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Original Research

A high-fat diet differentially regulates glutathione phenotypes in the obesity-prone mouse strains DBA/2J, C57BL/6J, and AKR/J

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ABSTRACT

The ubiquitous tripeptide glutathione (GSH) is a critical component of the endogenous antioxidant defense system. Tissue GSH concentrations and redox status (GSH/GSSG) are genetically controlled, but it is unclear whether interactions between genetic background and diet affect GSH homeostasis. The current study tested the hypothesis that a high-fat diet regulates GSH homeostasis in a manner dependent on genetic background. At 4 months of age, female mice representing 3 obesity-prone inbred strains—C57BL/6J (B6), DBA/2J (D2), and AKR/J (AKR)—were randomly assigned to consume a control (10% energy from fat) or high-fat (62% energy from fat) diet for 10 weeks (n = 5/diet per strain). Tissue GSH levels, GSSG levels, and GSH/GSSG were quantified, and hepatic expression of GSH-related enzymes was evaluated by quantitative reverse transcription polymerase chain reaction. The high-fat diet caused a decrease in hepatic GSH/GSSG in D2 mice. In contrast, B6 mice exhibited a decrease in GSSG levels in the liver and kidney, as well as a resultant increase in renal GSH/GSSG. AKR mice also exhibited increased renal GSH/GSSG on a high-fat diet. Finally, the high-fat diet induced a unique gene expression response in D2 mice compared with B6 and AKR. The D2 response was characterized by up-regulation of glutamate-cysteine ligase modifier subunit and down-regulation of glutathione reductase, whereas the B6 and AKR responses were characterized by up-regulation of glutathione peroxidase 1. Two-way analysis of variance analyses confirmed several diet-strain interactions within the GSH system, and linear regression models highlighted relationships between body mass and GSH outcomes as well. Overall, our data indicate that dietary fat regulates the GSH system in a strain-dependent manner.

Abbreviations: ANOVA, analysis of variance; Gclc, glutamate-cysteine ligase catalytic subunit; Gclm, glutamate-cysteine ligase modifier subunit; Gr, glutathione reductase; Gpx-1, glutathione peroxidase 1; GSH, glutathione (reduced); GSSG, glutathione disulfide.

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1. Introduction

Cellular defenses against a wide array of stressors are governed by the ubiquitous thiol tripeptide, glutathione (GSH). The protective capacity of this molecule is attributable in part to its abilities to directly neutralize reactive oxygen species and to serve as a cofactor for reactive oxygen species metabolizing enzymes [1]. Glutathione oxidation is coupled to the generation of the glutathione dimer (glutathione disulfide [GSSG]), and the subsequent decrease in GSH/GSSG serves as a quantitative measure of oxidative stress. Recent studies have indicated that genetic background is a critical determinant of tissue GSH levels and GSH/GSSG [2,3], and mapping efforts have identified novel genes and alleles associated with GSH homeostasis [3]. To support forward genetics approaches to define the genetic architecture underlying GSH levels and GSH/GSSG, it is important to determine whether environmental factors interact with genetic background to influence these phenotypes.

We predict that dietary fat regulates GSH homeostasis in a manner dependent on genetic background. Our rationale was informed by previous findings demonstrating that a high-fat diet increases hepatic GSSG levels and decreases GSH/GSSG ratios, and the effects are associated with the onset of morbidities such as nonalcoholic fatty liver disease in mice [4]. Those results align with the data from other studies, which have generally shown that diet-induced obesity perturbs GSH homeostasis, illustrated by a decline in GSH/GSSG and altered activity of GSH-related enzymes [5-10]. Importantly, the findings have clinical relevance as the GSH system has been linked to chronic conditions such as obesity [11] and diabetes [12] in humans as well.

The current study used 3 mouse strains that are established models of diet-induced obesity: C57BL/6J (B6), DBA/2J (D2), and AKR/J (AKR) [13-20]. We hypothesized that these strains would have different GSH redox responses to a high-fat diet, indicating that dietary fat regulates the GSH system in a strain-dependent manner. To test this hypothesis, we first sought to determine whether the GSH system of each strain was impacted, or not, by a high-fat diet and increased body weight. Second, we further explored potential interaction effects between strain and diet, as well as between strain and body weight, on GSH phenotypes. The effects of a high-fat diet on the GSH system were examined in the liver and kidney, because these tissues have the highest concentrations of GSH and were most likely to demonstrate strain effects. Expression of GSH-related enzymes was assessed to evaluate the potential origin of redox status changes, but gene expression analyses were limited to the liver because of the expectation that GSH redox effects would be most pronounced in this tissue.

2. Methods and materials

2.1. Animals

Female C57BL/6J (B6; JAX No. 000664), DBA/2J (D2; JAX No. 000671), and AKR/J (AKR; JAX No. 000648) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Female mice were used so that GSH data could be put into context of previous studies related to GSH heritability [3]. Five mice from each strain were randomly assigned to consume a control diet and an additional 5 mice were assigned to a high-fat diet. Mice were housed in an animal room on a 12-hour light/dark cycle. At the conclusion of the study, mice were humanely euthanized by cervical dislocation, and tissues were collected for analyses. All methods and experimental procedures for this study were approved by the University of Georgia Institutional Animal Care and Use Committee (A2013-08-011).

2.2. Diet

Mice were fed diets containing either 10% energy from fat (*control*; TestDiet 58Y2 [Land O'Lakes, Inc., Arden Hills, MN, USA]) or 62% energy from fat (*high-fat*; TestDiet 58Y1), which contains extra fat from lard. The composition of the experimental diets is described in Table 1. Dietary interventions were initiated when mice reached 4 months of age and were sustained for 10 weeks. During the study period, mice were fed ad libitum and given unrestricted access to water. Food intake and weights of the mice were measured on a weekly basis.

2.3. Assessment of total GSH, GSH, GSSG, and GSH/GSSG

Samples were prepared and analyzed as previously described with slight modifications [21]. Briefly, livers and kidneys were removed, rinsed in ice-cold phosphate-buffered saline (pH 7.4), and flash-frozen in liquid nitrogen. Within 24 hours after collection, tissues were homogenized in phosphate-buffered saline containing 10 mM diethylenetriaminepentaacetic acid

Table 1 – Composition of experimental diets

Ingredients	Control	High-fat
Sucrose	331.29	88.47
Dextrin	298.56	–
Casein	189.56	258.45
Powdered cellulose	47.39	64.61
Maltodextrin	33.17	161.53
Soybean oil	23.7	32.31
Lard	18.96	316.6
Potassium citrate, tribasic monohydrate	15.64	21.32
Calcium phosphate	12.32	16.8
DIO mineral mix	9.48	12.92
AIN-76 A vitamin mix	9.48	12.92
Calcium carbonate	5.21	7.11
L-Cystine	2.84	3.88
Choline bitartrate	1.9	2.58
Energy (kcal/g diet)	3.76	5.1
% Energy from fat	10.2	61.6
% Energy from carbohydrate	71.8	20.3
% Energy from protein	18	18.1

Values are reported as g/kg, except where otherwise noted. Diets were purchased from TestDiet.
The lipid sources in the control diet contained 18 ppm cholesterol, 13.9 g/kg linoleic acid, 1.9 g/kg linolenic acid, 1.9 g/kg omega-3 fatty acids, 13 g/kg monounsaturated fatty acids, and 11.4 g/kg saturated fatty acids.
The lipid sources in the high-fat diet contained 301 ppm cholesterol, 47 g/kg linoleic acid, 3.9 g/kg linolenic acid, 0.6 g/kg arachidonic acid, 3.9 g/kg omega-3 fatty acids, 140 g/kg monounsaturated fatty acids, and 136.8 g/kg saturated fatty acids.

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