Journal of Academia and Industrial Research (JAIR)
Volume 2, Issue 12 May 2014

ISSN: 2278-5213

RESEARCH ARTICLE

Periodic Effects of High-Fat Diet Exposure on Glucose Metabolism and Redox Status in C57BL/6 Mice

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Abstract

Metabolic disorders born as a result of high-fat diet (HFD) consumption are becoming a major challenge in our society, especially in industrialized world, with obesity plus its associated complications, hitting the unprecedented records. The aim of this study was to explore the changes in glucose metabolism and redox status depending on the period of HFD exposure in female C57BL/6 mice. Simultaneously, the antioxidant effects of quercetin were studied. Animals were randomly divided 3 groups: control, HFD and HFD fed quercetin (HFD+Q). Following euthanization, sacrifices were held after each 3, 6 and 13 weeks and by ELISA diagnostic kits, markers of oxidative stress in cortex (GSH/GSSG, TAOC, MDA, AOPP and 3-NT) were analyzed. Starting from 3 weeks, HFD group exhibited oxidative damage by significantly raising AOPP, 3-NT and MDA levels while lowering level of GSH/GSSG and TAOC ($P<0.05$). HFD dramatically raised body weight (35.39%) and significantly induced oxidative stress in HFD thereby up-regulating the expression. The antioxidant factor quercetin significantly reduced body weight (18.79%) and restored redox status.

Keywords: high-fat diet, obesity, glucose metabolism, quercetin, ELISA, oxidative stress.

Introduction

The modern lifestyle of an increased intake of palatable high-fat diet associated with decreased energy expenditure contributes to the current rising global prevalence of metabolic syndromes (Minehira and Tappy, 2002; Alberti *et al.,* 2006). Metabolic syndrome is often characterized by oxidative stress, a condition in which an imbalance results between production and inactivation of reactive oxygen species (ROS) (Aude *et al.,* 2004; Furukawa *et al.,* 2004; Ando and Fujita, 2009). It has been well documented that ROS play an important role in the pathogenesis of high energy intake-induced obesity, insulin resistance and cardiovascular disease (Roberts *et al.,* 2006; Matsuzawa *et al.,* 2008). Glucose metabolism disorder, thyroid hormones secretion imbalance and redox status disturbance are certainly the major causes of metabolic syndrome-born disease (Matsuzawa *et al.,* 2008; Pasupathi *et al.,* 2009). Obviously, free radicals and ROS would cause severe insults inside our body and unfortunately it is claimed that the brain is more susceptible to oxidative damage attributed to its high level of energy metabolism, unsaturated fatty acids and poor antioxidant defense system (Emerit *et al.,* 2004). Resveratrol, quercetin and lipoic acid however, scavenge most of ROS and restores body's normal activity (Bastiantto *et al.,* 2000; Arredondo *et al.,* 2010). Endless scientific endeavors were recently carried out in order to bring to our understanding about the association between consumption of high energy foods and metabolic syndromes, though few of them has completely made it clear about the changes that would occur in different periods of time. This study is aimed at analyzing the degree of metabolic changes in high-fat diet fed mice in different weeks as well as to get an insight on when HFD related diseases can be reversed during antioxidants resveratrol, quercetin and lipoic acid supplementation. CAT (Catalase), SOD (Sodium dismutase), MDA (Malondialdehyde), 3-NT (3-nitrotyrosine), TAOC (Total antioxidant capacity) and AOPP (Advanced oxidation protein products) was used to explore periodic changes in central redox state and their association to glucose metabolism behavior.

Materials and methods

Animals and experimental design: Female C57BL/6 mice (4 week old) were purchased from Shanghai Laboratory Animal Center, Chinese Academy Sciences. After being fed with normal diet for a week, the animals were randomly and averagely divided into three groups according to their body weight: (a) mice given a standard chow containing 5.34% fat (Control); (b) mice given a high-fat diet containing 19.57% fat (HFD); (c) mice given a HFD containing 0.01% Quercetin (HFD+Q) (Table 1). The mice were housed in a room under conditions of controlled temperature (23±1°C) and humidity (60%) with a 12-h light/12-h dark cycle. In addition, animals were given free access to water and food. The experimental protocol was developed according to the institution's guideline for the care and use of laboratory animals.

Table 1. Diet composition (%).

Ingredient	Content (%)	
	Normal diet	High-fat diet
Corn meal	53.00	31.00
Soybean meal	24.44	31.00
Wheat flour	9.00	12.24
Wheat bran	6.00	3.00
NaCl	0.20	0.20
$Ca(HCO_3)_2$	1.20	1.20
$CaCO_3$	1.60	1.60
Lard	2.80	18.00
Mineral mix	0.06	0.06
Lysine	0.28	0.28
Methionine	0.20	0.20
Vitamin mix	0.02	0.02
Sucrose	0.10	0.10
Choline chloride	0.10	0.10
Cholesterol	1.00	1.00

Diet composition

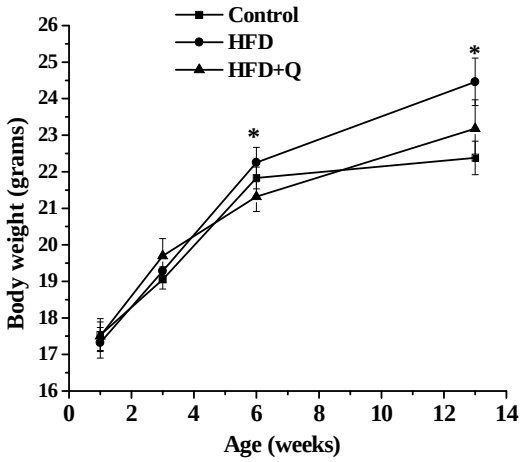
Corn meal: Corn meal contains 9.2% protein, 73.8% carbohydrate and 3.5% fat. Soybean meal contains 41.5% protein, 35% carbohydrate and 5% fat. Lard provides the following (g/100 g lard): 14:0, 2.0; 14:1, 0.3; 15:1, 0.1; 16:0, 26.5; 16:1, 3.7; 17:0, 0.5; 17:1, 0.4; 18:0, 12.1; 18:1, 42.5; 18:2(ω-6), 9.8; 18:3(ω-3), 0.7; 20:0, 0.2; 20:1, 0.6; 20:4(ω-6), 0.3.

Minerals (mg/1 kg feed): Fe, 53.7; Cu, 6.45; Mn, 53.7; Zn, 32.09; Se, 0.11; I, 0.32.

Vitamins (mg/1 kg feed): vitamin B_1, 6; vitamin B_2 (riboflavin), 6 vitamin B_3 (nicotinic acid), 30; vitamin B_5 (pantothenic acid), 16; vitamin B_6, 7; vitamin B_{11} (folic acid), 2; vitamin B_{12} (cobalamin), 0.01; vitamin A, 1.32; vitamin H (biotin), 0.2; vitamin D, 0.025; vitamin E, 50; vitamin K, 2; add multiple vitamin to 200 mg.

Tissue preparation and blood sampling: Experimental animals were treated keeping in mind our philosophy which states that animals must be managed and cared for through the application of uniform acceptable standards, in facilities designed for animal holding, in caging which provides for comfort and safety. As well the animal's social and behavioral needs were addressed: Unnecessary pain, stress, and anxiety were avoided according to Chinese Council of Medical Research. On the day of termination, the mice were slightly anesthetized and sacrificed by enucleation of eyeball after a 12-h fast. Blood was collected into centrifugal tubes containing heparin. Plasma samples were prepared from the blood and stored at -23°C until further analysis. The cortex tissue was immediately dissected, weighed and homogenized in a glass Teflon homogenizer with physiological saline to obtain 1:10 (weight/volume), whole homogenate which was stored at -80°C. A slice of liver, muscle, cortex and hypothalamus tissue was dissected and collected into centrifugal tubes containing 400 mL cell lysates (Biozol) then stored at -80°C for later RNA analysis.

Fig. 1. The effect of high-fat diet on mice's body weights with and without antioxidant factor at different weeks.



Values are expressed as mean ± SD for ten animals. *P<0.05 as compared to the respective value of control animals; **P<0.01 as compared to the respective value of control animals; #P< 0.05 as compared to the respective value of HFD animals. From day one of feeding only a HFD to 13 weeks, mice were remarkably overweight but were not considered obesity.

Measurement of GSSG/GSH, TAOC, MDA, AOPP and 3-NT: After the tissues homogenates were centrifuged at 11,000 g for 15 min at 4°C and the supernatant was taken for assaying GSH/GSSG, TAOC, MDA, AOPP, and 3-NT. Glutathione (GSH) and Glutathione disulfide (GSSG) were measured by fluorescence probe o-phthalaldehyde (OPA, Sinopharm, Shanghai, China) at 430 nm (Senft *et al.,* 2000).

Statistical analysis: All results were expressed as mean ± standard deviation. Comparisons across groups were performed by one-way analysis of variance. A difference of P<0.05 was considered statistically significant. Difference and correlation analysis was done with SPSS 17.0 (SPSS, Inc., Chicago, IL, USA).

Results and discussion

The change of body weight: Compared with control group, the body weight in HFD group increased at 7.83% in 13th week as shown on Fig. 1.

The level of markers of oxidative stress: As shown from Table 2, feeding high-fat diet for 6 weeks did not obviously bring about significant changes in the level of Glutathione/Glutathione disulfide (GSH/GSSG), Total antioxidant capacity (TAOC) and Malondialdehyde (MDA) in cortex (P>0.05). Normal central function is founded on the basis of redox homeostasis. Under physiological circumstances, central ROS-scavenging system and antioxidant enzymes can maintain the balance of the redox state. Nevertheless, the brain is more susceptible to oxidative damage compared with other tissues when insulted by external negative stimulus.

Table 3. The levels of GSH/GSSG ratio, TAOC and MDA: 3rd to 13th week.

Group	Control	HFD	HFD+Q
GSH/GSSG			
3 week	1.17±0.07	1.43±0.11	1.30±0.32
6 week	0.45±0.04	0.48±0.08	0.42±0.03
13 week	0.75±0.07[b]	0.63±0.04[a]	0.74±0.03[b]
TAOC			
3 week	0.66±0.06	0.65±0.05	0.67±0.05
6 week	0.62±0.08	0.58±0.07	0.56±0.07
13 week	1.17±0.16[c]	0.56±0.07[a]	0.86±0.09[b]
MDA			
3 week	3.28±0.38	3.12±0.30	3.15±0.26
6 week	4.63±0.43	4.64±0.46	4.09±0.38
13 week	2.59±0.31[a]	4.13±0.69[b]	3.05±0.48[a]

TAOC expressed in U/mg protein, MDA in nmol/mg protein. Different letters in the same row means P<0.05, having same letter means P>0.05.

The available data show that feeding high-fat diet for 13 weeks resulted in a significantly decrease in the level of GSH/GSSG and TAOC and an increase in the level of MDA in cortex (Table 2). Moreover, cortex of HFD group exhibited obvious oxidative damage with a lower level of GSH/GSSG and TAOC, but a higher level of AOPP, 3-NT and MDA. These findings suggested that consuming high-fat diet for more than 13 weeks impaired central antioxidant system and brought about protein oxidation and lipid peroxidation and quercetin was an antioxidant choice rather than resveratrol and lipoic acid. The outcome was coherent to several previous studies which indicated feeding high-fat diet could result in central oxidative stress (Zhang *et al.,* 2005; Bruce-Keller *et al.,* 2009). A deep study on glutathione proved that GSH works in concert with peroxides and free radicals to antagonize oxidative damage to the mercapto group and protect its membrane proteins (Meister and Anderson, 1983). Basically, under physiological circumstances, the level of GSH is much higher than GSSG and GSH/GSSG is stable with a certain percentage which defines cellular redox status. The normal content of GSH is affected by age, nutritional status and endocrine effects. In fact, the level of GSH would decrease in case of some pathological changes, such as hypothyroidism, liver disease and oxidative stress, which finally cause a decrease in the level of GSH/GSSG and impaired antioxidant capacity. MDA level shows how the body is under stress and it is used as measure of metabolic disorder susceptibility. It is also known as to be a byproduct of polyunsaturated fatty acid peroxide degradation and can potentially cause cytotoxicity to cross-linked polymers of protein including nucleic acid (Niehau and Samuelsso, 1968). However, after antioxidant factors intervened, the level of GSH/GSSG ratio and TAOC increased, MDA decreased significantly (P<0.05) and quercetin was reported the most prominent. AOPP was discovered in the plasma of patients with chronic renal failure (CRF) by Witko-Sarsat in 1996 whose main component is the product of serum albumin attacked by free radicals (Witko-Sarsat *et al.,* 1996) and since, then it is considered one of specific signs of protein oxidation. 3-NT is the product of tyrosine attacked by reactive nitrogen. Tyrosine residues, as a phosphorylated site, play an important role in keeping the normal function of the protein. Nitration in tyrosine residues leads to termination of phosphorylation process and destruction of protein spatial structure (Butterfield *et al.,* 2007). Same group of researcher continued our feeding up to 26 weeks and HFD group exhibited a significant lower level of GSH/GSSG and TAOC, but a higher level of MDA, Advanced oxidative protein products (AOPP) and 3- Nitrotyrosine (3-NT) in contrast to control animals (P<0.05) which is undisputable sign of a disturbed metabolism from HFD long-term exposure. As a result, nutrition-related metabolic disease, including obesity, insulin resistance and dyslipidemia, is becoming a major global public health problem (Miehira and Tappy, 2002; Alberti *et al.,* 2006). The antioxidant factor quercetin used in our study significantly raised the level of GSH/GSSG and TAOC, but lowered the level of MDA, AOPP and 3-NT (P<0.05).

Acknowledgements

The authors are grateful for the financial support from the 12th Five-Year Plan for Science and Technology Development of China (Grant No. 2012BAD33B05) for this study.

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