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Diminished gallbladder motility in rotund leptin-resistant obese mice

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Abstract

Background. Obesity is a risk factor for cholesterol gallstone formation, but the pathogenesis of this phenomenon remains unclear. Most human obesity is associated with diabetes and leptin-resistance. Previous studies from this laboratory have demonstrated that diabetic leptin-resistant (Lep^{db}) obese mice have low biliary cholesterol saturation indices, enlarged gallbladders and diminished gallbladder response to neurotransmitters. Recently, a novel leptin-resistant mouse strain $Lepr^{db\text{-}rtnd}$ (Rotund) has been discovered. Rotund mice are also obese, diabetic, and have an abnormal leptin receptor. Therefore, we tested the hypothesis that leptin-resistant obese Rotund mice would have large gallbladders and reduced biliary motility.

Methods. Eight-week-old control (C57BL/6J, $N=12$) and Rotund leptin-resistant ($Lepr^{db\text{-}rtnd}$, $N=9$) mice were fed a non-lithogenic diet for four weeks. Animals were fasted and underwent cholecystectomy. Gallbladder volumes were recorded, and contractile responses (N/cm^2) to acetylcholine (10^{-5} M), Neuropeptide Y ($10^{-8,-7,-6}$ M), and cholecystokinin ($10^{-10,-9,-8,-7}$ M) were measured. Results were analyzed using the Mann-Whitney Rank Sum Test.

Results. Compared to control mice, Rotund mice had larger body weights, higher serum glucose levels, and greater gallbladder volumes ($p<0.05$). Rotund gallbladders had less contractility ($p<0.05$) to acetylcholine and cholecystokinin than control mice. Responses to Neuropeptide Y were also less, but not statistically significant, in the Rotund mice.

Conclusions. These data suggest that leptin-resistant Rotund mice have (1) enlarged gallbladders with (2) diminished contractility compared to lean control mice. Therefore, this study confirms that leptin-resistance is associated with abnormal biliary motility and may lead to gallstone formation in leptin-resistant obesity.

Key Words: *Cholesterol, diabetes mellitus, gallbladder, gallstones, motility*

Introduction

Obesity has reached epidemic proportions in the United States and many westernized countries. More than 50 million US adults have a body mass index (BMI) greater than 30 [1]. Human obesity is associated with insulin-resistant diabetes mellitus, elevated serum lipids, and a 3.7 times greater risk of gallstone disease [2]. The majority of obese humans are resistant to leptin, a hormone produced by adipocytes that induces satiety and regulates energy expenditure. While short forms of the leptin receptor are known to exist, leptin is thought to work primarily on the long form receptor, which is highly prevalent in the hypothalamus but is also found throughout the gastrointestinal tract [3]. However, the exact relationship among obesity, leptin, and cholesterol gallstone disease is still unknown. In contrast, gallstone formation is known to require the interplay of three factors, cholesterol supersaturation of bile, cholesterol crystal pronucleators, and biliary stasis.

The long form of the leptin receptor is highly conserved between mice and humans and is defective in the leptin-resistant (Lep^{db}) mouse model [4]. Previous studies from our laboratory have shown that when leptin-resistant obese mice (Lep^{db}) are fed a standard, low cholesterol diet, they have enlarged gallbladders with diminished contraction to neurotransmitter stimulation [5]. Lep^{db} mice have an absent signal transcription and translation (STAT) region of the leptin receptor, but have a normal extracellular domain and leptin-binding capacity as well as a normal janus kinase (JAK) region [6]. Because JAK can phosphorylate and activate other pathways besides STAT [7], the Lep^{db} mouse model is not a true null leptin receptor model. Recently, a new leptin-resistant Rotund mouse has been characterized [6]. This mouse has a nucleotide deletion resulting in a premature stop

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codon and a severely truncated leptin receptor, which is devoid of all extracellular and intracellular domains. Therefore, using a true leptin receptor deficient model, we hypothesize that the Rotund mouse will also demonstrate elevated glucose levels, obesity, enlarged gallbladder volumes, and decreased gallbladder motility when compared to control mice.

Materials and methods

Animals and diets

To study gallbladder contraction, 12 lean control C57BL/6J and 9 Rotund 7-week-old female mice were obtained by special permission from a laboratory affiliated with Jackson Laboratory (Bar Harbor, ME). The mice were housed in cages up to 5 mice each in a light (6 am–6 pm) and temperature (22°C) controlled environment, and isolation precautions were observed. All mice received a standard low cholesterol CHOW diet (Ralston Purina, St. Louis, MO) for 4 weeks. At 12 weeks of age, all mice were fasted overnight. Upon study, mice were anesthetized with xylazine (15 mg/kg, Phoenix Pharmaceuticals, Burnsville, MN) and ketamine (50 mg/kg, Phoenix Pharmaceuticals, Burnsville, MN), weighed, and underwent cholecystectomy. Gallbladders were placed in ice cold, preoxygenated modified Krebs solution (in mmol/L: NaCl, 116.6; $NaCO_3$, 21.9; KH_2PO_4, 1.2; glucose, 5.4; $MgCl_2$, 1.2; KCl, 3.4; and $CaCl_2$ 2.5). Whole blood was obtained by aspiration from the right heart, and livers were removed and weighed.

Bile and Serum Glucose Analysis

Bile was aspirated from the fundus of intact gallbladders with a 30-gauge needle, placed into a microtube, centrifuged at 15,000 rpm for 5 min at room temperature (Micromax model, International Equipment Company, Needham Heights, MA), and measured with a micropipette. Whole blood was also centrifuged at 15,000 rpm for 5 min at room temperature to separate serum. Serum was warmed to 39°C, and glucose was measured with Freestyle glucose strips and glucometer (Therasense, Alameda, CA).

In-vitro *muscle bath*

Gallbladders were sutured with 7-0 polypropylene sutures at both ends and suspended longitudinally in 3 mL muscle bath wells filled with modified Krebs solution, warmed to 39°C, and oxygenated with 95% O_2 and 5% CO_2. Gallbladders were equilibrated at 0.025 grams of tension. Optimal length was then determined by stimulation with 10^{-5} M acetycholine (ACh, Sigma Chemical, St. Louis, MO) at 0.025 gram increments until maximal gallbladder contraction was obtained. Gallbladders were maintained at their optimal lengths while Neuropeptide Y (NPY, Sigmal Chemical) at 10^{-8}, 10^{-7}, and 10^{-6} M doses and cholecystokinin octapeptide (CCK, Sigmal Chemical) at 10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} M doses were added. Responses were measured with the Windaq/Ex computer software (Dataq Instruments, Inc., Akron, Ohio). After every neurotransmitter dosing and after every 15 minutes, gallbladders were rinsed with modified Krebs solution. Gallbladder lengths and weights were measured and used to calculate the cross-sectional area. Gallbladder contractile responses were normalized for area and were expressed as Newtons per centimeter squared (N/cm^2).

Statistical analysis

Data analyses were performed with SigmaStat Statistical Software (Jandel Corporation, San Rafael, CA). All data are expressed as mean $\pm$ SEM. Mouse body and liver weights, serum glucose, gallbladder volume, and neurotransmitter responses were analyzed by the Mann-Whitney Rank Sum Test. A p-value less than 0.05 was regarded as significant.

Results

Body and liver weights, serum glucose, and gallbladder volume

Data for body and liver weights, serum glucose levels and gallbladder volumes are shown in Table I and Figure 1. The body and liver weights of the Rotund mice were dramatically larger than the control animals ($p < 0.001$). In addition, the serum glucose levels of the Rotund mice were markedly greater than the glucose levels of the C57 control mice (421 and 160 mg/dL, respectively, $p < 0.01$). The gallbladder volumes (Figure 1) of the Rotund mice were 19.3 μL, which were significantly larger than 8.8 μL average gallbladder volume of the control mice ($p < 0.05$).

Muscle bath

Gallbladder responses to ACh are also shown in Figure 1. The contractile responses of leptin-resistant Rotund mice were significantly less than the responses of the control mice (0.04 versus 0.11 N/cm^2, $p < 0.05$). Gallbladder responses to NPY at the 10^{-8}, 10^{-7}, and 10^{-6} M concentrations are shown in Figure 2. Again,

Table I. Body weight, liver weight, and serum glucose levels for control and rotund mice

Strain	Body weight	Liver weight	Glucose
Control	17.2 $\pm$ 0.4	0.87 $\pm$ 0.05	160 $\pm$ 23
Rotund	40.0 $\pm$ 2.0*	2.16 $\pm$ 0.14*	421 $\pm$ 37*

Values are mean $\pm$ SEM, body and liver weights are shown in grams, and serum glucose levels are given as mg/dL.
* $p < 0.01$ versus Control.