Soluble fiber-enriched diets improve inflammation and oxidative stress biomarkers in Zucker fatty rats

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Running Title: Fiber improves inflammatory and oxidative state in Zucker rats.

Abstract

In this study we evaluated the effect of the administration of different soluble fiber enriched-diets on inflammatory and redox state of Zucker fatty rats. Four groups of ten 8 week-old female Zucker fatty rats were used. The four groups were respectively fed the following diets until the 15th week of life: standard diet (obese control), 10% high methoxylated apple pectin (HMAP), 5% soluble cocoa fiber (SCF), and 10% β-glucan enriched diets. A group of Zucker lean rats fed the standard diet as control for normal values of this rat model was also used. The plasma levels of tumoral necrosis factor-α (TNF-α), adiponectin, malondialdehyde (MDA) and reduced glutathione liver levels were measured at the end of treatment. TNF-α plasma levels decreased somewhat in Zucker fatty rats fed the different fibers, and MDA plasma levels significantly decreased in these animals. Nevertheless, adiponectin plasma levels increased in the Zucker fatty rats fed the SCF enriched diet, but did not change in the HMAP and the β-glucan group. The Zucker fatty rats fed the different fiber showed a trend towards increased the reduced glutathione liver levels, but significant differences with obese control levels were only obtained in the β-glucan group. The results obtained in this study suggest that the intake of the different soluble fiber-enriched diets that we have evaluated could prevent and/or attenuate the inflammatory and/or the prooxidative state of the metabolic syndrome.

Key words: Fiber, Inflammation, Obesity, Oxidative stress, Zucker rats

1 Introduction

The metabolic syndrome, which clusters different metabolic abnormalities such as central obesity, low concentrations of plasma high-density lipoprotein cholesterol, high levels of triglycerides, hypertension and hyperglycemia, together with insulin resistance [1,2], is associated with an increased risk of both cardiovascular disease [3,4] and type 2 diabetes [5]. Elevated levels of inflammatory biomarkers and proinflammatory cytokines evidenced the implications of ~~an~~ the inflammatory state in the metabolic syndrome [6-9].

Special attention is focussed on the effects of dietary fibers or high-fiber foods and the mechanisms by which they could regulate the production of inflammation markers involved in the pathogenic pathway of metabolic syndrome such as proinflammatory cytokines, acute-phase response markers and the anti-inflammatory adipocytokine adiponectin [10,11]. In addition, it has been also reported that dietary fiber intake may reduce the risk of several cardiometabolic diseases by mediating the proinflammatory process [12-14]. Two mechanistic hypotheses have emerged. Firstly, dietary fiber may decrease oxidation of glucose and lipids while maintaining a healthy intestinal environment. Secondly, dietary fiber may prevent inflammation by altering adipocytokines in adipose tissue and by increasing enterohepatic circulation of lipids and lipophilic compounds [15]. In this context, several researchers have recently reported an inverse association between dietary fiber intake and levels of the inflammatory biomarkers in hypertensive diabetic or obese people [16-19].

Zucker fatty rats can be considered as the most appropriate ~~best~~ experimental model of genetic obesity and resistance to insulin. These animals resemble in many ways the human metabolic syndrome. Additionally, Zucker fatty rats develop a proinflammatory response and oxidative stress [20]. Previous studies have shown that soluble fibers, such as high methoxylated apple pectin (HMAP), soluble cocoa fiber (SCF) and β-glucan attenuated some of the main clinical alterations that characterize the metabolic syndrome [21-23]. Therefore, the aim of this study was to evaluate the effect of the above mentioned fibers on the inflammatory and oxidative stress status of Zucker fatty rats. For this purpose, we assessed different biomarkers in plasma and tissues of Zucker fatty rats fed these fibers.

2 Material and Methods

Forty female 8 week-old Zucker fatty rats, weighing 260-275 g, and ten 8 week-old female Zucker lean rats, weighing 150-175 g, all purchased from Charles River Laboratories (Charles River Laboratories, Spain), were used in this study. The Zucker fatty rats were randomly divided into four groups of ten animals that were respectively fed the following diets until the 15th week of life: standard diet (obese control), 10% high methoxylated apple pectin (HMAP), 5% soluble cocoa fiber (SCF) and 10% β-glucan enriched diets. The Zucker lean rats were fed the standard diet. This group of animals was used as control for normal values of this rat model. The diets were prepared by Harlan Interfauna Ibérica (Barcelona, Spain). The standard diet (AIN-93M purified Rodent Diet) provides the nutrients required by adult rats according to the National Research Council guidelines (National Research Council, NIH Publication No. 85, Washington DC, 1985, p. 23). The other three diets contained either 10%

HMAP (apple pectin with 73% methylation degree, provided by Obipektin, Switzerland), 5% SCF (Soluble Cocoa Fiber product provided by Natraceutical Group, Spain), or 10% β-glucan [oat bran concentrate provided by Glambia Nutritionals, Belgium]. The four diets were prepared and formulated to provide the same amount of protein (14%), fat (4%) and carbohydrates (72%), and therefore, the same energy value. In particular, the β-glucan and SCF-enriched diets were formulated taking into account the amount of protein, fat and carbohydrates provided by oat bran concentrate and SCF. The composition of the four diets is presented in table 1.

The 10 lean Zucker rats were in turn fed the standard diet until the 15th week of life. During the experimental period the animals were maintained at a temperature of 23º C, with 12 h light/dark cycles and were fed *ad libitum* with free access to water. The values of solid and liquid diet intake, systolic and diastolic blood pressures, body weight, glucose, cholesterol and insulin of all these Zucker rats have been previously described in detail [21,22]. At the end of the experimental period (15th weeks of life), the rats were sacrificed by decapitation after an over-night fasting. Blood and liver samples were obtained to carry out the following biochemical determinations: plasma TNF-α, plasma adiponectin, plasma MDA and reduced glutathione in liver.

In this study, all the experiments were performed as authorized for scientific research (European Directive 86/609/CEE and Royal Decree 223/1988 of the Spanish Ministry of Agriculture, Fisheries and Food).

2.1 Plasma and tissue preparations

Blood samples from the sacrificed animals were collected into tubes containing lithium heparin as anticoagulant. These samples were centrifuged at 2500 g for 20 minutes at 4ºC to obtain the plasma which was divided into aliquots and kept frozen at -80ºC until analysis of TNF-α adiponectin and MDA. Livers were homogenized at 4 ºC in a Potter with PBS (0.01 M PBS, 0.15 M NaCl, pH 7.4), the homogenates were centrifuged at 5000 g for 15 min at 4ºC and the supernatant was recovered. The supernatants of the centrifuged samples were kept frozen at –80ºC until used for hepatic reduced gluthation evaluation. The protein content of the homogenates was determined by the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA), using bovine serum albumin as standard.

2.2 Plasma TNF-α determination

TNF-α concentration in plasma was determined using a rat TNF-α ELISA kit (Bender Medsystems, Austria) according to the manufacturer instructions. Spectrophotometric measurements were made at 450 nm using a spectrophotometer (Molecular Devices Thermo max Inc. Sunnyvale, USA). The plasma TNF-α values were expressed as pg/ml.

2.3 Plasma adiponectin determination

Plasma adiponectin concentration was determined using a mouse/rat adiponectin ELISA kit (B-Bridge International, USA). Spectrophotometric measurements were made at 450 nm with a spectrophotometer (Molecular Devices Thermo max Inc. Sunnyvale, USA). The plasma adiponectin values were expressed as μg/ml. The analytical sensitivity of the ELISA E091R yields < 0.081 ng/ml.

2.4 Malondialdehyde determination

Plasma malondialdehyde (MDA) levels were measured by a thiobarbituric acid assay [24].Plasma were mixed with 20% trichloroacetic acid in 0.6 M HCl (1:1, v/v), and the tubes were kept in ice for 20 min to precipitate plasma components and avoid possible interferences. Samples were centrifuged at 1500 g for 15 min before adding thiobarbituric acid (120 mM in Tris 260 mM, pH 7) to the supernatant in a proportion of 1:5 (v/v); then, the mixture was boiled at 97ºC for 30 min. Spectrophotometric readings at 535 nm were made at 20ºC. The plasma MDA values were expressed as µmol/mL MDA.

2.5 Glutathione determination

Reduced glutathione hepatic levels were determined by monochlorobimane fluorimetric method [25]. For this, 90 μl of liver homogenized supernatants were mixed with 10 μl of glutathione S-transferase solution (1U/ml) obtained from horse liver (Sigma-Aldrich, USA), and monochlorobimane (Fluka Biochemical, Switzerland) (100 mM). This reaction is catalysed by glutathione S-transferase. The levels of glutathione were quantified by a fluorimeter (Multiscan Asccent Labsystems, Spain) and were expressed as μmol/g tissue protein.

2.6 Statistical analysis

The results are expressed as mean values ± S.E.M. for a minimum of 8 rats. The data were analyzed by one-way ANOVA using GraphPad Prism 4 software. Differences between the groups were assessed by the Bonferroni test. Differences between the means were considered to be significant when $P < 0.05$.

3 Results

The results revealed that the TNF-α plasma levels in the Zucker fatty group fed the standard diet were the highest, whereas the Zucker lean rats showed the lowest levels of plasma TNF-α. Although the consumption of soluble fiber enriched-diets (HMAP, SCF or β-glucan) resulted in a slight trend to decrease of TNF-α plasma levels, no statistical differences were observed between these values and the values of this biomarker in the Zucker fatty rats fed the standard diet. Differences were neither observed between TNF-α plasma levels in the groups treated with fiber and TNF-α plasma levels in the Zucker lean rats (Figure 1).

No differences were observed between the Zucker fatty group and the Zucker lean group fed the standard diet. The Zucker fatty group fed the β-glucan-enriched diet showed the lowest value of adiponectin plasma levels, but no differences were observed between this group and the above mentioned groups. Differences were neither observed between The Zucker fatty rats fed the HMAP enriched diet nor the groups fed standard diet. Nevertheless, the Zucker fatty rats fed the SCF-enriched diet showed higher levels of plasma adiponectin when compared with the other groups (Figure 2).

MDA plasma levels were significantly higher in the Zucker fatty rats fed the standard diet than in the other Zucker rats. No differences were observed between the MDA plasma levels in the Zucker fatty groups fed fiber and in the Zucker lean rats. In addition, differences were neither observed in the levels of this metabolite between the three groups fed different fiber-enriched diets (Figure 3).

No differences were found between the liver glutathione levels of the Zucker fatty rats fed the standard diet and the levels of this biomarker in the Zucker lean rats. The Zucker fatty rats fed the different fiber showed a trend towards increased this variable, but significant differences with obese control levels were only obtained in the β-glucan group. However, no differences were observed between the liver glutathione levels in the animals fed the β-glucan enriched diet and those found in the animals fed the other fibers (Figure 4).

4 Discussion

The metabolic syndrome is associated with a long-term inflammatory condition and it causes an unusual production of cytokines [26], such as TNF-α [27,28]. This factor is over expressed in obesity and may regulate insulin resistance [27]. It has been also reported that Zucker fatty rats are characterized by TNF-α overproduction in adipose tissue [29, 30] and by a decreased plasma concentration of adiponectin, which contribute to insulin resistance [10]. In our study, the Zucker fatty rats fed the standard diet showed TNF-α plasma levels higher than those of the Zucker lean rats. The Zucker fatty rats fed the HMAP-, β-glucan- or the SCF- enriched diets showed a slight trend to decrease TNF-α plasma levels when compared to the Zucker fatty rats fed the standard diet. These results suggest that the intake of soluble fiber may improve the proinflammatory state that characterizes obesity. The results mentioned above are in agreement with the results reported by Galisteo et al., in 2005. These researchers demonstrated that the intake of a diet supplemented with husk of Plantago ovata, rich in soluble fiber, decreased TNF-α plasma levels in Zucker fatty rats [10]. In addition, several epidemiologic studies have shown an inverse association between the intake of fiber and TNF-α plasma levels [31] and also between the intake of fiber and other proinflammatoy mediators, such as C-reactive protein [31,32] and different interleukins [33].

In this study, the adiponectin plasma levels in the different groups of Zucker rats were also determined. It has been described that this adipocytokine have antiatherogenic and antidiabetic properties and may protect against insulin resistance [34]. Some studies have demonstrated that adiponectin levels are lower in diabetic than in healthy subjects. The decrease of adiponectin plasma levels in patients with type 2 diabetes was reported [35], and the levels of this cytokine also decreased in monkeys that develop type 2 diabetes when insulin sensibility decreased in these animals [36]. In our study, the Zucker fatty rats fed the SCF-enriched diet showed higher adiponectin plasma levels than the Zucker fatty rats fed the standard diet. However, no increase in the adiponectin levels was shown in the fatty animals fed HMAP, and slightly decreased in those fed β-glucan. These results suggest that SCF may protect against insulin resistance. In this context, the intake for 25 weeks of a diet supplemented with husk of Plantago ovata increased the adiponectin levels in Zucker fatty rats [10]. These researchers also concluded that the increase in adiponectin levels may be involved in the improvement of insulin resistance in this animal model [10]. It is worth noting that the adiponectin plasma levels observed in the Zucker fatty rats fed the standard diet were similar to those of the Zucker lean rats. Nevertheless, Oana et al, reported that adiponectin plasma levels in 17 week-old Zucker fatty rats were higher than in 17 week-old Zucker lean rats [37]. They concluded that the adiponectin receptor was less expressed in Zucker fatty rats than in Zucker lean rats. The levels of adiponectin in our study were obtained with 15 week-old Zucker fatty rats and the slightly increase in this biomarker was observed in the fatty animals when compared with 15 week-old Zucker lean animals. In view of all these data, we propose that the differences in adiponectin plasma levels between the fatty and the lean strain could increase with time, becoming significant in the aged rats.

It has been reported that the increase of the oxidative stress in the adipose tissue of obese patients could be one of the main mechanisms involved in the metabolic syndrome [38]. According to Furukawa et al., these patients exhibited an increase of oxygen reactive species and other free radicals and a decrease in the expression of antioxidant enzymes such as dismutase superoxide, reductase glutathione and catalase [39]. In addition, it is known that TNF-α overexpression activates nicotin-adenin dinucleotide phosphate oxidase in Zucker fatty rats. This activation promotes an increase in superoxide anion in these animals [40]. Accordingly, recent studies have suggested the potential therapeutic role of dietary antioxidant supplementation in the reduction of body weight, and its beneficial effect on several obesity related disorders [41].

Plasma MDA and reduced glutathione in the liver enabled us respectively to know the lipid peroxidation and the antioxidant protection degree of the animals. It is known that the oxidative stress is higher in the Zucker obese animals than in the lean animals [42]. Accordingly, in this study, Zucker fatty rats fed the standard diet showed higher MDA plasma levels than Zucker lean rats. In addition, our results indicated that the intake of the different soluble fibers resulted in a decreased lipid peroxidation. The consumption of cereal varieties, particularly rich in anthocyanins such as red or black rice, was reported to affect some oxidative stress biomarkers [43], and it should be noted that the SCF also contains polyphenols [22,23]. On the other hand, studies performed in rats fed whole-grain and refined wheat flours [44], in pigs fed wheat bran [45], and in human fed dietary supplements of wheat bran [46], did not show an effect on plasma MDA levels. It was suggested that the lack of effects may be due to the low antioxidant concentrations attained [44].

Reduced glutathione can scavenge reactive oxygen species and other free radicals. In our study, no changes were observed in the liver levels of this molecule when comparing the Zucker fatty rats fed standard diet and the Zucker lean rats. However, the reduced glutathione liver levels of Zucker fatty rats fed fiber enriched-diets were higher than those of Zucker lean rats. Nevertheless, there was only a significant difference in the β-glucan group. These results are in agreement with other studies. In fact, it was observed that the diet enriched in whole-grain and refined wheat flours produced an increase in liver glutathione in rats, and consequently improved the redox status in these animals [44]. Moreover, water-soluble corn bran hemicellulose suppressed the development of liver injury in rats [47]. It was suggested that the observed finding was partly due to the increase in glutathione concentration in this tissue. Therefore, we could also suggest that the soluble fibers used in this study may improve the redox state and attenuate the oxidative injury. In fact, the modification of the redox state in adipose tissue could be a therapeutic tool against obesity in the metabolic syndrome.

In conclusion, we have demonstrated that all the soluble fibers used in this study, and in particular SCF, improve the proinflammatory state associated with obesity and also protect from the oxidative stress that characterizes this pathology. Accordingly, we suggest that the studied fibres could be incorporated as ingredients in functional foods for beneficial use in obesity and other associated disorders. Nevertheless, human studies should be run to further confirm the findings of the present study in rats.

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Figure legends

Figure 1: TNF-α plasma levels in different groups of rats: Zucker lean fed standard diet (▩), Zucker fatty fed standard diet (□), Zucker fatty fed 10% HMAP-enriched diet (▥), Zucker fatty fed 5% SCF-enriched diet (⊟), Zucker fatty fed 10% β-glucan-enriched diet (■). The results are expressed as mean values ± SEM for a minimum of 8 rats. Different letters represent statistical differences ($p<0.05$).

Figure 2: Adiponectin plasma levels in different groups of rats: Zucker lean fed standard diet (▩), Zucker fatty fed standard diet (□), Zucker fatty fed 10% HMAP-enriched diet (▥), Zucker fatty fed 5% SCF-enriched diet (⊟), Zucker fatty fed 10% β-glucan-enriched diet (■). The results are expressed as mean values ± SEM for a minimum of 8 rats. Different letters represent statistical differences [$p<0.05$].

Figure 3: Malondialdehyde plasma levels in different groups of rats: Zucker lean fed standard diet (▩), Zucker fatty fed standard diet (□), Zucker fatty fed 10% HMAP-enriched diet (▥), Zucker fatty fed 5% SCF-enriched diet (⊟), Zucker fatty fed 10% β-glucan-enriched diet (■). The results are expressed as mean values ± SEM for a minimum of 8 rats. Different letters represent statistical differences [$p<0.05$].

Figure 4: Liver reduced glutathione in different groups of rats: Zucker lean fed standard diet (▩), Zucker fatty fed standard diet (□), Zucker fatty fed 10%

HMAP-enriched diet (▥), Zucker fatty fed 5% SCF-enriched diet (▤), Zucker fatty fed 10% β-glucan-enriched diet (■). The results are expressed as mean values ± SEM for a minimum of 8 rats. Different letters represent statistical differences [$p<0.05$].

Table 1. Composition of the diets [g/100 g dry weight].

	Standard diet	**5 % SCF**	**10% apple pectin**	**10% β-glucan**
PROTEIN	**14**	**14**	**14**	**14**
Casein	14	11.8	14	10.5
FAT	**4**	**4**	**4**	**4**
Soy Bean oil	4	3.36	4	3.63
CARBOHYDRATE	**72**	**72**	**72**	**72**
Sucrose	10	8.70	8.27	7.97
Dextrin	15.5	13.49	12.81	12.35
Corn starch	46.56	40.53	38.48	37.11
Powdered cellulose	5	5	5	5
SCF[1]	0	12.15	0	0
Brown Ribbon pure apple pectin[2]	0	0	12.5	0
Oat bran concentrate[3]	0	0	0	18.52
TBHQ	0.0008	0.0008	0.0008	0.0008
AIN 93 Mineral mixture	3.5	3.5	3.5	3.5
AIN 93 Vitamin mixture	1	1	1	1
L-Cystine	0.18	0.18	0.18	0.18
Choline bitartrate	0.25	0.25	0.25	0.25
ENERGY [kJ/100 g][4]	1589	1589	1589	1589

[1] SCF is Soluble Cocoa Fiber product that contains protein (17.92 %) and fat (5.26%); the exact amount of soluble cocoa fiber is 41.16%.

[2] Brown Ribbon pure apple pectin contains 80% of dietary fiber, 8-10% sugar, 8.7% water and 2-5% salts (viscosity = 188 mPas in 2% solution; density = 0.6-0.9 g/cm^3). The fiber is high methoxylated apple pectin (HMAP) with 73% methylation degree and a polygalacturonic acid content of 65%.

[3] Oat bran concentrate contains protein (19 %), fat (<2%) and carbohydrate (>77%); the amount of oat β-glucan is 54%.

[4] Energy value was determined by calculation in order Atwater system.

The carbohydrates substitution in both experimental diets was formulated at the expense of sucrose, dextrin and starch proportionally.

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Figure 1

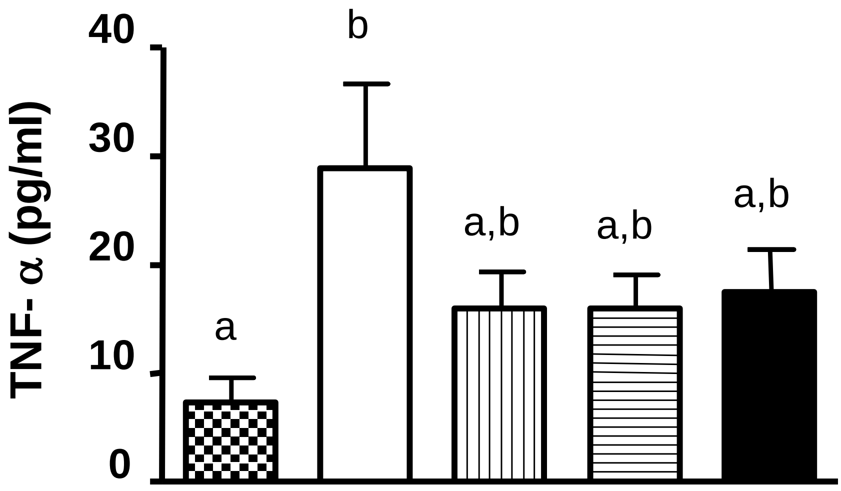

Figure 2

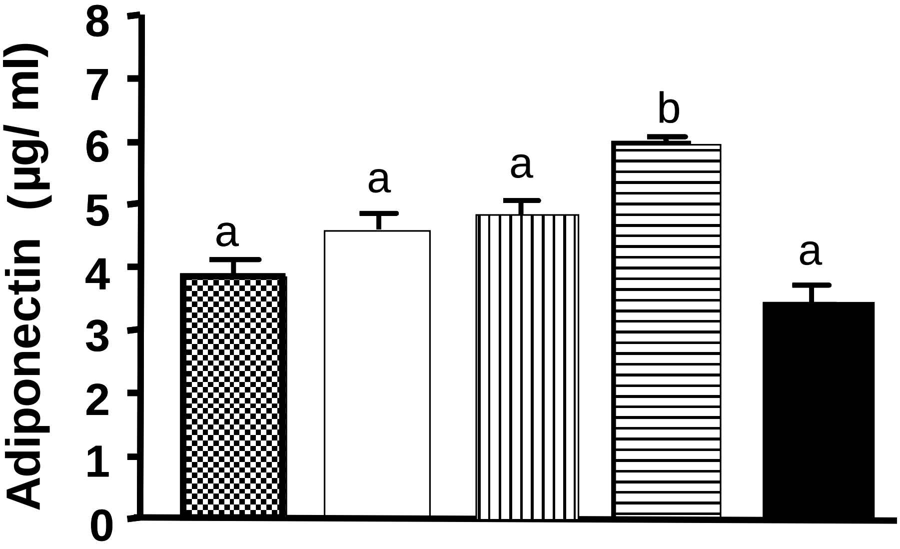

Figure 3

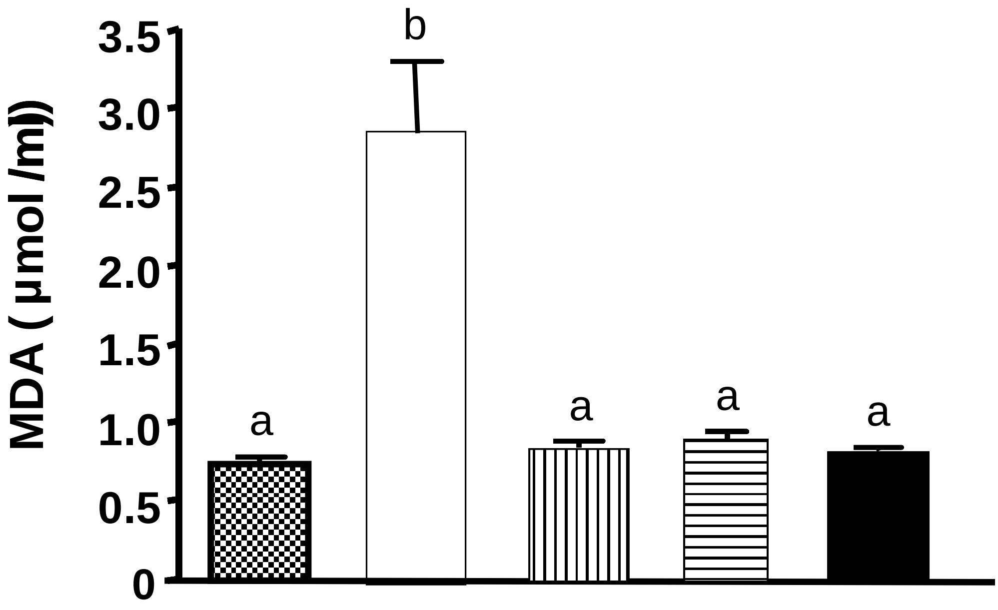

Figure 4