

Galantamine Alleviates Inflammation and Other Obesity-Associated Complications in High-Fat Diet-Fed Mice

Sanjaya K Satapathy,¹ Mahendar Ochani,² Meghan Dancho,² LaQueta K Hudson,² Mauricio Rosas-Ballina,² Sergio I Valdes-Ferrer,² Peder S Olofsson,² Yael Tobi Harris,^{2,3} Jesse Roth,² Sangeeta Chavan,² Kevin J Tracey,² and Valentin A Pavlov²

¹Division of Gastroenterology, North Shore-Long Island Jewish Health System, New Hyde Park, New York, United States of America,

²Laboratory of Biomedical Science, The Feinstein Institute for Medical Research, Manhasset, New York, United States of America, and

³Division of Endocrinology and Metabolism, North Shore- Long Island Jewish Health System, New Hyde Park, New York, United States of America

Obesity, a serious and growing health threat, is associated with low-grade inflammation that plays a role in mediating its adverse consequences. Previously, we have discovered a role for neural cholinergic signaling in controlling inflammation, and demonstrated that the cholinergic agent galantamine suppresses excessive proinflammatory cytokine release. The main objective of this study was to examine the efficacy of galantamine, a clinically-approved drug, in alleviating obesity-related inflammation and associated complications. After 8 wks on a high-fat diet, C57BL/6J mice were treated with either galantamine (4 mg/kg, intraperitoneally (i.p.)) or saline for 4 wks in parallel with mice on a low-fat diet and treated with saline. Galantamine treatment of obese mice significantly reduced body weight, food intake, abdominal adiposity, plasma cytokine and adipokine levels, and significantly improved blood glucose, insulin resistance and hepatic steatosis. In addition, galantamine alleviated impaired insulin sensitivity and glucose intolerance significantly. These results indicate a previously unrecognized potential of galantamine in alleviating obesity, inflammation and other obesity-related complications in mice. These findings are of interest for studying the efficacy of this clinically-approved drug in the context of human obesity and metabolic syndrome.

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INTRODUCTION

Obesity, a global epidemic, represents a major public health threat (1). Obesity is complicated by hyperinsulinemia, hyperglycemia, dyslipidemia, elevated blood pressure, low-grade systemic inflammation and fatty liver. This clustering of components, under the rubric of “the metabolic syndrome,” affects more than 50 million people in the United States alone (2–4). The metabolic syndrome significantly increases the risk of developing type 2 diabetes and cardiovascular diseases, and is linked directly to non-alcoholic steatohepatitis, cancer, stroke, sleep apnea, respiratory diseases, osteoarthritis and other disorders (3,5–8).

Current pharmacological options for the treatment of the metabolic syndrome are limited (3), and it would be useful to target a common pathophysiological mechanism for the treatment of this complex disorder.

Obesity is associated with low-grade chronic inflammation attributed to dysregulated production and release of cytokines and adipokines, including tumor necrosis factor (TNF), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), leptin, resistin and adiponectin in macrophage-infiltrated abdominal adipose tissue (9–11). Experimental evidence indicates that inflammation is an important contributor to the development of

insulin resistance and other pathophysiological conditions underlying the metabolic syndrome (2,9–12).

We recently discovered that galantamine, a centrally acting acetylcholinesterase inhibitor and a cholinergic drug in clinical use for the symptomatic treatment of Alzheimer’s disease, has significant antiinflammatory effects (13). Galantamine suppresses serum TNF and IL-6 levels and improves survival during lethal inflammation (13). These findings emanated from our work on the role of the nervous system in the regulation of inflammation (14–21). Accordingly, here, we reasoned that galantamine treatment might be explored to alleviate obesity-related inflammation and other complications. Galantamine significantly reduced the inflammatory state, decreased body weight and abdominal adiposity and alleviated insulin resistance and fatty liver in mice with high-fat diet-induced obesity.

Address correspondence and reprint requests to Valentin A Pavlov, Laboratory of Biomedical science, The Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, Phone: 516-562-2316, Fax: 516-562-2356, E-mail: vpavlov@nshs.edu.

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MATERIALS AND METHODS

Animals

Experiments with male C57BL/6J mice (5–6 months old, Jackson Lab, Bar Harbor, ME, USA) were performed in accordance with the National Institutes of Health (NIH) Guidelines under protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Feinstein Institute for Medical Research, North Shore-Long Island Jewish (LIJ) Health System, Manhasset, New York, United States of America.

Experimental Design

C57BL/6J mice initially were fed a regular chow for 10 d and then switched to a high-fat diet (D12492, 60% kcal from fat, Supplementary Table 1) or its corresponding isocaloric low-fat control diet (D12450B, 10% kcal from fat, Supplementary Table 2) (Research Diets, New Brunswick, NJ, USA) for 12 wks. After the first 8 wks, mice on the high-fat diet were divided into two groups and treated with saline (i.p., once daily) or galantamine (Galanthamine Hydrobromide, EMD Biosciences Inc., La Jolla, CA, USA) (4 mg/kg, i.p., once daily) for the remaining 4 wks of the study. In parallel, mice on the low-fat (control) diet were treated with saline. Body weight and food intake were determined on a weekly basis by trained personnel of the Center for Comparative Physiology at the Feinstein Institute, who were blinded to treatment groups. At the end of the experimental time period, mice were euthanized and abdominal adiposity, liver weight, cytokine and adipokine levels, metabolic profile, insulin resistance and liver histology were evaluated. In a separate experiment, groups of mice were fed and treated and body weights and food intake recorded as described above. At the end of the experimental time period, mice were subjected to the insulin sensitivity test or the glucose tolerance test as described below.

Blood Glucose Determination

Blood glucose during the study was measured by nicking the tails and using

the Contour blood glucose meter (Bayer, Elkhart, IN, USA) with Contour blood glucose test strips according to the manufacturer's recommendations. After blood collection the cuts were compressed with sterile gauze until bleeding termination.

Blood Collection and Tissue Harvesting

After an overnight fast and following body weight and blood glucose determination mice were euthanized by CO₂ asphyxiation. Heparinized blood was obtained by cardiac puncture and centrifuged within 30 min at 1,500g for 10 min. Visceral adipose tissues and liver were harvested, rinsed with saline, and weighed. One piece of liver (the biggest lobe) was fixed in formalin for further H&E staining. Plasma and visceral fats (frozen on dry ice) were transferred at –20°C prior to further manipulations.

Plasma Adipokine Determination and Other Blood Biochemistry Tests

Plasma samples were sent to Millipore, St. Charles, MO, USA, and plasma insulin, leptin, MCP-1, PAI-1, resistin, TNF and IL-6 levels were determined by using the Millipore's MILLIPLEX mouse adipokine panel assay with the following detection sensitivity limits: leptin 16.71 pg/mL; resistin 1.79 pg/mL; IL-6 1.89 pg/mL; TNF 4.39 pg/mL; MCP-1 14.4 pg/mL; PAI-1 16.4 pg/mL; insulin 42.2 pg/mL. Plasma samples were sent to IDEXX Laboratories, Westbrook, ME, USA, and plasma levels of total cholesterol and ALT were determined by using IDEXX's Olympus analyzers and spectrophotometric determination. Plasma adiponectin was measured by using the mouse adiponectin/Acrp30 immunoassay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations.

Insulin Resistance Evaluation

Insulin resistance was evaluated by using glucose and insulin level values and by applying the homeostatic model assessment-insulin resistance (HOMA-IR) formula (22).

Liver Histology and Hepatic Steatosis Assessment

Formalin fixed liver tissue was imbedded in paraffin, sliced and then liver tissue sections were subjected to hematoxylin and eosin (H&E) staining and microscopic slides prepared. Hepatic steatosis was determined by microscopic evaluation of H&E stained liver sections for lipid accumulation by using Zeiss Axiovert 200M (Carl Zeiss Microimaging, Inc, Thornwood, NY) and semiquantified by applying a previously implemented grading criteria: no fat accumulation (grade 0); less than 33% fat-containing hepatocytes (grade 1); less than 66% fat-containing hepatocytes (grade 2); more than 66% fat-containing hepatocytes (grade 3) (23).

Insulin Sensitivity and Glucose Tolerance Tests

Separate groups of mice were fed and treated and body weights and food intake recorded as described above in Experimental Design. At the end of the 12-wk period, mice from the three experimental groups were used in the insulin sensitivity and glucose tolerance tests. In the insulin sensitivity test, after an overnight (18 h) fast, mice (n = 10 per group) were weighed and administered i.p. with insulin (Humulin R; Lilly, Indianapolis, IN, USA; 0.5 units/kg). In the glucose tolerance test, after an overnight (21 h) fast, mice (n = 10 per group) were weighed and injected i.p. with glucose (10% D glucose solution; Sigma, St. Louis, MO, USA; 1g/kg). Glucose levels were determined at 0, 15, 30, 60 and 120 min after insulin or glucose administration in blood from the tail vein by using the Contour blood glucose meter (Bayer) with Contour blood glucose test strips.

Statistical Analysis

Data are expressed as mean ± SEM. Significant differences were assessed by using one way analysis of variance (ANOVA) followed by the Bonferroni post hoc test or by using a Student *t* test, when appropriate. Differences with *P* < 0.05 were considered statistically significant.

All supplementary materials are available online at www.molmed.org.

RESULTS

Galantamine Reduces Body Weight Gain, Food Intake and Abdominal Adiposity in High-Fat Diet-Fed Mice

To study the therapeutic efficacy of galantamine in the context of obesity, mice were fed a high-fat diet for 8 wks prior to drug treatment. The high-fat diet for 8 wks resulted in a gradual body weight increase that reached a difference of 10 g ($P < 0.05$) as compared with the body weight of control, low-fat diet-fed mice (Figure 1A). High-fat diet-fed mice were then divided into two groups with equal average body weight (see Figure 1A) and blood glucose levels (after 6-h fast) of 229.7 ± 11.3 mg/dL and 222.12 ± 9.79 mg/dL, respectively, for treatment with either galantamine or vehicle (saline, i.p.) daily for 4 wks. In parallel, after 8 wks on a low-fat diet, mice (with an average blood glucose level of 175 ± 6.8 mg/dL) were treated with saline (one i.p. daily injection) for 4 wks. As shown in Figure 1A, mice on the high-fat diet and treated with saline (HFD-S) continued gaining weight. In contrast, the weight of mice on the high-fat diet and treated with galantamine (HFD-G) was lower after the first week of treatment (at week 9 as compared with week 8) and then sustained until the end of the investigative time period. At week 11 and 12 the weight of HFD-G mice was lower than the weight of HFD-S mice (see Figure 1A). The weight of mice on the low-fat diet and treated with saline was not altered significantly during these 4 wks (see Figure 1A). Food intake, which did not differ significantly among the groups of mice for the first 8 wks preceding treatment (Figure 1B). Moreover, galantamine treatment was associated with additional suppression of food intake in the HFD-G group as compared with the HFD-S group (see Figure 1B). Mice on the high-fat diet (HFD-S) had increased abdominal adiposity as compared with mice on the

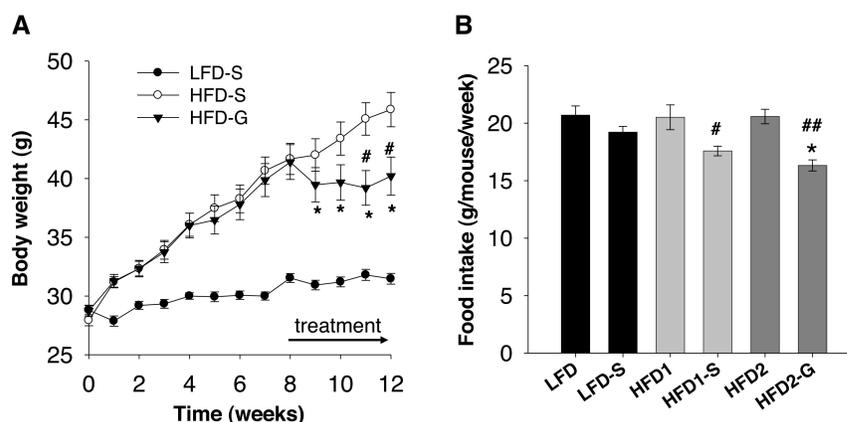


Figure 1. Galantamine treatment lowers body weight and suppresses food intake of high-fat diet-fed mice. (A) Body weight of mice on a low-fat diet and treated with saline (LFD-S), high-fat diet and treated with saline (HFD-S) and high-fat diet and treated with galantamine (HFD-G) ($*P < 0.05$ versus week 8; $\#P < 0.05$ versus HFD-S at the corresponding weeks) (B) Food intake in groups of mice on a different diet regimen prior to and during treatments: mice on a low-fat diet prior to treatment with saline (LFD); mice on a low-fat diet and treated with saline (LFD-S); mice on a high-fat diet prior to treatment with saline (HFD1); mice on a high-fat diet and treated with saline (HFD1-S); mice on a high-fat diet prior to treatment with galantamine (HFD2); mice on a high-fat diet and treated with galantamine (HFD2-G). ($\#P < 0.05$ versus HFD1; $\#\#P < 0.05$ versus HFD2; $*P < 0.05$ versus HFD1-S).

low-fat diet (LFD-S) (Table 1). Galantamine treatment significantly reduced mesenteric and retroperitoneal/perirenal adipose tissue weight (HFD-G group, Table 1). Together these results demonstrate a sustained suppressive effect of galantamine on body weight gain during high-fat diet-induced obesity accompanied by decreased food intake and associated with selectively reduced abdominal adipose tissue accumulation.

Galantamine Alters Systemic Cytokine and Adipokine Levels in High-Fat Diet-Fed Mice

Plasma IL-6 and leptin levels were elevated significantly in the HFD-S mice as compared with the LFD-S mice (Figure 2A, B). Galantamine reduced these

levels significantly (in the HFD-G mice) (see Figure 2A, B). Plasma MCP-1 levels were elevated (not statistically significant) in the HFD-S mice as compared with the LFD-S mice and galantamine significantly reduced MCP-1 levels in the HFD-G mice (Figure 2C). Plasma resistin levels were significantly higher in the HFD-S mice as compared with the LFD-S mice and galantamine significantly lowered these levels in the HFD-G animals (Figure 2D). Plasma plasminogen activator inhibitor-1 (PAI-1) levels were higher in the HFD-S mice as compared with the LFD-S and HFD-G mice, but none of these differences were statistically significant (Figure 2E). Plasma TNF levels were below the sensitivity limit (4.39 pg/mL) of the assay used; only one value in the LFD-S group, two values in

Table 1. Effect of galantamine on abdominal adiposity in the context of mouse obesity.

| | LFD-S | HFD-S | HFD-G |
|--|-----------------|------------------------------|--------------------------------|
| Mesenteric adipose tissue (g) | 0.07 \pm 0.01 | 1.07 \pm 0.12 ^a | 0.72 \pm 0.11 ^{a,b} |
| Retroperitoneal/perirenal adipose tissue (g) | 0.19 \pm 0.02 | 0.99 \pm 0.06 ^a | 0.75 \pm 0.08 ^{a,b} |
| Epididymal adipose tissue (g) | 0.67 \pm 0.08 | 2.36 \pm 0.15 ^a | 2.17 \pm 0.18 ^a |

^a $P < 0.05$ vs. LFD-S.

^b $P < 0.05$ vs. HFD-S.

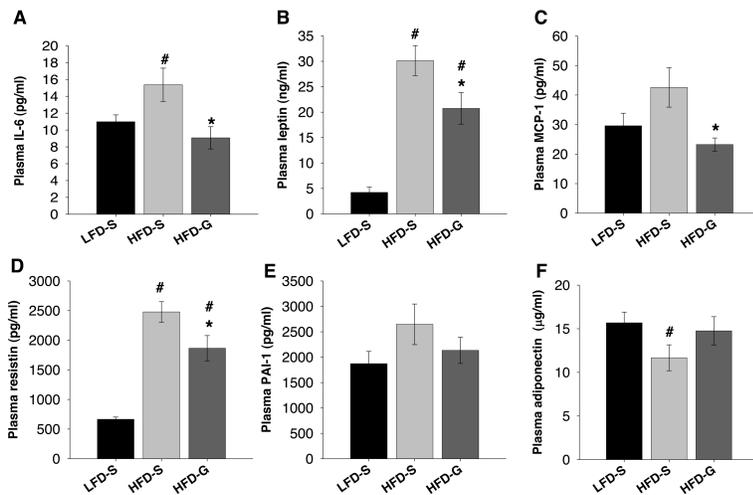


Figure 2. Galantamine alters circulatory cytokine and adipokine levels in high-fat diet-fed mice. Plasma cytokine/adipokine levels were determined in mice fed a low-fat diet and treated with saline (LFD-S), high-fat diet and treated with saline (HFD-S), or high-fat diet and treated with galantamine (HFD-G). Plasma levels of: (A) IL-6; (B) leptin; (C) MCP-1; (D) resistin; (E) PAI-1; (F) adiponectin ([#]*P* < 0.05 versus LFD-S, ^{*}*P* < 0.05 versus HFD-S).

the HFD-S group and none in the HFD-G group above this limit were detected. Adiponectin levels have been shown previously to be correlated negatively with obesity and insulin resistance, and experimental evidence points to its anti-inflammatory function (24). As shown in Figure 2F, plasma adiponectin levels were lower in HFD-S mice as compared with LFD-S mice. Galantamine treatment resulted in a certain increase in these levels (see Figure 2F). In addition, adiponectin protein levels in abdominal adipose (mesenteric, retroperitoneal/perirenal, epididymal) tissues were generally higher in the LFD-S group as compared with the HFD-S group and galantamine treatment (in the HFD-G group) did not result in significant alterations (Supplementary Figure 1). Together, these data reveal that galantamine inhibits proinflammatory cytokines and adipokines implicated in the development of insulin resistance.

Galantamine Lowers Fasting Blood Glucose and Plasma Insulin, Alleviates Insulin Resistance and Decreases Plasma Cholesterol Levels in High-Fat Diet-Fed Mice

We observed significantly higher fasting blood glucose and plasma insulin

levels in HFD-S mice as compared with LFD-S mice; galantamine significantly reduced these levels (HFD-G mice) (Figure 3A, B). Applying the homeostatic model assessment insulin resistance

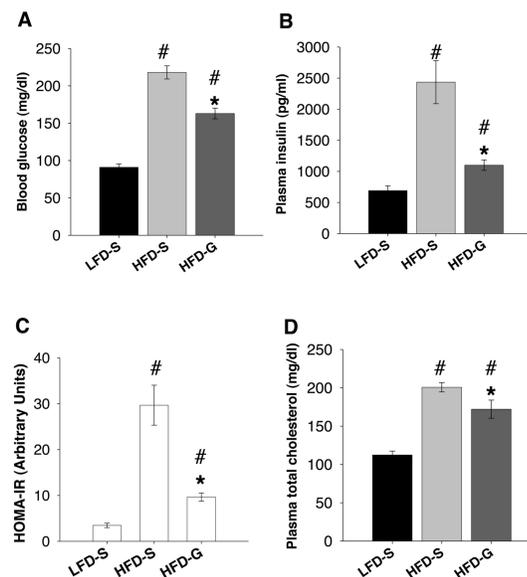


Figure 3. Galantamine lowers circulatory glucose, insulin and cholesterol in high-fat diet-fed mice. Blood glucose and plasma insulin and cholesterol levels were determined in mice fed a low-fat diet and treated with saline (LFD-S), high-fat diet and treated with saline (HFD-S) or high-fat diet and treated with galantamine (HFD-G). (A) Blood glucose levels, (B) plasma insulin levels, (C) insulin resistance as determined by applying the HOMA-IR formula, (D) plasma total cholesterol levels. ([#]*P* < 0.05 versus LFD-S, ^{*}*P* < 0.05 versus HFD-S).

(HOMA-IR) formula revealed increased insulin resistance in HFD-S mice as compared with the LFD-S group, and galantamine reversed this insulin resistance (Figure 3C). Plasma total cholesterol levels also were elevated significantly in HFD-S mice as compared with LFD-S mice and galantamine significantly decreased these levels in HFD-G mice (Figure 3D). Together, these results indicate that galantamine significantly alleviates fasting hyperglycemia, hyperinsulinemia, insulin resistance and hypercholesterolemia.

Galantamine Suppresses Fatty Liver Manifestations in High-Fat Diet-Fed Mice

Obesity is frequently accompanied by increased plasma aminotransferase levels and lipid accumulation in the liver, a condition known as fatty liver. This ectopic lipid accumulation contributes to insulin resistance, dyslipidemia and insulinemia. At the end of the 12-wk study-time period plasma alanine aminotransferase (ALT) levels were elevated significantly in the

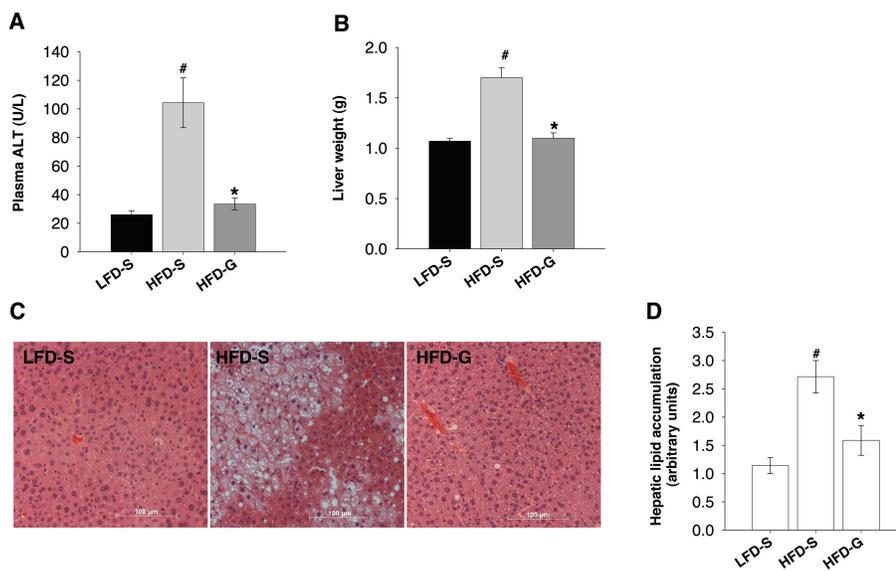


Figure 4. Galantamine reduces alanine aminotransferase levels, liver weight and hepatocyte lipid accumulation in mice with high-fat diet-induced obesity. Plasma ALT levels and liver weights were determined in mice fed a low-fat diet and treated with saline (LFD-S), high-fat diet and treated with saline (HFD-S) or high-fat diet and treated with galantamine (HFD-G). Following H&E staining of liver tissue slides, lipid accumulation was determined by microscopic histological evaluation and applying grading criteria as described in Materials and Methods. (A) Plasma ALT levels, (B) liver weight, (C) Representative photomicrographs ($\times 20$) of H&E-stained liver section slides in: LFD-S mice; HFD-S mice; and HFD-G mice. (D) Hepatocyte lipid accumulation in the three groups of mice ($n = 7$ per group with representative average body and liver weights). ($\#P < 0.05$ versus LFD-S, $*P < 0.05$ versus HFD-S).

HFD-S mice as compared with the LFD-S mice and galantamine significantly decreased ALT levels (in HFD-G mice) (Figure 4A). These alterations were consistent with an increased liver weight in the high-fat diet-fed (HFD-S) mice, as compared with the low-fat diet-fed (LFD-S) animals, and a significant liver weight decrease following galantamine treatment (in the HFD-G mice) (Figure 4B). The fluctuations in liver weight were associated with notable differences in liver gross appearance in the three groups of mice (Supplementary Figure 2). Microscopic observations demonstrated increased hepatocyte fat accumulation (steatosis) in HFD-S mice, as compared with LFD-S mice, and galantamine decreased lipid accumulation in HFD-G mice (Figure 4C). Semiquantitative analysis revealed that lipid accumulation in livers from galantamine-treated (HFD-G) mice was reduced significantly as compared with HFD-S mice (Figure 4D). These observations indicate that galantamine al-

leviates fatty liver in high-fat diet-induced obesity.

Galantamine Suppresses Body Weight Gain and Food Intake and Alleviates Impaired Insulin Sensitivity and Glucose Intolerance

To further examine the effects of galantamine on obesity-related insulin resistance and impaired glucose homeostasis, in a separate experiment we employed the same experimental design of high-fat diet-induced obesity prior to subjecting mice to an insulin sensitivity test (IST) and a glucose tolerance test (GTT). As shown in Figure 5A, at 8 wks, the average body weight of mice on a high-fat diet was 11.25 g ($P < 0.05$) higher than the body weight of mice on a low-fat diet. Importantly, while body weight of mice on the high-fat diet and treated with saline (HFD-S) continued to increase, the weight of mice on the high-fat diet and treated with galantamine (HFD-

G) was decreased (see Figure 5A). Food intake suppression was statistically significant in the HFD-S and HFD-G groups of mice as compared with the prior-to-treatment values (Figure 5B). In addition, the food intake in the galantamine-treated (HFD-G group) mice was significantly lower as compared with the HFD-S group of mice (see Figure 5B).

At the end of the 12-wk experimental period, mice were fasted overnight for IST and GTT. In the IST, following insulin i.p. administration, blood glucose in the LFD-S mice reached its lowest levels at 30 min, and then increased, as determined at the 120-min time point (Figure 5C). Impaired insulin sensitivity in the HFD-S group of mice was indicated by higher blood glucose levels at the 15-min and 30-min time points as compared with the LFD-S mice (see Figure 5C). Moreover, blood glucose in the HFD-S mice reached lower levels at 60 min, followed by no recovery up to the 120-min time point (see Figure 5C). In contrast, blood glucose levels in the HFD-G mice decreased sharply to their lowest levels at 30 min, remained unchanged at 60 min, and increased at 120 min (see Figure 5C). These values were significantly lower at 15 min and 30 min as compared with the HFD-S mice, and at 120 min as compared with the LFD-S mice (see Figure 5C). In the GTT test, the initial increase in blood glucose levels (after glucose administration) was higher in the HFD-S group of mice as compared with the LFD-S group of mice (Figure 5D,a). Galantamine treatment (HFD-G group) suppressed blood glucose levels as compared with the HFD-S group of mice (see Figure 5D,a). Accordingly, the area under the curve analysis of the GTT data showed higher values in the HFD-S mice as compared with the LFD-S mice (Figure 5D,b), thus indicating increased (whole-body) insulin resistance. These values were significantly lower in the HFD-G mice as compared with the HFD-S mice (see Figure 5D,b). Together these results indicate that galantamine attenuates impaired insulin sensitivity, glucose intolerance and insulin resistance in high-fat diet-fed obese mice.

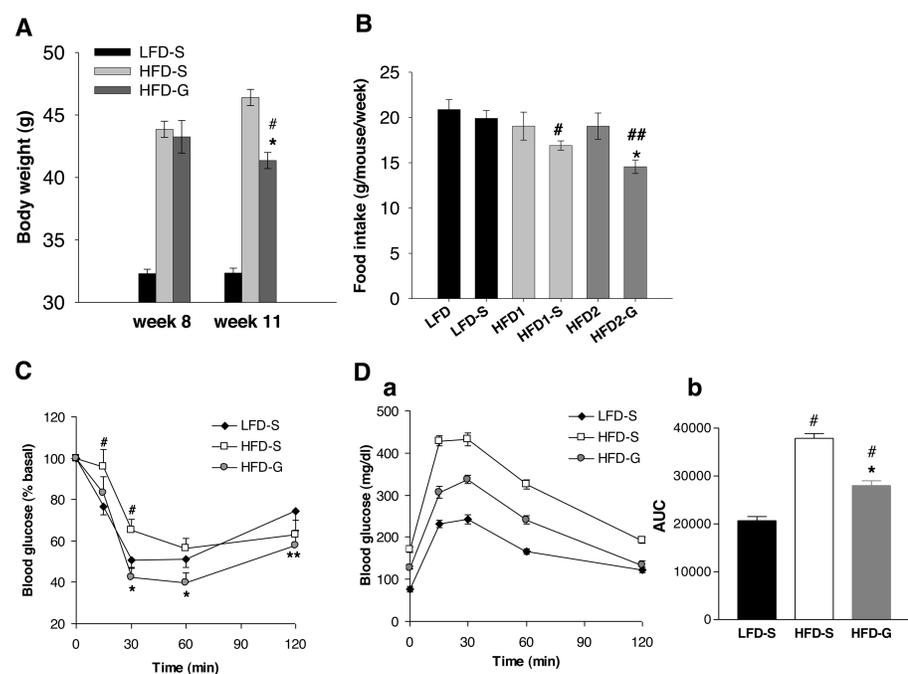


Figure 5. Galantamine treatment lowers body weight and food intake and alleviates impaired insulin sensitivity and glucose intolerance in high-fat diet-fed mice. Following 12 wks on a different diet and treatment regimens, mice on a low-fat diet and treated with saline (LFD-S), high-fat diet and treated with saline (HFD-S) and high-fat diet and treated with galantamine (HFD-G) were subjected to the insulin sensitivity test or the glucose tolerance test. (A) Body weight of mice on a low-fat diet and treated with saline (LFD-S), high-fat diet and treated with saline (HFD-S) and high-fat diet and treated with galantamine (HFD-G) before the beginning of treatments (wk 8) and after treatments (wk 11) (* $P < 0.05$ versus HFD-G prior to treatment (wk 8); # $P < 0.05$ versus HFD-S after treatment (wk 11)) (B) Food intake prior to and during treatments: mice on a low-fat diet prior to treatment with saline (LFD); mice on a low-fat diet and treated with saline (LFD-S); mice on a high-fat diet prior to treatment with saline (HFD1); mice on a high-fat diet and treated with saline (HFD1-S); mice on a high-fat diet prior to treatment with galantamine (HFD2); mice on a high-fat diet and treated with galantamine (HFD2-G) (# $P < 0.05$ versus HFD1; ## $P < 0.05$ versus HFD2; * $P < 0.05$ versus HFD1-S) (C) Insulin sensitivity test (# $P < 0.05$ versus LFD-S at the same time point, * $P < 0.05$ versus HFD-S at the same time points, ** $P < 0.05$ versus LFD-S at the same time point) (D,a) Glucose tolerance test (D,b) Area under the curve assessment of the glucose tolerance test (# $P < 0.05$ versus LFD-S, * $P < 0.05$ versus HFD-S).

DISCUSSION

Here we report that administration of the clinically-approved acetylcholinesterase inhibitor galantamine to mice with high-fat diet-induced obesity significantly attenuates the inflammatory state, reduces body weight and abdominal adiposity and alleviates hyperglycemia, hyperinsulinemia, hypercholesterolemia, insulin resistance and fatty liver. These findings indicate a previously unrecognized potential of galantamine in alleviating obesity and obesity-associated

complications and suggest the possibility of utilizing this drug in the treatment of obesity and the metabolic syndrome.

A major causative factor for the obesity epidemic is the increased consumption of high-energy, high-fat foods (25). Therefore, in this study, we utilized high-fat diet-induced obesity in C57BL/6J mice, an experimental model that has been used widely in preclinical studies of obesity and the metabolic syndrome (26,27–30). Galantamine in the range 1–10 mg/kg (daily treatment) has been used exten-

sively in preclinical investigations (31–34). In this study, we utilized treatment with galantamine dose (4 mg/kg) within this range, which we have shown previously to cause antiinflammatory effects in acute settings of murine endotoxemia (13).

The body weight-lowering and abdominal adiposity-decreasing effects of galantamine treatment shown here can be related partially to a food intake suppressing effect of this cholinergic drug. The regulation of feeding behavior and appetite is complex, and a role for central cholinergic signaling in this regulation has been reported previously. For instance, it has been shown that presynaptic, $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR)-mediated cholinergic mechanisms modulate melanin-concentrating hormone regulation of appetite in the lateral hypothalamus (35). This modulation has been suggested as a possible mechanism of the appetite-suppressing effect of nicotine (35,36). In addition, a recent paper reported that a selective $\alpha 7$ nAChR agonist significantly inhibited food intake in obese mice (37). We have shown previously that the galantamine antiinflammatory mechanism is centrally mediated and requires the $\alpha 7$ nAChR (13). Moreover, in addition to being an acetylcholinesterase inhibitor, galantamine is a positive allosteric modulator of nicotinic receptors, including $\alpha 7$ nAChR (38). Therefore, it is possible that a central, nicotinic receptor-mediated cholinergic appetite modulation is implicated in the food intake suppressing effect of galantamine.

Decreasing body weight and abdominal adiposity could be contributing factors to alleviating the inflammatory state in galantamine-treated obese mice. However, while galantamine treated obese (HFD-G) mice were still significantly heavier and with higher abdominal adiposity than lean (LFD-S) mice, galantamine treatment reduced systemic IL-6 and MCP-1 to levels detected in lean mice. These results suggest a specific antiinflammatory effect of galantamine that cannot be attributed simply to reduced body weight and abdominal adiposity. We have shown recently that the antiinflammatory effects of galanta-

mine are centrally mediated and require vagus nerve signaling (13), and galantamine also stimulates efferent vagus nerve activity (39). In addition, it has been reported that abdominal adipose tissues, an important source of cytokines and adipokines in obesity, receive vagus nerve innervation (40). Therefore, it is possible that the centrally acting acetylcholinesterase inhibitor galantamine activates vagus nerve-mediated cholinergic signaling to adipose tissues that plays a role in regulating cytokine and adipokine release. MCP-1, IL-6 and resistin are implicated directly in mediating macrophage infiltration into adipose tissue, and the pathophysiology of insulin resistance and hepatic steatosis and their systemic levels are elevated in obesity (24,41–43). In contrast, decreased adiponectin levels in obese individuals inversely correlate with insulin resistance and hepatosteatosis (24,41). Leptin plays an essential role as a regulator of energy homeostasis (24). Weight gain triggers the release of leptin that suppresses food intake and stimulates energy expenditure. This function is compromised in obese individuals and elevated leptin levels (leptinemia) are associated with leptin resistance that occurs in parallel with insulin resistance. It is possible that lowering circulating levels of IL-6, MCP-1, leptin, and resistin and elevating adiponectin levels in obese mice by galantamine may, in turn, mediate beneficial effects on metabolic pathways implicated in insulin resistance, food intake and liver pathophysiology.

Elevated markers of inflammation, increased visceral adiposity and ectopic fat accumulation in the liver (hepatic steatosis) are accompanied frequently by elevated plasma ALT and cholesterol levels, and have been linked to insulin resistance, dyslipidemias and cardiovascular risk (42,44,45). A recent large, national, population-based study has shown an association between trunk fat accumulation and increased ALT levels and has indicated the possibility that liver injury can be induced by metabolically active intraabdominal fat (46). ALT levels have been associated closely with the develop-

ment of incident metabolic syndrome and type 2 diabetes (47). Notably, we show here that galantamine treatment of obese mice significantly suppressed serum ALT levels and liver enlargement to levels determined in lean mice. In addition, galantamine treatment significantly decreased the ectopic fat accumulation in the liver (hepatic steatosis). These beneficial effects of galantamine on liver pathophysiology, which could be associated with its anti-inflammatory effects, characterize the potential of this drug in alleviating fatty liver disease, the hepatic manifestation of the metabolic syndrome (42,44,45).

Cholinergic mechanisms play a role in the regulation of hepatic glucose production, a major determinant of fasting blood glucose (48–51). Central inhibition of lipid oxidation, insulin or leptin activate a brain hypothalamic to the liver pathway that is mediated through efferent vagus nerve cholinergic output and results in inhibition of hepatic glucose production and increased insulin sensitivity (52–54). Brain cholinergic signaling also regulates hepatic glycogen synthesis mediated by muscarinic receptors and efferent vagus nerve activity (55). It is likely that these glucose homeostasis-controlling vagus nerve-mediated cholinergic mechanisms become suppressed or dysfunctional during obesity, as indicated by autonomic dysfunction and lower vagal tone in obese individuals (56,57). Therefore, it is possible that galantamine, acting through a central muscarinic receptor-mediated mechanism and stimulation of the efferent vagus nerve as found previously (13,39) reinstates and activates these pathways, resulting in anti-hyperglycemic effects and alleviation of glucose intolerance.

Galantamine has been tested repeatedly in humans and is in clinical use for treating patients with Alzheimer's disease. This previous experience should facilitate clinical development of galantamine in treating obesity and the metabolic syndrome in humans. Such a clinical application of galantamine may be particularly appropriate in individuals who have limited possibilities to benefit from dieting and exercising, for example,

elderly or patients with antipsychotic medication-induced obesity. Intriguingly, elevated serum markers of inflammation, including IL-6, in elderly people with the metabolic syndrome have been linked to significant cognitive impairment (58). Moreover, higher serum levels of systemic proinflammatory markers have also been associated with cognitive deterioration during the progression of Alzheimer's disease (59,60). Therefore, based on our current data and a previous study (13), one may speculate that ameliorating inflammation can be an important factor contributing to the beneficial effect of galantamine in patients with Alzheimer's disease.

In conclusion, our results demonstrate the efficacy of cholinergic stimulation by galantamine in alleviating obesity-associated inflammation, obesity and other components of the metabolic syndrome in mice and suggest a rationale for further development of this clinically used drug in the treatment of these epidemics.

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DISCLOSURE

KJ Tracey and VA Pavlov are inventors on patents related to the content of this paper. KJ Tracey is a consultant to SetPoint Medical, Inc.

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