26 $NaHCO_3$, 10 glucose, 1.3 $MgSO_4$, 2.5 $CaCl_2$, saturated with 95% $O_2$ and 5% $CO_2$ at 37 °C. Hypothalamic ARC neurons were whole-cell patched under a current-clamp configuration with the internal solution containing (in mM)130 K-gluconate, 10 KCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.5 GTP-Na2, 10 phosphocreatine-$Na_2$ (pH = 7.25 and osmolality = 290 mOsm), and a change in the spontaneous firing frequency was monitored before and after the application of DMSO or SHQA (4.3 ng/μl).

**Biochemical assays**

For glucose tolerance test, we intraperitoneally injected 10 ml glucose per kg of body weight into experimental mice after 8 hour fasting. The blood glucose was measured from mice's tails at 0, 15, 30, 60, and 120 min after the glucose injection. The plasma triglyceride and total cholesterol were measured using pharmaceutical enzymatic kits according to the manufacter's instructions (Asan Pharm, Seoul, South Korea). Free fatty acids in plasma were asseseed with acyl-CoA synthetase–acyl-CoA oxidase (ACS-ACOD) with the NEFA-HR (non-esterified

fatty acids) reagent according to the manufacter's instruction Wako, Tokyo, Japan).

**Total RNA isolation, cDNA synthesis, and quantitative real-time PCR**

The total RNA was extracted from the brown adipose tissue, white adipose tissue, and hypothalamus using Trizol reagent (Invitrogen Life Technologies, USA) according to the manufacturer's instructions. For cDNA synthesis, SuperScript®III First-Strand Synthesis System for RT-PCR Kit (Invitrogen Life Technologies, USA) was used to reverse-transcribe total RNA (5 μg). cDNA expression was quantified by real-time PCR using the PowerSYBR®Green PCR Master Mix (Appliedbiosystems) on the QuantStudio™3 Real-Time PCR System (Appliedbiosystems, USA), and primers with the following sequences: *Ucp1*, 5'-AGGCTTCCAGTACCATTAGGT-3' and 5'-CTGAGTGAGGCAAAGCTGATTT-3'; *Pgc1a*, 5'-TATGGAGTGACATAGAGTGTGCT-3' and 5'-CCACTTCAATCCACCCAGAAAG-3'; *Prdm16*, 5'-CCAAGGCAAGGGCGAAGAA-3' and 5'-AGTCTGGTGGGATTGGAATGT-3'; *Ppara*, 5'-AGAGCCCCATCTGTCCTCTC-3' and 5'-ACTGGTAGTCTGCAAAACCAAA-3'; *Pparg*, 5'-TCGCTGATGCACTGCCTATG-3' and 5'-GAGAGGTCCACAGAGCTGATT-3'; *Ucp2*, 5'-ATGGTTGGTTTCAAGGCCACA-3' and 5'-CGGTATCCAGAGGGAAAGTGAT-3'; *Cpt1*, 5'-GCACACCAGGCAGTAGCTTT-3' and 5'-CAGGAGTTGATTCCAGACAGGTA-3'; *Tfam*, 5'-ATTCCGAAGTGTTTTTCCAGCA-3' and 5'-TCTGAAAGTTTTGCATCTGGGT-3'; *Acc*, 5'-CCTTTGGCAACAAGCAAGGTA-3' and 5'-AGTCGTACACATAGGTGGTCC-3'; *lepRb*, 5'-TGGTCCCAGCAGCTATGGT-3' and 5'-ACCCAGAGAAGTTAGCACTGT-3'; *lepRb2*, 5'-GTCTTCGGGGATGTGAATGTC-3' and 5'-ACCTAAGGGTGGATCGGGTTT-3'; *Crhr1*, 5'-GGAACCTCATCTCGGCTTTCA-3' and 5'-GTTACGTGGAAGTAGTTGTAGGC-3'; *Thr*, 5'-GAACAGCTCAAGAATGGTGGC-3' and

5’-GAATCGAACTCTGCACTTCTCTC-3’; *Hcrtr1*, 5’-GAGGATTCCCTCTCTCGTCG-3’ and 5’-GGTGTAGGTATTCCCTCCACA-3’; *Hcrtr2*, 5’-GAGGATTCCCTCTCTCGTCG-3’ and 5’-GGTGTAGGTATTCCCTCCACA-3’; *Ptpn2*, 5’-CAGCAACTACTGAAAGAAGCCC-3’ and 5’-AGGATAGATTTTGTCGGCCTTG-3’; *Bdnf*, 5’-TCATACTTCGGTTGCATGAAGG-3’ and 5’-AGACCTCTCGAACCTGCCC-3’; *Gabra1*, 5’-AAAAGTCGGGGTCTCTCTGAC-3’ and 5’-CAGTCGGTCCAAAATTCTTGTGA-3’; *Gabra2*, 5’-GGACCCAGTCAGGTTGGTG-3’ and 5’-TCCTGGTCTAAGCCGATTATCAT-3’; *Gabrb1*, 5’-TCCCGTGATGGTTGCTATGG-3’ and 5’-CCGCAAGCGAATGTCATATCC-3’; *Gabrb2,* 5’-ATGTCGCTGGTTAAAGAGACG-3’ and 5’-CTGCCACTCGGTTGTCCAAA-3’; *Gabrg*1, 5’-GAAGCTGAAAAACAAGACTTCGG-3’ and 5’-ATGCTGTTCATGGGAATGAGAG-3’; *Gabrg2*, 5’-AGAAAAACCCTCTTCTTCGGATG-3’ and 5’-GTGGCATTGTTCATTTGAATGGT-3’; *Vgat*, 5’-ACCTCCGTGTCCAACAAGTC-3’ and 5’-CAAAGTCGAGATCGTCGCAGT-3’ *Actb*, 5'-GGCTGTATTCCCCTCCATCG-3' and 5'-CCAGTTGGTAACAATGCCATGT-3'. PCR results were normalized to those of the control genes encoding β-actin (*Actb*).

**Statistical analysis**

Experimental values were shown as mean ± standard error of the mean (S.E.M.) and evaluated with one-way ANOVA by Tukey’s test. The statistical analysis was performed using the GraphPad PRISM software (GraphPad Prism Software Inc., version 8, CA, USA). P-values of $<0.05$ were deemed significant.

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**Author contriibution**

D.K. co-designed and performed mice surgery, chemical injection, energy expenditure, biochemical assay and qPCR; H.K. isolated and identified SHQA from *Sargassum serratifolium*; Y.L. performed initial experiemnts of extracts from *Sargassum serratifolium*; H.H. and H.R. performed electrophysiology and data analysis; C.C., B.L.and M.S.K. performed data analysis and wrote the paper; B.L. and M.S.K. conceived of the hypothesis and designed the project; and all authors participated in discussions.

**Competing financial interest**

The authors declare no competing financial interests.

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**Figure legends**

**Figure 1. SHQA increases the frequency of spontaneous firing in ARC neurons.**

Hypothalamic ARC neurons were whole-cell patched under a current-clamp configuration and a change in the spontaneous firing frequency was monitored before and after the application of DMSO or SHQA (4.3 ng/μl). Data are expressed as mean ± S.E.M. (n=5). Statistical differences between no treatment (NT), DMSO, and SHQA were determined using one-way ANOVA followed by the Tukey test: *p<0.05.

**Figure 2. Hypothalamic SHQA injection ameliorates HFD-induced obesity.**

(A) The chemical structure of SHQA. (B) Mice were fed each diet shown in the figure with or without the hypothalamic injection of SHQA for 12 weeks. Body weight was measured every week. (C) Accumulated 24 hours - food intake of normal diets (ND) and (D) chronic (2 month) food intake were measured. (E) Adipose tissue weight was measured at the end of the experiment. Data are expressed as mean ± S.E.M.(n=6–7). Statistical differences between CON, HFD+Veh, HFD+SHQA/L (SHQA, 20ng/μl), and HFD+SHQA/H (SHQA, 100ng/μl) groups were determined using one-way ANOVA followed by the Tukey test: *p<0.05, ** p<0.01, *** p<0.001.

**Figure 3. Hypothalamic injection of SHQA elevates energy expenditure and thermogenesis.**

Indirect calorimetry was applied to measure the energy expenditure of the mice fed a chow diet or HFD with or without SHQA injection during the light and dark cycle. (A) 24 h metabolic

rate (kcal/kg/h) was automatically calculated by the machine based on oxygen consumption and carbon dioxide production of mice. (B) The values of the metabolic rate were averaged and converted to the bar graphs. (C) To investigate adaptive thermogenesis, a rectal thermometer was applied to mice fed a chow diet or HFD with or without SHQA injection and the temperature was recorded in a cold room at 4°C for 34 h. Data are expressed as mean ± S.E.M. (n=6–7). Statistical differences between CON, HFD+Veh, HFD+SHQA/L (SHQA, 20ng/μl), and HFD+SHQA/H (SHQA, 100ng/μl) were determined using one-way ANOVA followed by the Tukey test: *p<0.05, *** p<0.001.

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(A) A glucose tolerance test was performed using 12 wk-old mice fed a chow diet or HFD with or without SHQA injection. (B) The area under the curve was calculated using the values from (A). (B) Total triglycerides, (C) Total cholesterols, and (D) fatty acids in the blood were determined using commercially available kits. Data are expressed as mean ± S.E.M.(n=6–7). Statistical differences between CON, HFD+Veh, HFD+SHQA/L (SHQA, 20ng/μl), and HFD+SHQA/H (SHQA, 100ng/μl) were determined using one-way ANOVA followed by the Tukey test: *p<0.05, *** p<0.001.

**Figure 5. SHQA injection alters gene expression signature in white and brown adipose tissues of mice.**

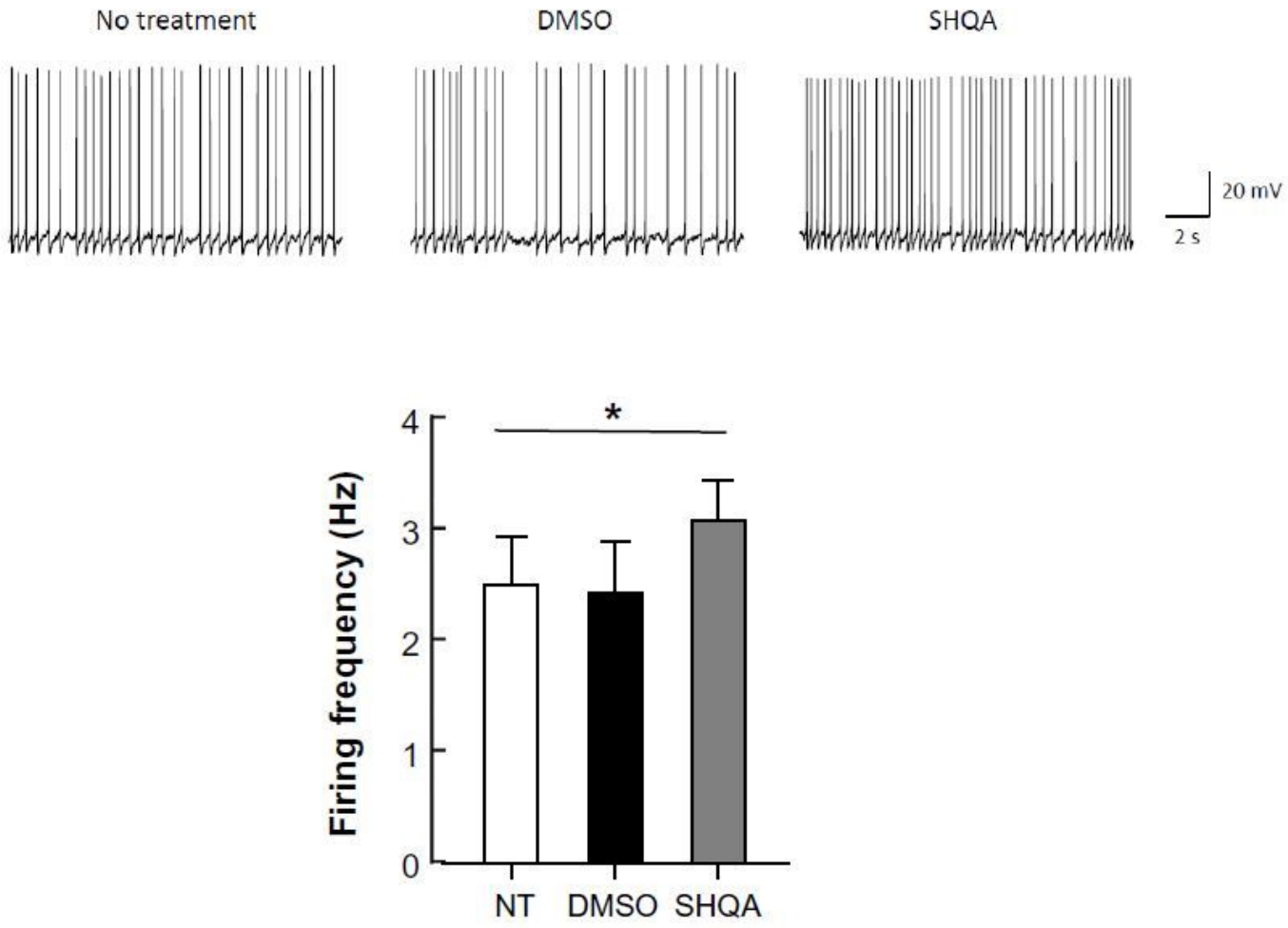
mRNA expression levels of genes related to adipose tissue browning and thermogenesis were measured in (A) brown adipose tissues and (B) subcutaneous white adipose tissue of 12 wk-

old mice fed a chow diet or HFD with or without SHQA injection. Data are expressed as mean ± S.E.M. (n=6–7). Statistical differences between CON, HFD+Veh, HFD+SHQA/L (SHQA, 20ng/μl), and HFD+SHQA/H (SHQA, 100ng/μl) were determined using one-way ANOVA followed by the Tukey test: *p<0.05, ** p<0.01.

**Figure 6. SHQA injection alters gene expression profile in the hypothalamus.**

mRNA expression levels of genes related to (A) sympathetic outflow and (B) GABAnergic signaling pathways were measured in the hypothalamus of 12 wk-old mice fed a chow diet or HFD with or without SHQA injection. Data are expressed as mean ± S.E.M. (n=6–7). Statistical differences between CON, HFD+Veh, HFD+SHQA/L, and HFD+SHQA/H were determined using one-way ANOVA followed by the Tukey test: *p<0.05, *** p<0.001.

# Figures

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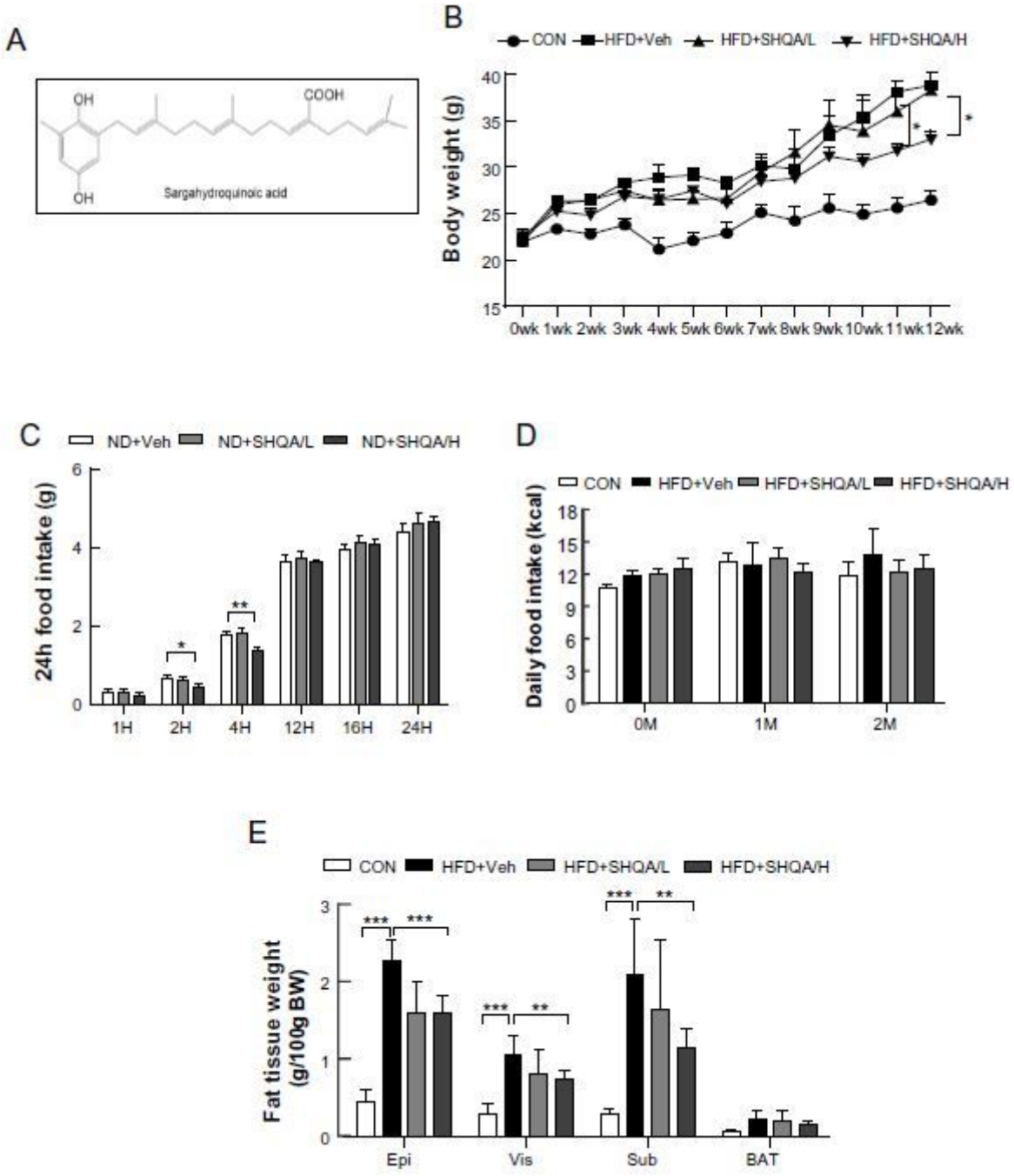


## Figure 2

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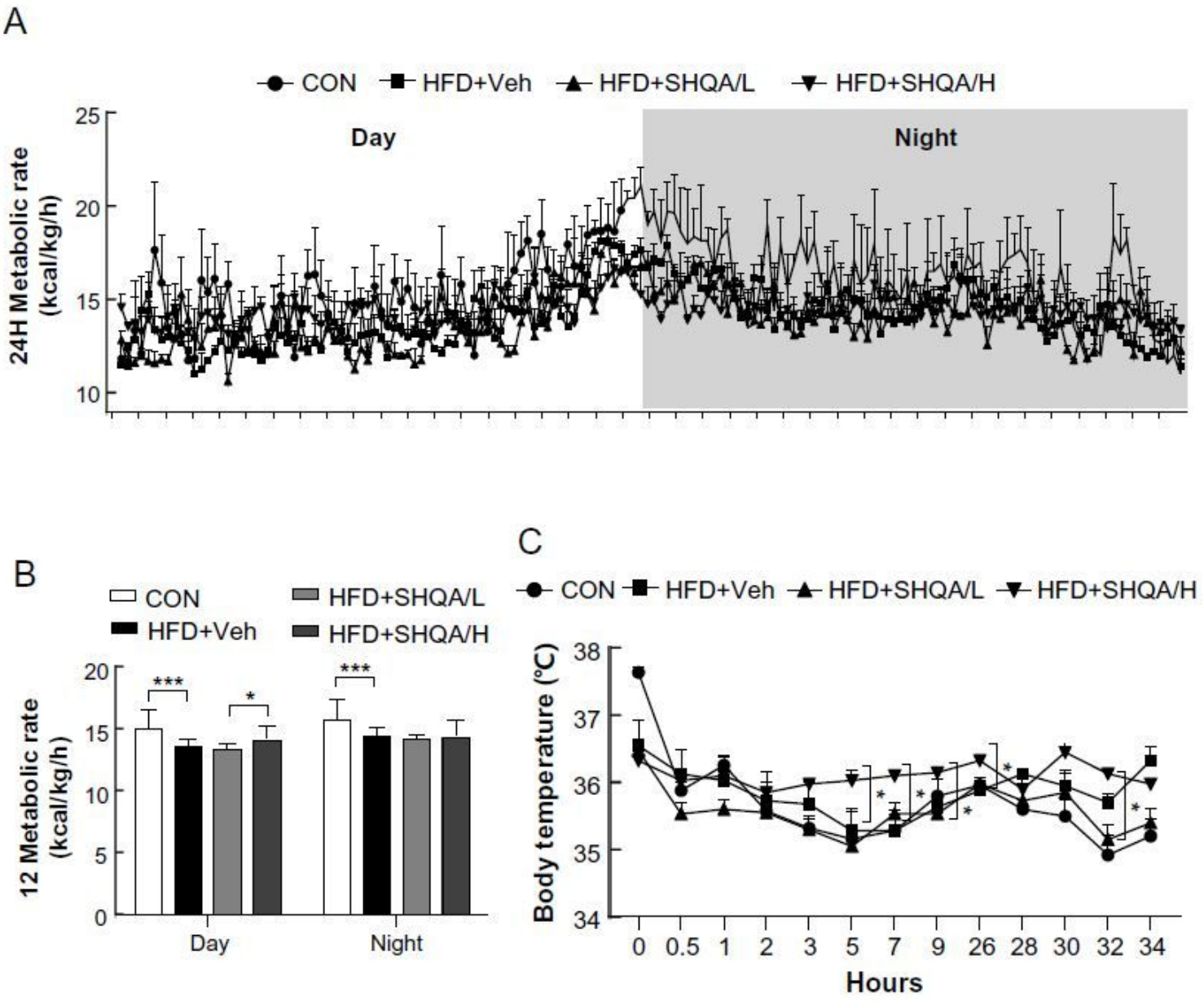


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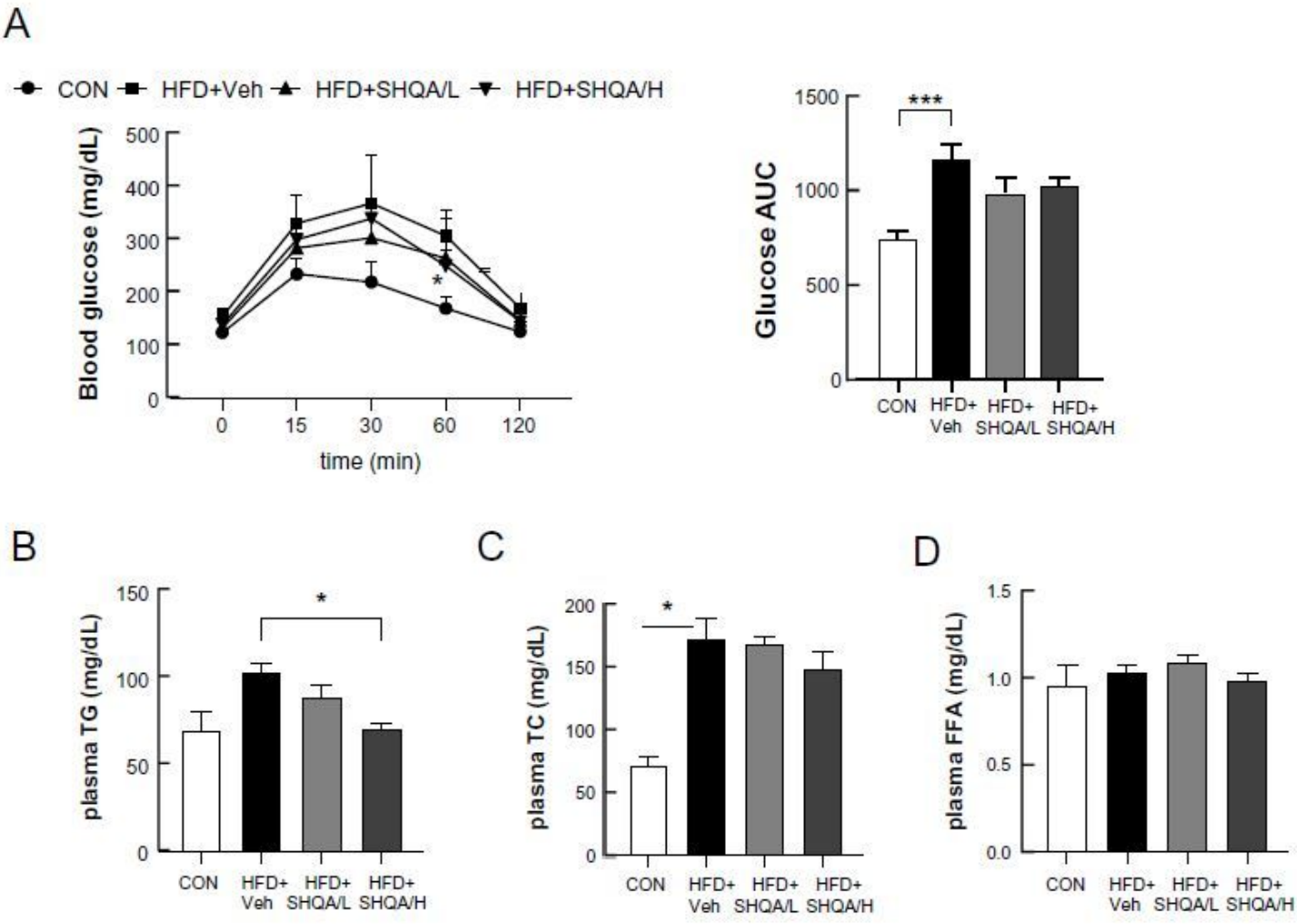


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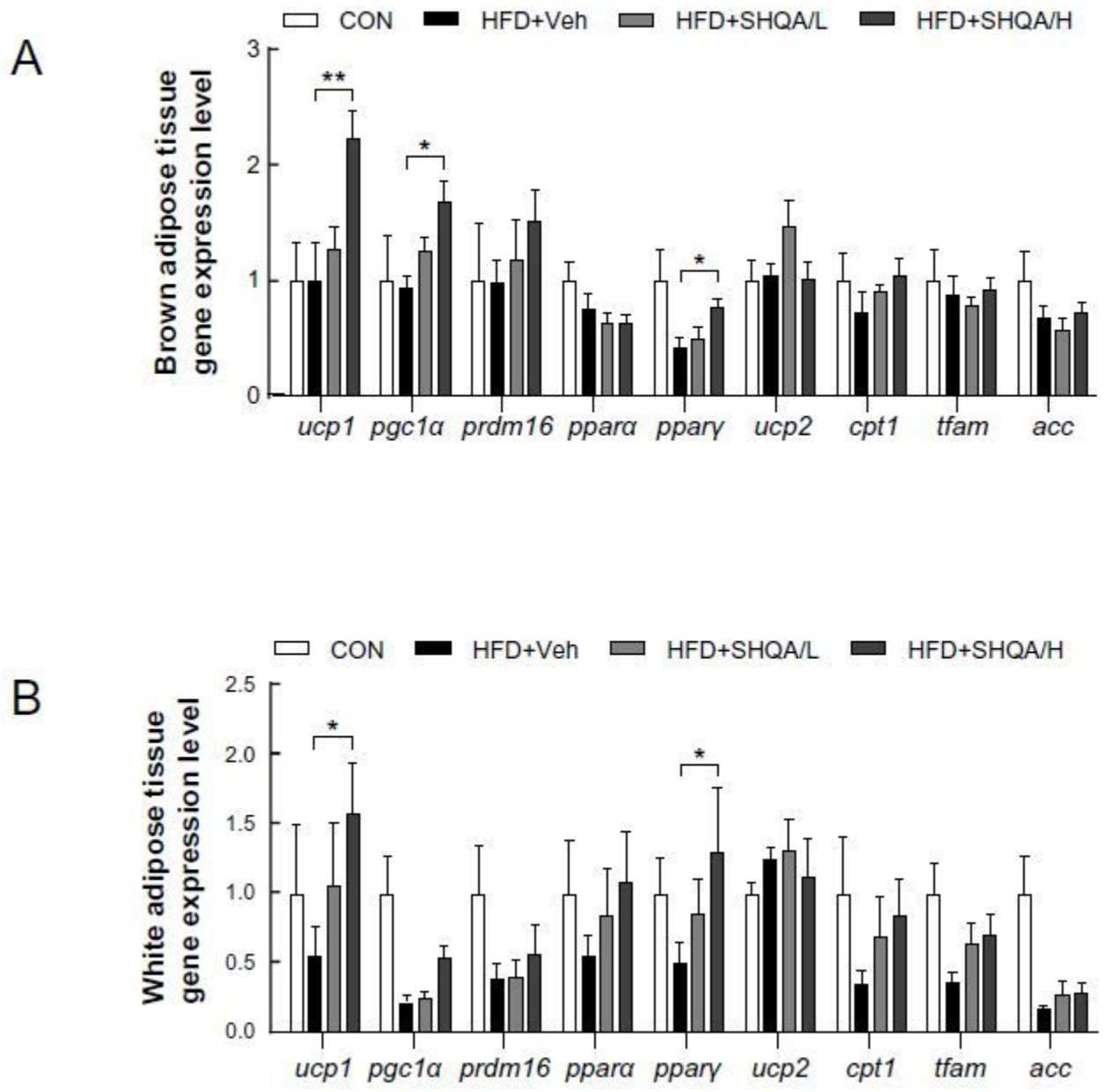


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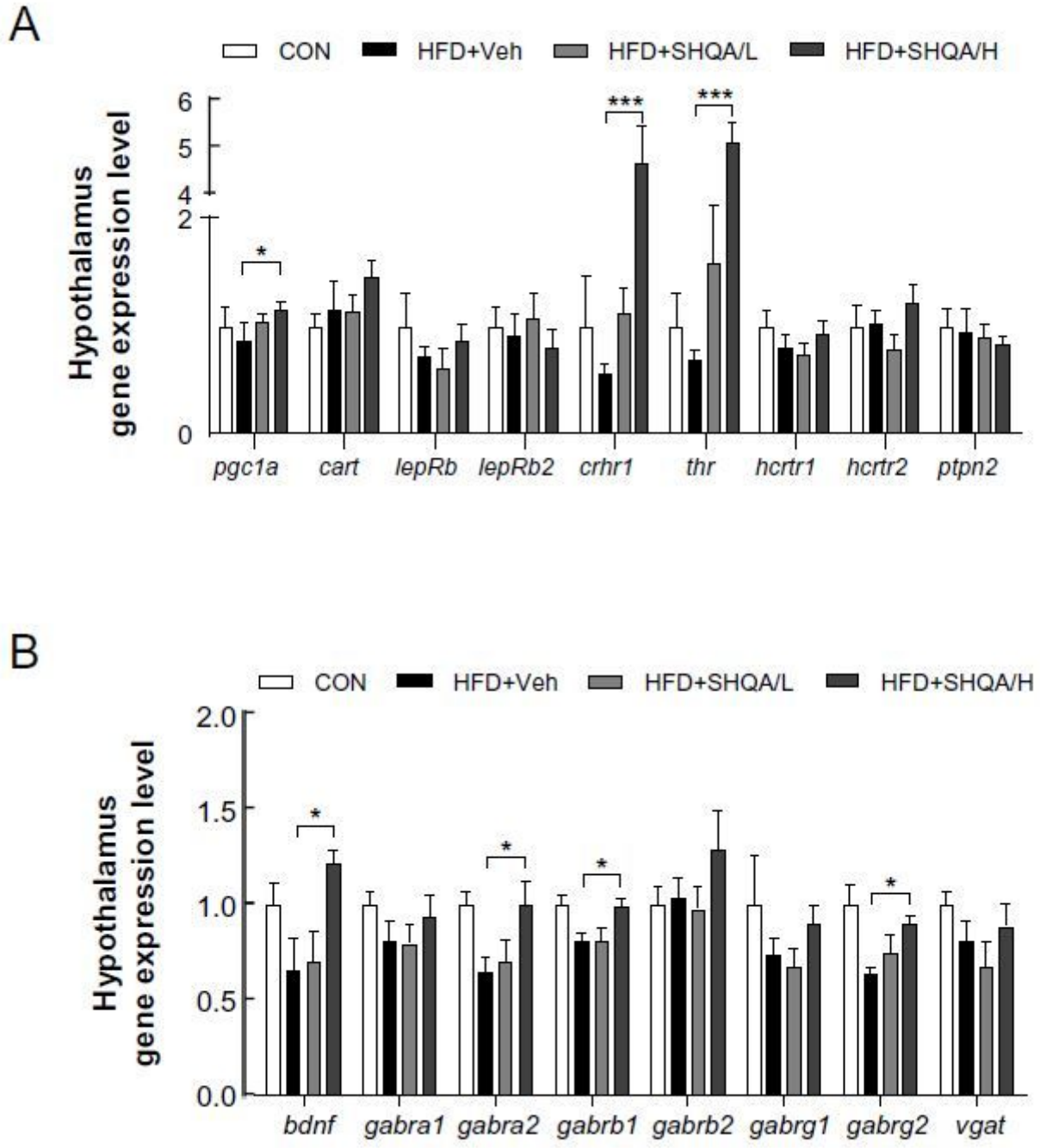


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