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Original Contribution

DIET-INDUCED CHANGES IN SOME PARAMETERS OF OXIDATIVE STATUS IN WISTAR RATS – COMPARISON BETWEEN THREE DIFFERENT DIETS AND EFFECTS OF REPLACING DIETARY SUCROSE WITH STEVIOL GLYCOSIDES

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ABSTRSACT

PURPOSE: The aim of the study was to evaluate the effects of a high-fat diet, high-carbohydrate diet and a combined high-fat high-carbohydrate diet on some parameters of oxidative status in a rat experimental model, and to test the potential positive effects of replacing sucrose with stevia extract in some of the diets. METHODS: The following parameters of oxidative status were measured: advanced oxidation protein products (AOPP), catalase, glutathione and ferric reducing ability of plasma (FRAP). Fifty-six male Wistar rats were used in the study. Rats were divided into seven groups: 1) group BD (before diet), in which parameters were measured before beginning of the diet; and six more groups in which parameters were measured after 5 weeks on the respective dietary regimen, as follows: 2) group SD (standard diet); 3) group HFD (high-fat diet); 4) group HCHD (high-carbohydrate diet); 5) group HFHCHD (high-fat high-carbohydrate diet); 6) group SD-S (standard diet with added stevia extract); 7) group HFD-S (high-fat diet with added stevia extract). RESULTS: Diets enriched with fats and carbohydrates induce significant changes in some of the oxidative status parameters. CONCLUSIONS: A five-week experimental diet enriched with fats and carbohydrates has the potential to disrupt pro-oxidant / antioxidant balance in rats.

Key words: oxidative stress, AOPP, FRAP, catalase, glutathione, high-fat diet, high-carbohydrate diet

INTRODUCTION

The pro-oxidant / antioxidant balance is an important part of bodily homeostasis. It is determined by the equilibrium between exogenous and endogenous pro-oxidants and the antioxidant systems (1). Generally, pro-oxidants generate reactive oxygen species (ROS) or inhibit the antioxidant systems. While antioxidants, on the other hand, are such compounds which can either inhibit chemical reactions producing free radicals or directly scavenge and neutralize free radicals (2). In the state of homeostasis, the production of reactive oxygen species is a regulated physiological process. Uncontrolled overproduction of ROS exceeding the inhibiting and neutralizing capacity of the antioxidant systems leads to an imbalance known as oxidative stress, which may potentially result in damage of proteins, lipids and cellular DNA (3). Oxidative stress is involved in the pathogenesis of many diseases such as: atherosclerosis, chronic obstructive pulmonary disease, Alzheimer's disease, complications of diabetes and cancer, thus emphasizing the various mechanisms by which ROS contribute to cellular damage (4, 5). Recently, oxidative stress has been recognized as a key pathogenic factor decreasing insulin sensitivity by changing insulin receptor signal transduction at different levels in peripheral tissues. As a result, the expression of GLUT4 in the cellular membrane is being decreased (6). Various parameters have been widely used to assess the oxidative status in clinical samples – oxidative stress index, serum levels of malondialdehyde, levels of ROS, AOPP,

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catalase, glutathione, FRAP and various exogenous antioxidants (7).

It is also generally accepted that insulin resistance and obesity are related. Adipose tissue has been suggested to secrete pro-inflammatory cytokines and to induce mitochondrial dysfunction, which is also involved in the pathogenesis of insulin resistance. Moreover, recently studies have demonstrated that obesity can induce insulin resistance through oxidative stress (8-10).

Different experimental animal models have been intensively used in insulin resistance, diabetes, metabolic syndrome and obesity related research (11). Such models are helpful tools in studying the pathogenesis of various disorders but are also useful in testing new treatment approaches (12). In terms of obesity, diet-induced models involving mice or rats are most commonly used. Diet-induced models of obesity are based either on a single type of diet or a combination of diets, such as high-fructose, high-glucose, high-sucrose, high-fat, high-fructose/high-fat, or high-sucrose/high-fat diets, with the high-fat diet being most commonly used. Many research teams have used experimentally various models of high-fat diets with different amount of fat – fats supplying between 20 and 60 % of total energy of the diet. The fats included in the diet can be either of plant origin (corn, sunflower or olive oil) or animal-derived fats (beef tallow or lard). High-fat diets and other diets with high calorie content have been used specifically to induce experimental obesity in small rodent animals (13-15). High-fat diets and other high-calorie diets have been experimentally proven to induce hyperglycemia, insulin resistance, dyslipidemia and increased free fatty acids in the blood (15, 16). More recently, scientific research has focused on the relation between high-fat / high-carbohydrate dietary intake and oxidative stress (17, 18). The growing awareness of diet-related diseases has also prompted scientific research on the use of natural sweeteners like stevia extracts. Several studies have demonstrated potential antioxidant, anti-inflammatory and antidiabetic effects of steviol glycosides (19, 20).

The aim of the present study was to evaluate the effects of a high-fat diet, high-carbohydrate diet and a combined high-fat high-carbohydrate diet on some parameters of oxidative status in a rat experimental model and to test the potential positive effects of replacing sucrose with stevia extract in some of the diets.

MATERIALS AND METHODS

Animals

Fifty-six male Wistar rats, aged 9-10 weeks, were used in the study. Rats were housed indoors at a constant ambient temperature of 22 ± 2ºC, controlled humidity – 55 ± 10 %, 12:12 h light-dark photoperiod and had access to water and food ad libitum.

All experimental procedures were in accordance with the ethical standards (Permit № 335 of the Bulgarian Food Safety Agency; Statement of the Ethics Committee № 251 of 19.10. 2022).

Experimental design

Rats were divided into seven equal groups – six of the groups were submitted to different dietary regimens for a period of five weeks, and one pool group was used to measure the initial levels of laboratory parameters before the beginning of the diets.

Groups: 1) group BD (before diet), in which parameters were measured before beginning of the dietary regimens; 2) group SD (standard diet) – rats were fed a standard pellet diet for laboratory rats (Melhran, LTD, Bulgaria); 3) group HFD (high-fat diet) – rats from this group were fed the same standard pellet diet supplemented with lard in such an amount that 40% of the energy intake was supplied by fat; 4) group HCHD (high-carbohydrate diet) – animals from this group were fed the standard pellet diet supplemented with sucrose in such an amount that 75% of the energy content was supplied by carbohydrates; 5) group HFHCHD (high-fat high-carbohydrate diet) – rats were fed the standard pellet diet supplemented with lard and sucrose (30% of energy coming from fat and 57% from carbohydrates); 6) group SD-S (standard diet with added stevia extract) – rats from this group were fed the standard pellet diet supplemented with stevia extract equivalent to the amount of sucrose in the HCHD, according to the instructions of the manufacturer (Balgarska Stevia, LTD, Bulgaria); 7) group HFD-S (high-fat diet with added stevia extract) – rats from this group were fed the standard pellet diet supplemented with lard (same amount as in HFHCHD) and stevia extract (equivalent to the amount of sugar in HFHCHD).

Stevia extract (Balgarska Stevia, LTD, Bulgaria): steviol glycosides (96.8 %) – rebaudioside A (61.06 %), stevioside (30.36 %), rebaudioside C (12.42 %), dulcoside A (2.49 %), steviolbioside (0.2 %), rebaudioside B (0.16 %), rubusoside (0.12 %).

Laboratory parameters

The parameters of oxidative status were measured in plasma, serum and red blood cells lysate. The following parameters were measured: advanced oxidation protein products (AOPP – μmol/L) – by the method of Hanasand *et al*. (21); catalase (CAT – kU/L) – by the method of Goth (22); glutathione (GSH – mmol/L) – by the method of Beutler *et al*. (23); ferric reducing ability of plasma (FRAP – mmol/L) – by the method of Benzie and Strain (24).

Statistical analysis

Results are presented as mean ± SD. Data was submitted to the one-way ANOVA test and Tukey`s post hoc test (Graph Pad InStat3). Differences were considered statistically significant at the $p<0.05$ level.

RESULTS

Baseline AOPP levels were 87.29±11.4 μmol/l. After 5 weeks of application of the respective dietary regimes, the values of this indicator increased in the HFD group, and the changes were statistically significant compared to BD ($p<0.01$), as well as compared to SD and SD-S groups ($p<0.001$) and HCHD, HFD-S groups ($p<0.05$). The values in the HCHD and HFD-S groups were significantly higher than those in the SD group ($p<0.01$). In the HFHCHD group, the increase was significant as compared to SD ($p<0.001$) and to SD-S ($P<0.01$), (**Figure 1**).

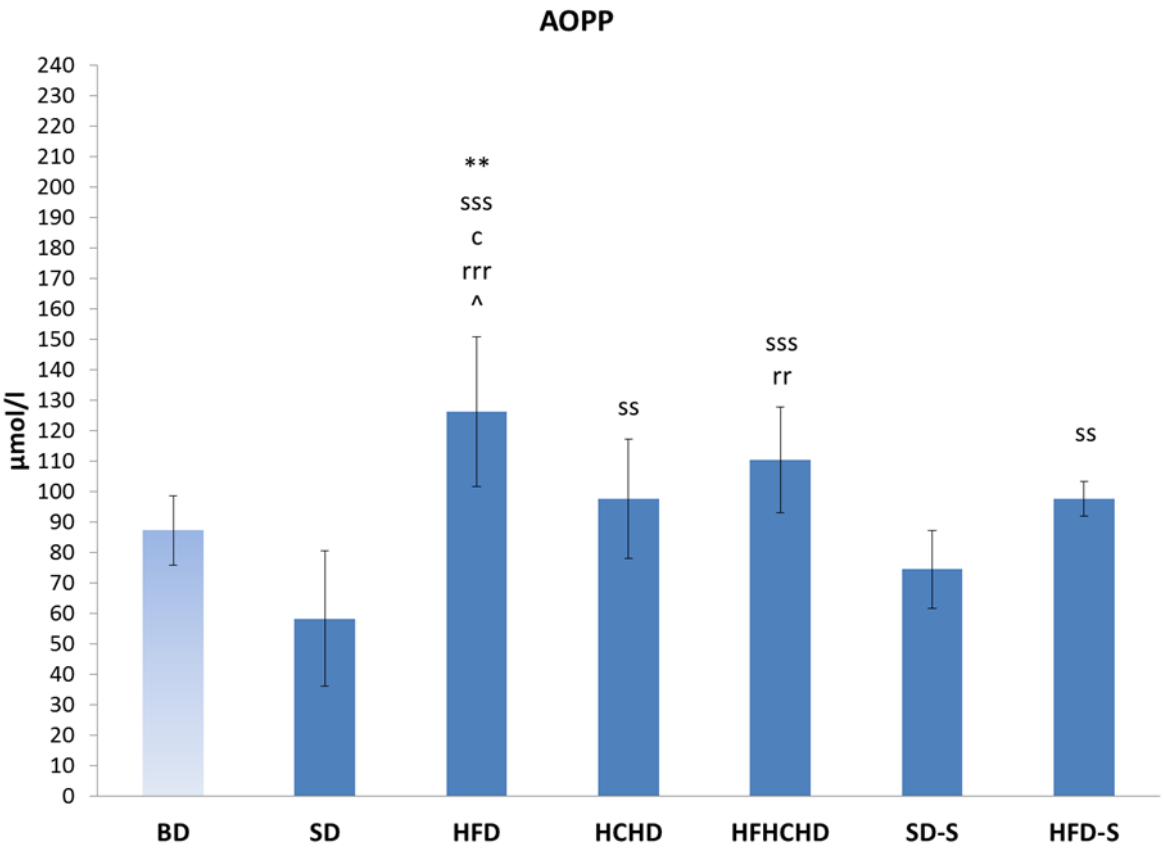

Figure 1. AOPP levels in rats (n=8) before diet (BD) and in groups SD (n=8), HFD (n=8), HCHD (n=8), HFHCHD (n=8), SD-S (n=8), HFD-S (n=8). Results are presented as mean ± SD. Statistically significant differences are indicated as follows: ****** $p<0.01$ compared to BD; **rr** $p<0.01$ compared to SD-S; **rrr** $p<0.001$ compared to SD-S; **ss** $p<0.01$ compared to SD; **sss** $p<0.001$ compared to SD; **c** $p<0.05$ compared to HCHD; ^ $p<0.05$ compared to HFD-S.

Baseline catalase levels were 109.3±32.8 kU/l. After application of the respective diets for 5 weeks, catalase levels increased significantly in the HFD and HCHD groups compared to the SD-S group ($p<0.05$), (**Figure 2**).

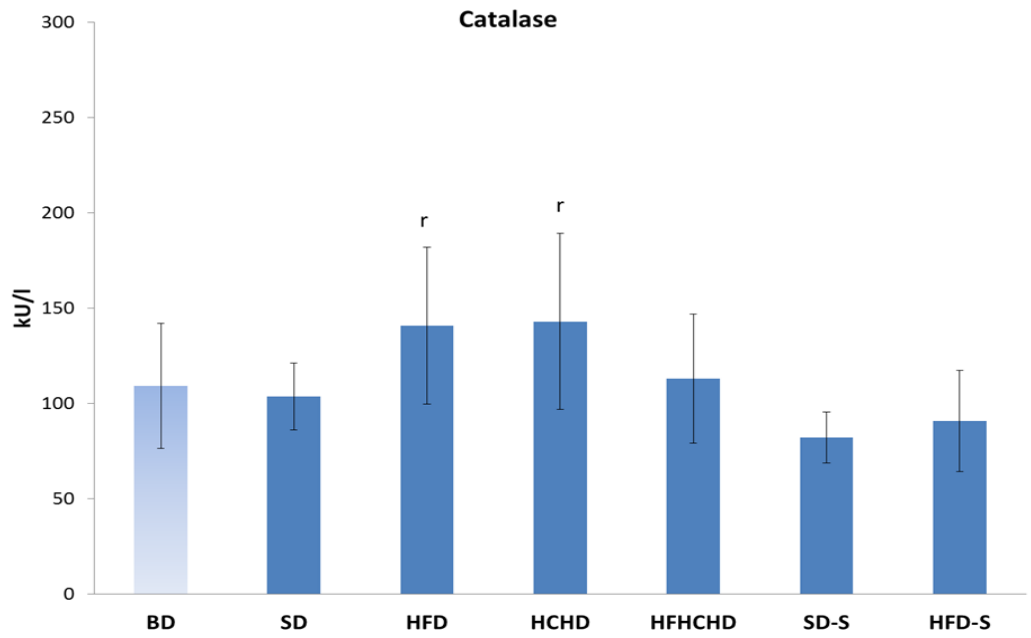

Figure 2. Catalase levels in rats (n=8) before diet (BD) and in groups SD (n=8), HFD (n=8), HCHD (n=8), HFHCHD (n=8), SD-S (n=8), HFD-S (n=8). Results are presented as mean ± SD. Statistically significant differences are indicated as follows: **r** p<0.05 compared to SD-S.

Baseline glutathione concentrations were 1.16±0.27 mmol/l. After the application of the respective dietary regimes for a period of 5 weeks, the values of this indicator increased in the HFD group, and the increase was significant compared to the HCHD group (p<0.05) and compared to the SD-S and HFD-S groups (p<0.001). In the SD group, significantly higher values were also recorded compared to the HCHD group (p<0.05) and compared to the SD-S and HFD-S groups (p<0.01), (**Figure 3**).

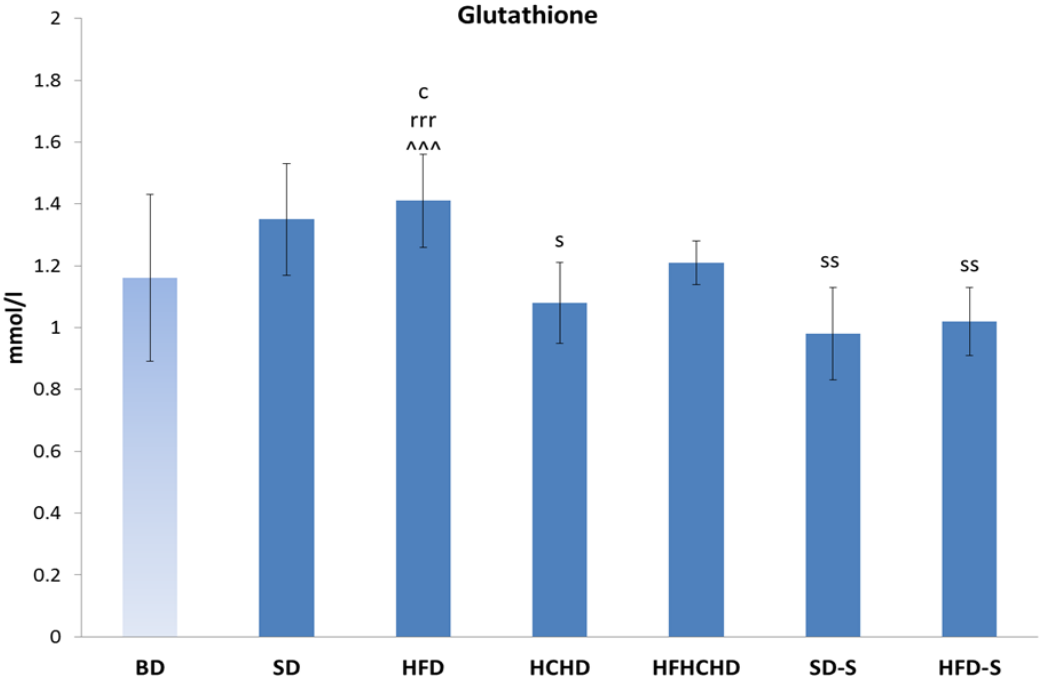

Figure 3. Glutathione levels in rats (n=8) before diet (BD) and in groups SD (n=8), HFD (n=8), HCHD (n=8), HFHCHD (n=8), SD-S (n=8), HFD-S (n=8). Results are presented as mean ± SD. Statistically significant differences are indicated as follows: **rrr** p<0.001 compared to SD-S; **s** p<0.05 compared to SD; **ss** p<0.01 compared to SD; **c** p<0.05 compared to HCHD; ^^^ p<0.001 compared to HFD-S.

Baseline FRAP values were 0.22±0.05 mmol/l. After 5 weeks of application of the respective dietary regimens, the values of this indicator increased statistically significantly in all groups compared to the initial level ($p<0.001$). Significantly lower values were recorded in the HFHCHD group compared to the HCHD group ($p<0.05$), (**Figure 4**).

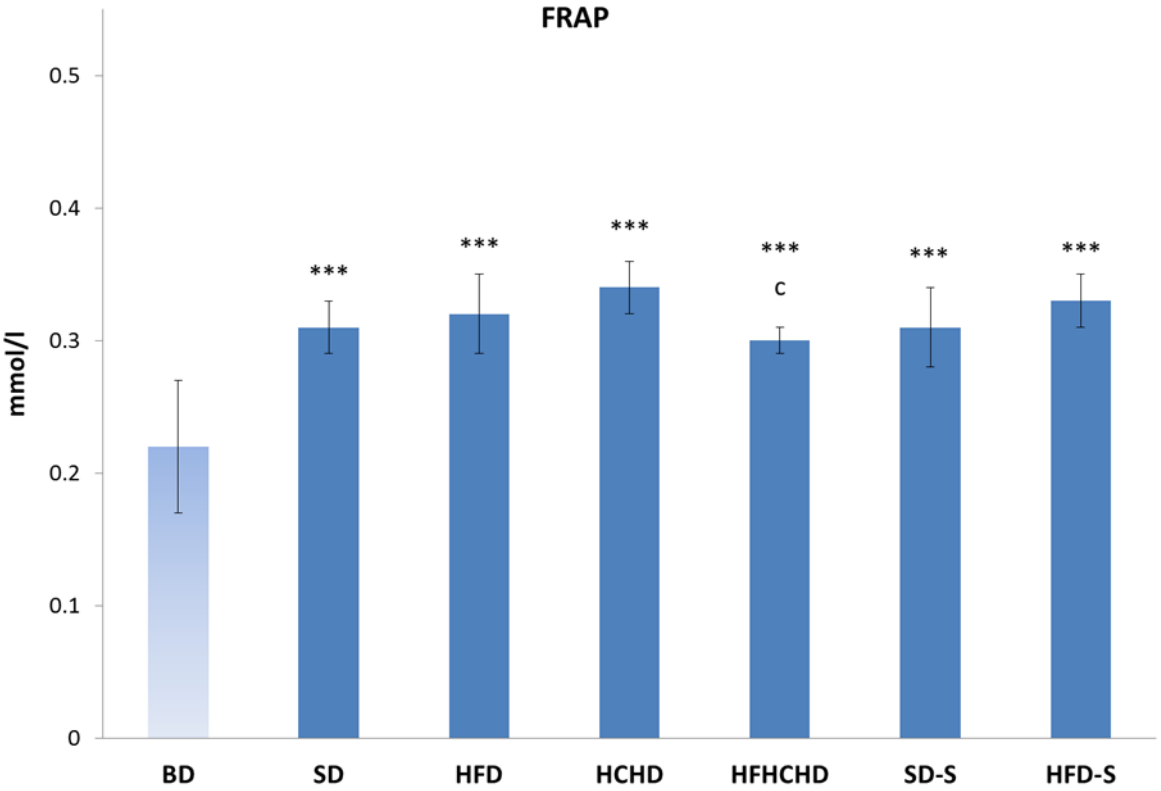

Figure 4. FRAP levels in rats (n=8) before diet (BD) and in groups SD (n=8), HFD (n=8), HCHD (n=8), HFHCHD (n=8), SD-S (n=8), HFD-S (n=8). Results are presented as mean ± SD. Statistically significant differences are indicated as follows: ******* $p<0.01$ compared to BD; **c** $p<0.05$ compared to HCHD.

DISCUSSION

Oxidative stress is characterized as a state of imbalance between oxidants and antioxidants, in which oxidants predominate. Reactive oxygen species (ROS) have the potential to oxidize proteins and lipids, which in turn leads to disorders of energy metabolism, disorders of signaling between and within cells, disorders of nutrient transport and eventually cellular dysfunction and damage (25). Thus, oxidative stress is generally recognized to be the basis for the development of a variety of diseases and disorders – vascular disorders, insulin resistance, diabetes type 2, metabolic syndrome, age-related degenerative diseases, etc. (26). ROS includes both free radical and non-free radical oxygen intermediates (peroxides), like superoxide, singlet oxygen, and hydroxyl radical. Hydrogen peroxide (H_2O_2) is not as reactive as the previously mentioned chemicals, but is easily activated and is also classified as ROS. These molecules are generated from diatomic oxygen (O_2) by membrane enzymatic systems (such as NADPH oxidases). Lipid metabolism within the peroxisomes and various cytosolic enzymes are also sources of ROS (27). Although all these sources generate oxidants, the vast majority of ROS (approximately 90%) is generated in mitochondria as a result of the electron transport chain and oxidative phosphorylation (28). Although hydrogen peroxide is a relatively stable molecule, most of the ROS have a very short life (less than a second) and their direct measurement is difficult. For this reason, indirect measurement of ROS by evaluating the damage they cause to lipids and proteins is a preferable approach to assess oxidative stress in biological samples (7). Measurement of protein carbonyl content is a valuable source of information about the oxidative status of an organism. Protein carbonyls are modified proteins generated by the oxidation of some amino acids by ROS (29). Measurement of

advanced oxidation protein products (AOPP) is another useful method to evaluate the oxidative status of the organism (30). In accordance with other similar studies (31), our research demonstrates that a high-fat diet, high-carbohydrate diet and combined (high-fat high-carbohydrate) diet have the potential to induce oxidative stress, which is confirmed by the statistically significant increase of AOPP after experimental rats have been on the specific dietary regimens for a period of 5 weeks. High-fat and high-carbohydrate diets induce oxidative stress through increased fatty acid metabolism within peroxisomes and mitochondria. In addition, high calorie diets can also cause obesity, which leads to low-grade inflammation through the accumulation of white adipose tissue that secretes pro-inflammatory factors. Several studies have demonstrated that fat tissue derived pro-inflammatory factors can activate immune cells, which produce ROS in very high concentrations (32). Our research supports the statement that replacing sucrose in the high-carbohydrate diet with stevia extract seems to be useful in avoiding oxidative stress. As stevia extract has zero calories, the overall reduction of calorie intake seems to be a reasonable explanation for this. Nevertheless, replacing sucrose with stevia extract in the high-fat high-carbohydrate diet is not efficient in reducing dietary induced oxidative stress. The high content of fats alone seems to be sufficient to activate excessive ROS generation, which supports the statement that steviol glycosides simply reduce the calorie intake without exerting any pharmacological effects.

Our research also supports the generally accepted statement that oxidative stress activates endogenous antioxidants. We have recorded increased levels of catalase in the high-fat and high-carbohydrate groups. Catalase is known to degrade hydrogen peroxide into water and oxygen, thus protecting the cells from ROS induced damage (33). Moreover, some studies have suggested that catalase induction is an immediate antioxidant response to a high fat diet, which is at least partly regulated at the transcription level (34). Our study has also demonstrated that a high-fat diet increases glutathione levels as part of the antioxidant enzyme systems. Although some researchers have demonstrated that continuous high-calorie intake may deplete the GSH stores (35), our study obviously did not have that long duration needed for GSH depletion. Many studies have suggested that measurement of ferric reducing ability of plasma is a useful method for assessment of the antioxidant power of biological fluids (24, 36). Nevertheless, some researchers have stated that FRAP may not be useful as a measure of antioxidant power for some specific disorders (37). Our study failed to record any significant changes in FRAP induced by the dietary regimens, except for the lower initial levels, which could be related to the age difference.

CONCLUSIONS

A five-week experimental diet enriched with fats and / or carbohydrates has the potential to disrupt pro-oxidant / antioxidant balance in rats. Diets with high fat content have the highest potential to induce oxidative stress.

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Conflict of interests

The authors declare no conflict of interests.

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