Glutathione Depletion Prevents Diet-Induced Obesity and Enhances Insulin Sensitivity

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Excessive accumulation of reactive oxygen species (ROS) in adipose tissue has been implicated in the development of insulin resistance and type 2 diabetes. However, emerging evidence suggests a physiologic role of ROS in cellular signaling and insulin sensitivity. In this study, we demonstrate that pharmacologic depletion of the antioxidant glutathione in mice prevents diet-induced obesity, increases energy expenditure and locomotor activity, and enhances insulin sensitivity. These observations support a beneficial role of ROS in glucose homeostasis and warrant further research to define the regulation of metabolism and energy balance by ROS.

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Chronic oxidative stress has been implicated in the development of type 2 diabetes and its associated cardiovascular complications (1,2). Reactive oxygen species (ROS) accumulate in obesity and considerable experimental and clinical data have linked oxidative stress to insulin resistance and β-cell dysfunction (1,3). At the cellular level, chronic oxidative stress resulting from prolonged exposure to high concentrations of ROS alters insulin-stimulated glucose update, an effect that is thought to be mediated by impaired insulin signaling (2,3). However, considering that certain ROS may also serve as important second messengers and signaling intermediates (4), the causal contribution of physiological concentrations of ROS to insulin resistance and obesity remains controversial. For example, transient and low dose concentrations of H_2O_2 enhance insulin sensitivity (5,6), indicating that the role of ROS in glucose metabolism may depend on the ROS concentration and the mechanisms of their generation. Since glutathione peroxidase (GPx) constitutes the principal antioxidant defense system to scavenge physiological concentrations of H_2O_2 in mammals (6), we investigated in this study the role of pharmacological glutathione depletion on diet-induced obesity and insulin sensitivity. Surprisingly, depletion of endogenous glutathione protected mice from obesity, preserved insulin sensitivity, and increased energy expenditure, pointing to a more complex role of endogenous ROS in diabetes and energy balance than previously anticipated.

METHODS AND PROCEDURES

Animal experiments

C57BL/6 mice were obtained from The Jackson Laboratory. All mice were housed in plexiglas-ventilated cages within a pathogen-free barrier facility that maintained a 12-hour light/12-hour dark cycle. Mice had access to autoclaved water and pellet food *ad libitum*. Prior to 11 weeks of age, all mice were fed a standard rodent chow diet containing ~5% kcal fat (Harlan Teklad). At 11 weeks of age, all mice were fed a high-fat diet (HFD) containing 45% kcal from fat (Research Diets, New Brunswick, NJ). During this period *L*-buthionine-(*S,R*)-sulfoximine (BSO, Sigma-Aldrich, St Louis, MO) was administered in the drinking water of the treatment group at a concentration of 30 mmol/l. BSO was dissolved in diH_2O and filtered before use. Weight gain was monitored weekly.

Body composition

Body composition was analyzed after 6 weeks of HFD feeding using quantitative magnetic resonance imaging (EchoMRI, Echo Medical Systems, Houston, TX) as described (7).

Metabolic measurements

Glucose tolerance tests were performed in HFD-fed animals after an overnight fasting period (n = 10 and n = 9 for HFD + BSO, respectively). Following an intraperitoneal injection of glucose dissolved in water (2 g/kg body weight), blood glucose levels were analyzed before and 15, 30, 60, 90, and 120 min after injection using a Freestyle Flash Glucometer (Abbott Laboratories, Chicago, IL). Insulin sensitivity was analyzed in fed animals (n = 10 for each group). Following an intraperitoneal bolus injection of recombinant human regular insulin (0.4 U/kg body weight) (Novolin R; Novo Nordisk, Princeton, NJ), blood glucose concentrations were measured before and 30, 60, 90, and 120 min after injection.

Analysis of energy expenditure and locomotor activity

Energy expenditure, food intake, water intake, and locomotor activity were analyzed using a calorimetry system (LabMaster; TSE Systems, Chesterfield, MO). Mice were fed a HFD with or without BSO treatment (30 mmol/l) in the drinking water (n = 7 for each group). After 3 weeks, the mice were placed in the calorimetry system, adapted for 24 h, and cumulative recordings were collected over the following 72 h.

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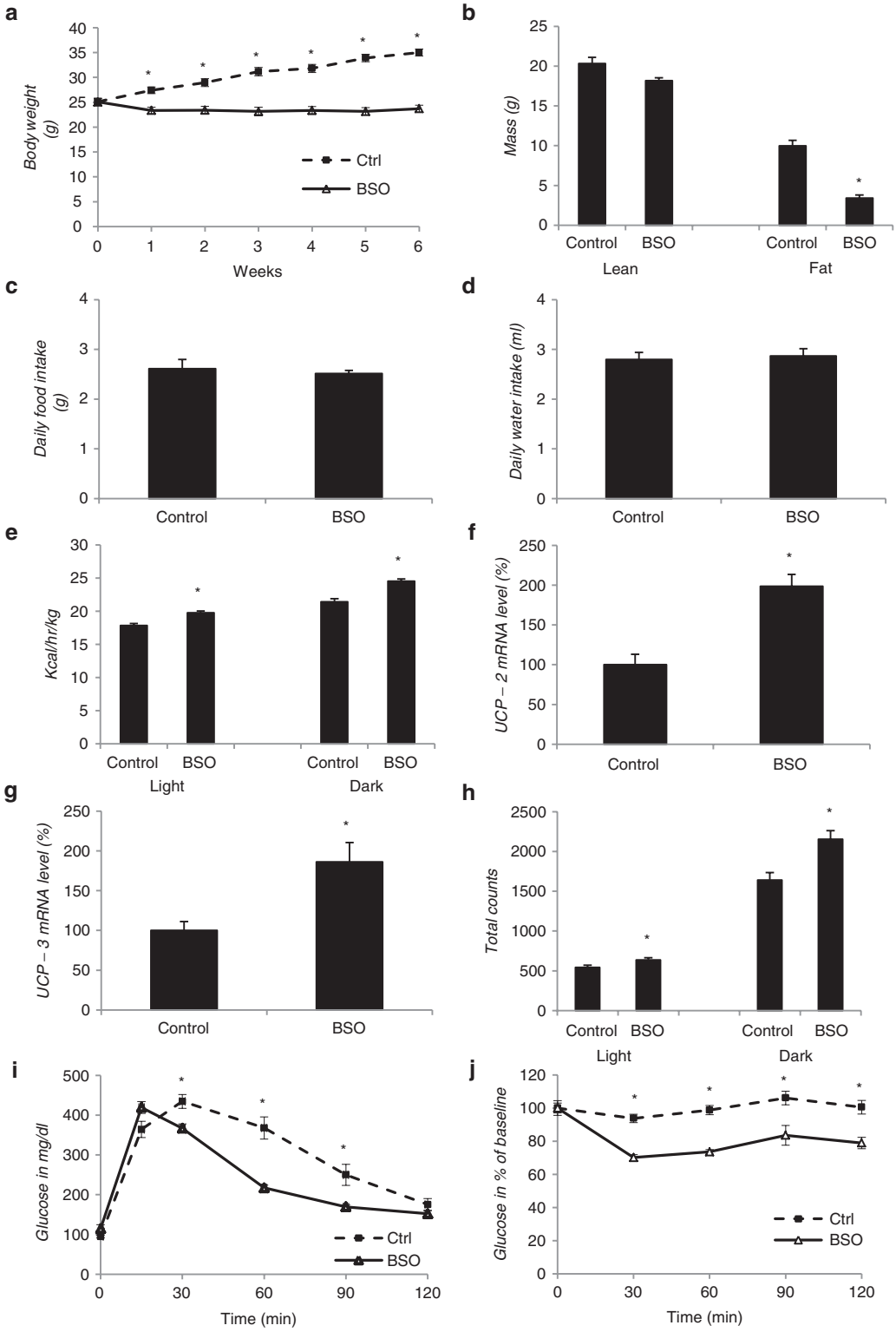

Figure 1 Glutathione depletion prevents diet-induced obesity, increases energy expenditure and enhances insulin sensitivity. (**a**) C57BL/6 mice were fed a high-fat diet for 6 weeks and treated with 30 mmol/l BSO provided in the drinking water. (**b**) Body composition was measured after 6 weeks of high-fat diet using EchoMRI. Lean and fat mass is presented in gram. (**c**,**d**) Daily food and water intake were monitored over 72 h in a calorimetry system. (**e**) Energy expenditure was measured after 3 weeks of high-fat diet and BSO treatment. Cumulative results of three different light or dark phases are presented. (**f**,**g**) mRNA expression of UCP-2 and -3 was measured in epididymal white adipose tissue after 6 weeks of high-fat diet and BSO treatment. (**h**) Locomotor activity was measured after 3 weeks of high-fat diet and BSO treatment. Cumulative results of three different light or dark phases are presented. (**i**) Glucose tolerance was determined after 6 weeks of high-fat diet and BSO treatment following an intraperitoneal injection of 2 g glucose/kg body weight. (**j**) Insulin tolerance was determined following an intraperitoneal injection of 0.4 U insulin/kg body weight. All results are presented as mean ± s.e.m. (*$P < 0.05$). BSO, *L*-buthionine-(*S,R*)-sulfoximine

RNA isolation and quantitative real-time polymerase chain reaction

RNA from epididymal adipose tissue was isolated and reverse transcribed using TRIzol and Superscript II (Invitrogen, Carlsbad, CA). Quantitative real-time polymerase chain reaction analysis of target gene expression was performed using an iCycler and SYBR Green I system (Bio-Rad, Hercules, CA). Each sample was normalized to mRNA expression of the housekeeping gene *β-actin.* Primer sequences are available on request.

Statistics

ANOVAs using one-way or two-way ANOVA with Bonferroni's *t*-test for *post hoc* analysis and paired or unpaired *t*-test were performed for statistical analysis as appropriate. Data were reported as means ± s.e.m. P values < 0.05 were considered statistically significant.

RESULTS

Glutathione depletion protects from diet-induced obesity

To investigate whether glutathione depletion modulates diet-induced obesity *in vivo*, we challenged C57BL/6J mice with a HFD containing 45% kcal from fat and treated mice with vehicle or BSO (30 mmol/l). At this dose, BSO has previously been shown to deplete endogenous glutathione in mice resulting in increased levels of ROS in metabolically active tissues including liver, muscle, and adipose tissue (8–10). Surprisingly, BSO-treated mice fed a HFD were completely protected from diet-induced obesity (**Figure 1a**), despite similar food intake and water consumption (**Figure 1c,d**). Analysis of body composition in mice fed a HFD diet confirmed significantly decreased fat mass in BSO-treated mice without significant differences in lean body mass, indicating that the difference in body weight was due to reduced fat mass in BSO-treated mice (**Figure 1b**).

Glutathione-depleted mice exhibit increased energy expenditure and locomotor activity

The observation that BSO treatment prevented diet-induced obesity in mice without affecting food intake raised the question as to whether glutathione depletion might modulate energy balance. To address this possibility, we next analyzed energy expenditure activity in BSO-treated mice. As shown in **Figure 1e**, BSO treatment of HFD-fed mice increased energy expenditure during both light and dark phase, which was associated with a significant increase in the expression of the uncoupling proteins UCP-2 and UCP-3 in adipose tissue (**Figure 1f,g**). Furthermore, BSO-treated mice displayed increased locomotor activity during both light and dark phase (**Figure 1h**). In concert, these data indicate that BSO treatment increases energy expenditure and locomotor activity in mice, which may contribute to the protection from diet-induced obesity by glutathione depletion.

Glutathione depletion enhances insulin sensitivity

To further characterize the effect of glutathione depletion on glucose homeostasis, we analyzed glucose tolerance and insulin sensitivity in mice treated with BSO. As expected, control mice fed a HFD displayed considerably impaired glucose clearance and insulin resistance. In contrast, mice fed a HFD and treated with BSO displayed less glucose intolerance and preserved insulin sensitivity (**Figure 1i,j**). This data suggest that glutathione depletion enhances insulin sensitivity in HFD-fed mice.

DISCUSSION

Abundant evidence has demonstrated the accumulation of oxidative stress during obesity and suggests a causal participation of pathological ROS concentrations in the development of insulin resistance (1,2). For instance, nutrient excess increases mitochondrial production of H_2O_2, which attenuates insulin signaling (3). Conversely, antioxidant treatment or overexpression of antioxidant enzymes can prevent diet-induced insulin resistance in mice (11). However, despite these apparent deleterious effects of pathological ROS concentrations on insulin sensitivity, it is well established that physiological levels of ROS enhance cellular signaling (4). Particularly H_2O_2 has been implicated in the activation of protein phosphorylation-dependent pathways, including insulin signaling and can exert insulin-mimicking effects through the inactivation of oxidation-sensitive protein tyrosine phosphatases (3). In mammalian cells, the primary antioxidant system to scavenge H_2O_2 is the glutathione system, which converts H_2O_2 to water through the activity of GPx using reduced glutathione as an electron donor (6). Using a pharmacological approach, our findings demonstrate that glutathione depletion enhances insulin sensitivity and, interestingly, prevents diet-induced obesity by increasing energy expenditure and locomotor activity. These observations complement prior studies using genetic modulation of glutathione peroxidase in mice. GPx1-deficient mice are protected from high-fat diet-induced insulin resistance (6) while mice overexpressing GPx1 are insulin resistant due to altered insulin signaling (12). Moreover, increased ROS levels in GPx1-deficient mice protected against diet-induced obesity and increased energy expenditure (6), which is consistent with our data obtained through pharmacological glutathione depletion. Although the molecular mechanisms underlying increased energy expenditure in glutathione-depleted mice remain unknown, is it possible that the observed increase in the expression of UCP-2 and UCP-3 in BSO-treated mice induced mitochondrial uncoupling, which has previously been described in response to increased levels of ROS (13). Alternatively, the enhanced energy expenditure in BSO-treated mice might be the result of increased locomotor activity. In skeletal muscle, ROS are necessary for optimal contractile function, force production, and exercise-induced adaptations (14). Furthermore, particularly H_2O_2 is increasingly recognized as a potent neuromodulator (15). It is therefore conceivable, that glutathione depletion may lead to activity-stimulating changes in the redox environment of muscle or brain.

Collectively, our observations presented in this study provide further evidence to support a differential role of ROS in obesity and diabetes, likely depending on the mechanisms of induction and the concentrations of ROS. While high levels of ROS induced through pathological responses are likely to contribute to insulin resistance, physiological low levels may be beneficial to increase energy expenditure and augment insulin sensitivity;

however, further studies are warranted to corroborate this notion and to define the role of ROS in obesity and diabetes.

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DISCLOSURE

The authors declared no conflict of interest.

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