Central inflammation and leptin resistance are attenuated by ginsenoside Rb1 treatment in obese mice fed a high-fat diet

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Publication Details

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Central Inflammation and Leptin Resistance Are Attenuated by Ginsenoside Rb1 Treatment in Obese Mice Fed a High-Fat Diet

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Abstract

A low-grade pro-inflammatory state is at the pathogenic core of obesity and type 2 diabetes. We tested the hypothesis that the plant terpenoid compound ginsenoside Rb1 (Rb1), known to exert anti-inflammatory effects, would ameliorate obesity, obesity-associated inflammation and glucose intolerance in the high-fat diet-induced obese mouse model. Furthermore, we examined the effect of Rb1 treatment on central leptin sensitivity and the leptin signaling pathway in the hypothalamus. We found that intraperitoneal injections of Rb1 (14 mg/kg, daily) for 21 days significantly reduced body weight gain, fat mass accumulation, and improved glucose tolerance in obese mice on a HF diet compared to vehicle treatment. Importantly, Rb1 treatment also reduced levels of pro-inflammatory cytokines (TNF-α, IL-6 and/or IL-1β) and NF-κB pathway molecules (p-IκBα and p-κBβ2) in adipose tissue and liver. In the hypothalamus, Rb1 treatment decreased the expression of inflammatory markers (IL-6, IL-1β and p-IκBα) and negative regulators of leptin signaling (SOCS3 and PTP1B). Furthermore, Rb1 treatment restored the anorexic effect of leptin in high-fat fed mice as well as leptin pSTAT3 signaling in the hypothalamus. Ginsenoside Rb1 has potential for use as an anti-obesity therapeutic agent that modulates obesity-induced inflammation and improves central leptin sensitivity in HF diet-induced obesity.

Introduction

Obesity has reached epidemic proportions and is an important risk factor for the development of type 2 diabetes, cardiovascular disease and cancer. It is generally accepted that a low-grade pro-inflammatory state is at the pathogenic core of obesity and type 2 diabetes [1,2]. This inflammatory response includes elevated levels of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and interleukin 6 (IL-6), and activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, including inhibitor kappa B alpha (IkBα) and IkB kinase (IKK) [3,4]. The activation of pro-inflammatory cytokines and NF-κB signaling pathway mediate the transcription of the suppressor of cytokine signaling 3 (SOCS3) and protein-tyrosine phosphatase 1B (PTP1B), negative regulators of insulin and leptin signaling, which induce insulin and leptin resistance in peripheral tissues and the central nervous system [5,6,7]. Obesity associated inflammation in white adipose tissue and the liver leads to glucose intolerance, insulin resistance and metabolic dysfunction [2,3,9]. Over-nutrition and obesity also leads to hypothalamic inflammation and stimulation of local pro-inflammatory NF-κB signaling, resulting in the dysfunction of hypothalamic neurons [4,5]. Furthermore, recent studies have shown that induction of inflammation in the hypothalamus results in experimental obesity, resistance to the anorexigenic hormone leptin, peripheral insulin resistance and defective regulation of food intake and energy expenditure [10,11,12].

Targeted deletion of certain genes important for mediating inflammatory responses protect against the development of hyperglycemia, insulin resistance and obesity in obese mouse models. Disruption of the gene encoding IKK and the innate immune system receptor Toll-like receptor (TLR)-4 in mice confers protection from insulin and leptin resistance, and obesity in mouse models [5,13]. Also, inhibition of NF-κB signaling using high-dose salicylates confers protection from obesity-induced inflammation and insulin resistance in mice [14]. Activation of hypothalamic NF-κB by central injection of a constitutively active IKKβ lentiviral vector interrupts central leptin and insulin signaling, while genetic or viral vector mediated suppression of IKK within the mediodasal hypothalamus protects against obesity and glucose intolerance in mice [5]. Therefore, compounds that attenuate the peripheral and hypothalamic inflammation associated with obesity may prove useful in the management of patients with obesity and type 2 diabetes.

Some plant-derived triterpenoids are anti-inflammatory and inhibit the NF-κB signaling pathway [15]. The tetracyclic triterpenoid ginsenoside Rb1 (Rb1) is the major bioactive compound extracted from ginseng [16,17]. This compound inhibits inflammation in in vitro and in vivo models, including anti-inflammatory effects on aortic smooth muscle exposed to...
TNF-α [18], the colon of colitis mice [19], and brain tissue in a cerebral ischemia animal model [20]. In high-fat diet-induced obese rats, Rb1 significantly reduces food intake and body weight gain [21]. A study by Lin [22] shows that in high-fat diet-induced obese mice, Rb1 significantly reduces weight gain, blood glucose and total cholesterol. However, it is unknown whether Rb1 can improve obesity-associated inflammation and central leptin resistance.

Materials and Methods

Animal care and treatment
C57Bl/6 male mice (6 weeks old, average body weight of 19.6±1.4 g) were obtained from the Animal Resources Centre (Perth, Western Australia) and housed in environmentally controlled conditions (temperature 22°C, 12 hour light/dark cycle). All animals were fed a lab chow (LC) diet (5% fat, Vella Stock Feeds, Doonside, NSW, Australia) ad libitum for one week and then fed a high-fat (HF) diet for 16 weeks (The HF diet contained 40% of energy as fat, with the fat content consisting of half lard and half sunflower oil). The proportion of saturated fat, n-6 polyunsaturated fat, n-3 polyunsaturated fat and monounsaturated fat were 12%, 16%, 0.4% and 11% respectively. SF11-095, Stock Feeds, Doonside, NSW, Australia) and housed in environmentally controlled conditions (temperature 22°C, 12 hour light/dark cycle). All animals were fed a lab chow (LC) diet (5% fat, Vella Stock Feeds, Doonside, NSW, Australia) ad libitum for one week and then fed a high-fat (HF) diet for 16 weeks (The HF diet contained 40% of energy as fat, with the fat content consisting of half lard and half sunflower oil). The proportion of saturated fat, n-6 polyunsaturated fat, n-3 polyunsaturated fat and monounsaturated fat were 12%, 16%, 0.4% and 11% respectively. SF11-095, Stock Feeds, Western Australia). After 16 weeks of HF diet, obese mice (average body weight of 44.18±2.60 g) were randomized into two groups (n = 16 per group) and treated with either daily intraperitoneal (ip) injections of Rb1 (14 mg/kg, based on a Rb1 dose (10 mg/kg) described previously in rats [21], and using a body surface area ratio of 0.14 from rat to mouse [23]) or vehicle (saline) for 21 days. This study also included a parallel control group of age-matched mice fed a LC diet. Rb1 purified by high-performance liquid chromatography (HPLC) to ≥98% was purchased from Jilin University in China. During Rb1 treatment the animal’s food intake and body weight were recorded daily. All procedures were approved by the Animal Ethics Committee of the University of Wollongong, NSW, Australia, and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. The approval ID for this study is AE10/08.

Glucose tolerance test (GTT)
On day 18 of Rb1 treatment, the mice were injected intraperitoneally with glucose at a dose of 0.5 g/kg after an overnight fast. Blood samples were taken from the tail vein, and blood glucose concentration determined using a glucometer (Freestyle; Abbott Diabetes Care, Alameda, CA) at 0 (fasting), 30, 60 and 120 minutes after glucose injection.

Central leptin sensitivity
Central leptin sensitivity was examined in moderate and severely obese mice. For moderate obesity, mice were fed a HF diet for 8 weeks followed by an acute treatment of Rb1 (14 mg/kg/day, ip) for 2 days. For severe obesity, the mice were fed a HF diet for 16 weeks and then administered Rb1 (14 mg/kg/day, ip) for 21 days. The central leptin sensitivity test was performed as follows. Mice were anesthetized by isoflurane inhalation and placed in a stereotactic device. An intracerebroventricular (icv) cannula was implanted into the right lateral brain ventricle (0.25 mm posterior and 1.0 mm lateral relative to Bregma and 2.5 mm below the surface of the skull) [24]. Five days after implantation the mice were fasted for 6 hours, and either leptin (0.1 μg/3 μl) or saline (3 μl) was injected into the lateral ventricle through the cannula. Food intake and body weight were measured for 24 hours after the leptin or vehicle injection.

Blood and Tissue collection
Following a further four day interval after examining central leptin sensitivity, mice were fasted for 6 hours, administered an icv injection of either leptin (0.1 μg/3 μl) or saline (3 μl) and then euthanized 1 hour later for tissue collection. Blood, white adipose tissue, liver and brain tissue were collected. The plasma from mice receiving saline icv injections was collected after centrifugation at 3000rpm for 15 minutes. Plasma and other tissues were stored at −80°C for further analyses.

Using a standard mouse brain atlas [24], 500 μm frozen brain sections were cut from Bregma -1.22 mm to -2.72 mm using a cryostat at a temperature of −18°C. The mediobasal hypothalamus was dissected and then collected using a Stoelting Brain Punch (#57401, 0.5 mm diameter, Wood Dale, Stoelting Co, USA) in an overlapping pattern over the 3rd ventricle [25].

Determination of plasma leptin, insulin, peptide YY (PYY) and adiponectin
Plasma leptin, insulin and PYY were measured using the mouse metabolic magnetic bead panel kit (Merck Millipore, MA), and adiponectin was assayed with the mouse single plex adiponectin kit (Merck Millipore).

Histological analysis and morphometry
Epididymal fat was fixed in 10% buffered formaldehyde and then embedded in paraffin. Tissue sections (5 μm) were cut and mounted onto polylysine slides. The sections were stained with hematoxylin and eosin and photographed at 100× magnification. Using the image analysis software ImageJ 1.46r (http://rsweb.nih.gov/ij/download.html), two fields per section and six sections per fat mass were analyzed to quantify the area and number of adipocytes.

Western blot analysis
As described in our previous study [26], tissue protein was extracted using NP-40 Lysis Buffer. The following antibodies were used: TNF-α (sc-8301), IL-1β (sc-7884), and IL-6 (sc-7920) from Santa Cruz Biotechnology (Dallas, TX); and p-IκBα (#2859), p-IKK (#2697), p-STAT3 (#9145), SOCS3 (#2932), and p-FOXO1 (#9461) from Cell Signaling Technology (Beverly, MA). Bands corresponding to the proteins of interest were analyzed using the automatic imaging analysis system Quanntity One (Bio-Rad Laboratories, Hercules, CA). All quantitative analyses were normalized to β-actin as described in our previous study [26].

Quantitative real-time PCR (qPCR)
Total mediobasal hypothalamic RNA was extracted using the Aurum total RNA mini kit (Bio-Rad Laboratories, Hercules, CA) and reverse-transcribed to first-strand complementary DNA with the high-capacity cDNA reverse transcription kit (AB Applied Biosystems, Carlsbad, CA), according to the manufacturer’s instructions. qPCR was performed in a 20 μl final reaction volume using SYBR green I master in a Lightcycler 480 (F. Hoffmann-La Roche Ltd, Basel, Switzerland). Primers used are listed in Table S1. Amplification was carried out with 45 cycles of 95°C for 10 seconds, 60°C for 30 seconds and 72°C for 30 seconds. The mRNA expression levels for hypothalamic neuropeptides were normalized to gamma actin, which served as the internal control. Experiments were performed in triplicate. The level of expression for each gene was calculated using the comparative threshold cycle value (Ct) method, using the formula $2^{-\Delta\Delta Ct}$ as described previously [27,28].
Statistical analysis

Data were analyzed using the SPSS 19 statistical package (SPSS, Chicago, IL). The two-tailed student’s t-test was used to compare food intake, adipose tissue histology and weight, inflammatory markers in epididymal adipose tissue and liver, and hypothalamic neuropeptides. One-way analysis of variance (ANOVA) was followed by the post hoc Tukey–Kramer honestly significant difference (HSD) test was used to analyze final body weight gain, plasma cytokines, central inflammatory markers, and central leptin sensitivity. A $p<0.05$ was regarded as statistically significant, and $p<0.10$ were considered a trend. Values are expressed as mean ± SEM.

Results

Rb1 treatment lowered food intake and prevented weight gain and fat deposition in obese mice on a HF diet

Overall, Rb1 treatment reduced average food intake by 11% ($p<0.05$) in HF diet fed mice, and a reduction of food intake was
observed on days 7, 8, 10, 12, 14, 19 and 20 of Rb1 treatment (all \( p < 0.05 \), Fig. 1A) compared with HF control group. Rb1 treatment significantly reduced body weight gain (Fig. 1C) and visceral and subcutaneous (inguinal) fat deposition (Fig. 1D and Table 1) in mice maintained on a HF diet. Rb1 treatment also decreased the size of adipocytes (an indication of fat storage), with adipocytes from epididymal visceral fat pads being significantly smaller in response to Rb1 treatment (Fig. 1E and F). The distribution of adipocytes by cell surface area showed a higher proportion of small-sized cells (1,000 \( \mu \text{m}^2 \)) and a lower proportion of larger-sized cells (5,000–7,000 \( \mu \text{m}^2 \)) in the Rb1-treated group compared to the HF group (Fig. 1G).

<table>
<thead>
<tr>
<th>Table 1. Weight of fat pads in HF diet-induced obese mice with and without ginsenoside Rb1 treatment.</th>
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<tbody>
<tr>
<td>Visceral fat (g)</td>
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<tr>
<td>0.85±0.06</td>
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<tr>
<td>Perirenal fat (g)</td>
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<tr>
<td>Mesenteric fat (g)</td>
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<tr>
<td>0.85±0.06</td>
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<tr>
<td>3.78±0.17</td>
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<tr>
<td>0.85±0.06</td>
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<tr>
<td>Inguinal fat (g)</td>
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<tr>
<td>HF: high-fat diet–induced obese mice; Rb1: high-fat diet–induced obese mice treated with ginsenoside Rb1. Visceral fat includes epididymal, perirenal and mesenteric. (* p&lt;0.05 ) vs. HF group. Data are presented as Mean±SEM. doi:10.1371/journal.pone.0092618.t001</td>
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</table>

Figure 2. Chronic administration of Rb1 improved plasma profiles and glucose tolerance in obese mice fed a HF diet for 16 weeks. Rb1 decreased plasma leptin (A) and insulin (B), improved glucose tolerance (C) and AUC (D), and increased adiponectin (E) and PYY (F) in obese mice (\( n=8 \)) fed a HF diet for 16 weeks. \(* p<0.05 \) vs. HF control group; \# \( p<0.05 \) vs. LC diet control group. Data are presented as mean ± SEM. Area under the curve for glucose (AUC glucose) was calculated using the trapezoidal rule. doi:10.1371/journal.pone.0092618.g002
Rb1 treatment improved blood hormone profiles for energy balance regulation

HF diet-induced hyperleptinemia was significantly decreased by Rb1 treatment (Fig. 2A). Plasma insulin was elevated in HF diet-induced obese mice, but Rb1 did not significantly reverse hyperinsulinemia in these animals (Fig. 2B). To evaluate the functional outcome of Rb1 treatment on glucose homeostasis, we conducted a glucose tolerance test (GTT). Blood glucose was reduced by Rb1 treatment at the 30 and 60 minute time points of the GTT (Fig. 2C). The blood glucose area under the curve (AUC) after glucose injection was reduced in Rb1-treated mice compared to HF mice without Rb1 treatment (Fig. 2D). Rb1 also increased plasma adiponectin in HF diet-induced obese mice (Fig. 2E). Circulating concentrations of the anorexigenic peptide PYY were significantly increased in the Rb1 treatment group compared with HF mice (Fig. 2F).

Rb1 treatment decreased inflammation in adipose tissue and the liver

Given the anti-inflammatory properties of Rb1 in aortic smooth muscle, colon and brain [18,19,20], we investigated whether Rb1 could reduce low-grade inflammation of adipose and liver tissue in HF diet-induced obese mice. In the epididymal adipose tissue of HF mice treated with Rb1, we found significantly reduced expression of pro-inflammatory cytokines (TNF-α, -44%; IL-6, -25%; IL-1β, -30%; \( p<0.05 \)), as well as the inflammatory signaling molecule p-IKK (\( p=0.001 \)), compared to HF control mice (Fig. 3A). In a statistical trend, Rb1 treatment lowered p-IkBα expression in epididymal adipose tissue (\( p=0.06, \) Fig. 3A). For the liver of HF mice treated with Rb1, the expression of TNF-α and IL-6 (\( p<0.05 \)) was also significantly reduced compared to HF mice without Rb1 treatment (Fig. 3B). Rb1 treatment lowered SOCS3 expression in the liver (\( p<0.05, \) Fig. 3B) compared to HF control mice, in a statistical trend. However, no difference was found in the hepatic expression of IL-1β and p-IKK in HF mice with or without Rb1 treatment (Fig. 3B).

Rb1 treatment attenuated hypothalamic inflammation and negative regulators of leptin signaling

A HF diet stimulates pro-inflammatory cytokine mRNA expression in the hypothalamus of rodents [4], and here we investigated if Rb1 treatment could attenuate this inflammation. Using western blot analysis, we confirmed that protein levels of IL-6, TNF-α and p-IKK increased in the mediobasal hypothalamus of HF diet-induced obese mice compared with LC diet mice (Fig. 4A, C and D). The protein levels of SOCS3 and PTP1B, negative regulators of leptin signaling, also increased in the mediobasal hypothalamus of HF fed mice (Fig. 4E and F). Importantly, Rb1 treatment significantly decreased the expression of IL-6, IL-1β, p-IKK, SOCS3 and PTP1B (-14%, -31%, -15%, -20% and -14% respectively; \( p<0.05 \); Fig. 4) in the hypothalamic compared with HF control mice.

Rb1 treatment improved central leptin sensitivity and leptin signaling

To evaluate if Rb1 treatment improved central leptin sensitivity in conjunction with the inhibition of hypothalamic inflammation, central leptin sensitivity was examined at two stages in the development of obesity, at 8 and 16 weeks of HF diet. First, we demonstrated that icv injection of leptin decreased energy intake (-31%; \( p<0.05 \), Fig. 5A) and body weight gain (\( p<0.05 \), Fig. 5D) compared with saline injection in lean LC fed mice. Second, after 8 weeks of HF diet leptin did not suppress energy intake and body weight gain in HF control mice (Fig. 5B and E), while acute Rb1 treatment (2 days) restored leptin sensitivity, evidenced by a 41% reduction in energy intake and a very significant reduction in body weight gain following leptin icv injection compared to saline icv injection (\( p<0.05 \), Fig. 5B and E). Furthermore, acute Rb1 treatment did not significantly suppress overall food intake and body weight (Table S2). In severely obese control mice fed a HF diet for 16 weeks, leptin icv injection did not significantly decrease energy intake and body weight gain compared with saline (\( p>0.05 \), Fig. 5C and F). With the addition of chronic Rb1 treatment, icv leptin injections significantly decreased energy intake by -22% and decreased body weight gain by -251% compared to leptin injections in obese mice not treated with Rb1 (\( p<0.05 \), Fig. 5C and F). This suggests that the Rb1 chronic treatment increased the ability of leptin to inhibit energy intake and body weight gain.

To clarify the mechanism by which chronic Rb1 treatment improved leptin sensitivity, protein expression of the leptin signaling molecules p-STAT3 and p-FOXO1 was measured in the mediobasal hypothalamus. Icv injection of leptin increased p-STAT3 (+55%, \( p<0.05 \)) in mice fed LC diet, while these responses were not observed in HF diet-induced obese mice (Fig. 6A). After
Rb1 treatment, the response of leptin signaling molecules was restored, with a 42% increase in p-STAT3 (\(p<0.001\)) following leptin administration to Rb1-treated HF mice (Fig. 6A). Leptin also increased phosphorylation of FOXO1 in the mediobasal hypothalamus of LC diet fed mice, a response that was blunted in HF mice (Fig. 6B). However, in this case Rb1 treatment did not restore the leptin-induced increase in p-FOXO1 (Fig. 6B). Therefore, in the mediobasal hypothalamus, Rb1 acted on the STAT3 pathways rather than the FOXO1 pathway to restore leptin signaling.

Rb1 treatment affected the hypothalamic neuropeptides regulating energy balance

The effect of Rb1 treatment on hypothalamic neuropeptides expression was examined to investigate the mechanisms by which this compound suppressed food intake and body weight gain. Rb1 treatment significantly increased anorexigenic pro-opiomelanocortin (POMC, +75%; \(p<0.05\)) and decreased orexigenic agouti-related protein (AgRP, -24%; \(p<0.05\)) mRNA expression in the mediobasal hypothalamus of HF mice, but had no effect on orexigenic neuropeptide Y (NPY) mRNA levels (Table 2).

**Discussion**

In the current study, Rb1 prevented body weight gain and reduced fat mass in obese mice fed a HF diet. Rb1 also decreased average food intake during the course of this study. This is similar to the study by Xiong and colleagues, which showed that Rb1 has an anti-obesity effect in rats and its suppression of food intake is not due to malaise, as attested by a conditioned taste aversion test [21]. Importantly, our study extends the mechanism of Rb1 in suppressing food intake. Rb1 treatment increased the anorexigenic hormone, peptide YY (YY), in the blood and modulated...
hypothalamic neuropeptides, specifically by increasing anorexi-
genomically POMC and decreasing orexigenic AgRP mRNA expression
in HF diet-induced obese mice. Rodent models of HF diet-induced
obesity are characterized by inflammation in both peripheral
tissues and in the hypothalamic regions critical for energy
homeostasis [4,8], which is considered an important mechanism
linking obesity to glucose intolerance, insulin resistance and leptin
resistance. This study has demonstrated that ginsenoside Rb1
treatment provides an anti-obesity-associated inflammatory effect,
with the pronounced reduction of peripheral and hypothalamic
inflammation and improvement of glucose tolerance and central
leptin resistance in HF diet-induced obese mice.

Overnutrition and obesity induce inflammatory responses in
peripheral metabolic tissues, which decreases insulin sensitivity in
target cells (adipocytes and hepatocytes) and contributes to glucose
intolerance and the development of type 2 diabetes [2,8,9,29]. For
example, in obese rodents fed a HF diet macrophages infiltrate the
liver and increase the mRNA expression of the pro-inflammatory
cytokines TNF-α, IL-1β and IL-6 [2,9]. These cytokines activate
NF-κB signaling in hepatocytes, causing hepatic insulin resistance
and glucose intolerance [2,3]. In the current study, Rb1 decreased
the level of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6)
and the inflammatory signaling molecule p-IKK in the adipose
tissue and liver, which may have contributed to the improved
glucose tolerance observed in Rb1 treated diet-induced obese mice.
Furthermore, it is well-documented that adiponectin levels after Rb1 treatment may contribute to the
improved glucose tolerance and reduced fat accumulation that we
observed in obese mice treated with this compound.

Our study demonstrated that chronic treatment with Rb1
suppressed inflammation in the mediobasal hypothalamus of diet-
induced obese mice, as shown by decreased protein expression of
IL-6, IL-1β and p-IKK/NFκB mRNA expression in mouse brain tissue, and inhibited morphological
activation of microglia following intraperitoneal injections of
lipopolysaccharide endotoxin [33]. Recently, Thaler and col-
leagues demonstrated that mice and rats fed a HF diet had
increased TNF-α and IKK/NFκB mRNA expression in the
hypothalamus [4]. Hypothalamic inflammation is considered a key
pathology of obesity in rodents and humans [34], leading to
central leptin resistance through activation of the negative
regulators of leptin signaling, SOCS and PTP1B [5,12]. Our
results demonstrate that Rb1 decreased the upregulation of
SOCS3 and PTP1B in the hypothalamus of HF diet-induced
obese mice. Therefore, the inhibition of SOCS3 and PTP1B and
attenuation of hypothalamic inflammation, contributes to the
therapeutic effect of Rb1 on central leptin resistance observed in
our mouse model.

The adipocyte-derived hormone leptin promotes negative
energy balance through various signaling pathways (STAT3 and
FOXO1) in the hypothalamus. Constitutive activation of the
inflammatory signaling molecule IKKβ in the hypothalamus of
mice impaired STAT3 phosphorylation in response to central

![Figure 5. Chronic Rb1 treatment improved central leptin sensitivity in obese mice fed a HF diet for 16 weeks.](image-url)

Energy intake (A–C) and
body weight gain (D–F) for 24 hours after the icv injection of leptin or saline in mice fed a LC diet (n = 7–8), in obese mice fed a HF diet for 8 weeks with or without acute treatment of Rb1 (14 mg/kg ip daily for 2 days) (n = 7–8), in obese mice fed a HF diet for 16 weeks with or without chronic
treatment of Rb1 (14 mg/kg ip daily for 21 days) (n = 7–8), *p<0.05 vs. (Vehicle + Saline) group; **p<0.05 vs. (Vehicle + leptin) group; and ***p<0.05 vs.
(Rb1 + Saline) group. Data are presented as mean ± SEM.
doi:10.1371/journal.pone.0092618.g005

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LC mice, but not in HF mice with central leptin resistance. The central leptin injections stimulated FOXO1 phosphorylation in agouti-related protein (AgRP) expression [37]. In our study, genic pro-opiomelanocortin (POMC) and inhibiting orexigenic to neuropeptide promoters, stimulating transcription of anorexi-
results in its export from the nucleus and allows p-STAT3 to bind activity in the hypothalamus [35,36]. Phosphorylation of FOXO1 FOXO1 phosphorylation and degradation, decreasing FOXO1 [35,36]. Leptin signaling through the PI3K/Akt pathway induces hypothalamus and contributes to anorexigenic effect of leptin [38]. In the present study, the increased plasma PYY in Rb1 treated HF diet-induced obese mice may have contributed to the negative energy balance, lower body weight gain and fat accumulation in these animals. The mechanism by which Rb1 treatment increased circulating PYY levels remains to be determined. However, PYY is predominantly secreted by intestinal L cells located in the distal gastrointestinal tract [40], and it has been reported that the PYY levels are decreased in patients with inflammatory bowel disease [41]. In addition, three days of oral Rb1 treatment potently inhibited the expression of TNF-κ and IL-1β in the inflamed colon of mice with colitis [19]. Since the colon of obese mice overexpresses pro-inflammatory cytokines [42], an anti-inflammatory effect of Rb1 in the gastrointestinal tract may have increased PYY secretion in obese mice.

The melanocortin system comprises anorexigenic POMC expressing neurons and orexigenic AgRP expressing neurons in the arcuate nucleus of the mediobasal hypothalamus [43]. α-melanocortin-stimulating hormone (α-MSH), a post-translational product of the POMC gene, binds to the melanocortin receptor 4 (MC4R) and triggers an anorectic signal in the hypothalamus, while AgRP (an inverse agonist of MC4R) prevents α-MSH binding to MC4R. Chronic Rb1 treatment significantly increased POMC and inhibited AgRP mRNA expression in high-fat diet fed mice with central leptin resistance [5]. In our study, leptin induced phosphorylation of STAT3 was restored after Rb1 treatment in HF mice, suggesting the anti-inflammatory properties of Rb1 (inhibition of p-IKK in the hypothalamus) may contribute to this effect, FOXO1, a member of forkhead box of obese mice overexpresses pro-inflammatory cytokines [42], an anti-inflammatory effect of Rb1 in the gastrointestinal tract may have increased PYY secretion in obese mice.

**Table 2.** Relative mRNA expression of neuropeptides in the mediobasal hypothalamus in obese mice fed a HF diet for 16 weeks with or without Rb1 chronic treatment.

<table>
<thead>
<tr>
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<th>HF</th>
<th>Rb1</th>
<th>p-value</th>
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<tbody>
<tr>
<td>POMC</td>
<td>1.00±0.14</td>
<td>1.75±0.23*</td>
<td>0.034</td>
</tr>
<tr>
<td>AgRP</td>
<td>1.00±0.05</td>
<td>0.76±0.07*</td>
<td>0.045</td>
</tr>
<tr>
<td>NPY</td>
<td>1.00±0.04</td>
<td>0.90±0.09</td>
<td>0.464</td>
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HF: high-fat diet-induced obese mice; Rb1: high-fat diet-induced obese mice treated with ginsenoside Rb1. *p<0.05 vs. HF group. Data are presented as Mean ± SEM.

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mice, implying that Rb1 exerts its anorexigenic action at least partially by targeting the melanocortin system, the POMC and AgRP neurons. It is known that these neurons located in the mediobasal hypothalamus receive and integrate the signaling of various gut and adipostatic hormones, including PYY, leptin and insulin. In our study, the effect of Rb1 treatment on the hypothalamic melanocortin system may be due to increased plasma PYY, and the improvement of hyperleptinemia and central leptin sensitivity after Rb1 treatment.

In summary, this study has demonstrated that ginsenoside Rb1 treatment inhibits inflammation in the adipose tissue, liver and hypothalamus of HF diet induced obese mice. With Rb1 resulted in the improvement of glucose tolerance, central leptin sensitivity and hypothalamic leptin signaling (p-STAT3). We have also shown that Rb1 treatment increased the circulating concentrations of the anorexogenic hormone PYY and regulated melanocortin POMC/AgRP neuropeptides in the mediobasal hypothalamus, which contribute to negative energy balance. Ginsenoside Rb1 has the potential for use as an antiobesity therapeutic agent that functions by modulating obesity-induced inflammation and improving central leptin sensitivity in HF diet-induced obesity.

References


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Author Contributions

Conceived and designed the experiments: YZW YHY. Performed the experiments: YZW YHY. Analyzed the data: YZW YHY. Contributed reagents/materials/analysis tools: YZW YHY. Wrote the paper: YZW YHY. AZ MH XFH.


