Research Article

Chardonnay grape seed procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers

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Studies reported the effects of polyphenols but not for grape polyphenols towards obesity. We analysed the effects of a polyphenolic grape seed extract (GSE) on obesity and oxidative stress in hamsters receiving a high-fat diet (HFD). Three groups of hamsters received a standard diet (STD), or a HFD plus a daily gavage with water (Control, HFD) or a solution of GSE (HFD + GSE) for 12 wk. Plasma glucose, triglycerides (TG), insulin, leptin and adiponectin were measured. Oxidative stress was assessed by cardiac production of superoxide anion and NAD(P)H oxidase expression. After 12 wk, HFD increased abdominal fat as compared with standards. GSE avoided this feature. HFD led to higher plasma glucose, TG, insulin and greater insulin resistance (HOMA-IR) values. GSE prevented in part these effects, reducing insulinemia and leptinemia by 16.5 and 45%, respectively, whereas adiponectin level increased by 61% compared with obese controls. GSE lowered glycemia and HOMA-IR and strongly prevented cardiac production of superoxide by 74% and NAD(P)H oxidase expression by 30%. This is the first time that chronic consumption of grape phenolics is shown to reduce obesity development and related metabolic pathways including adipokine secretion and oxidative stress.

Keywords: Antioxidant / Grape seed proanthocyanidins / Hamster / Obesity / Oxidative stress

Received: April 29, 2008; revised: May 29, 2008; accepted: June 12, 2008

1 Introduction

According to the International Obesity Task Force, an estimated 300 millions adults worldwide are obese. Obesity, particularly that caused by visceral fat accumulation, is an important risk factor for sedentary lifestyle-related diseases, such as type 2 diabetes mellitus, coronary heart diseases and cerebrovascular events, hyperlipidemia, hypertension and cancer [1–4]. Oxidative stress could be a potential link between fat accumulation and obesity-related morbidity such as diabetes and cardiovascular diseases. Indeed, this pathology can contribute to an increased susceptibility to reactive oxygen species (ROS). In the general population, several groups at risk of high oxidative stress are identified and more particularly the obese subjects, in relation to reduced antioxidant defences and/or a strong free radicals production.

High fat feeding has commonly been used to induce visceral obesity in rodent animal models [5] because of the similar pathogenesis of obesity to that found in humans [6]. Preventive or therapeutic strategies to control most of human obesity should target these abnormalities. Antiobesity foods and food ingredients may avert the condition,

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Abbreviations: GSE, grape seed extract; **GSHPx,** glutathione peroxidase; **HFD,** high-fat diet; **HOMA-IR,** homeostatic model assessment for insulin resistance; **ROS,** reactive oxygen species; **SOD,** superoxide dismutase; **STD,** standard diet; **TG,** triglyceride

possibly leading to the prevention of lifestyle-related diseases, if they are effective in reducing the visceral fat mass [7]. It has been reported that the body weights of rats and their plasma triglycerides (TG), cholesterol and LDL-cholesterol have been significantly reduced by feeding Oolong, black and green tea leaves. Epigallocatechin gallate (EGCG) purified from green tea when given to mice in diet decreased diet-induced obesity by decreasing energy absorption and increasing fat oxidation [8]. Supplementation with tea catechins resulted in a significant reduction of high-fat diet (HFD)-induced body weight gain, adipose tissue mass, liver fat content and the development of hyperinsulinemia and hyperleptinemia in C57BL/6J mice [9]. Recently, the continuous ingestion of a green tea extract high in catechins by humans led to a reduction in body fat, systolic blood pressure and LDL cholesterol, suggesting that the ingestion of such an extract contributes to a decrease in obesity and cardiovascular disease risks [10]. More recently, Wolfram *et al.* [11] showed that supplementation with Teavigo®, a green tea extract containing about 94% EGCG, abolishes diet-induced obesity and claimed that this was a valuable natural treatment option for obesity. Nakagawa *et al.* [12] demonstrated the abdominal fat lowering and hypoglycemic effects of licorice hydrophobic flavonoids in obese diabetic mice and elucidated their mechanisms of action by showing that they induce differentiation of human adipocytes. Sugiyama *et al.* [13] suggested that oligomeric procyanidin was the main contributor to the effect of apple polyphenol extract on inhibiting TG absorption in mice and humans. Apple procyanidins may relieve obesity *via* a lipase inhibiting activity and may be effective for obesity-related diseases. To the best of our knowledge, the antiobesity effects of grape seed catechins have not been examined yet. Only Vadillo *et al.* [14] showed that moderate red wine intake can prevent the increase of body weight by modulating energy intake in a rat diet-induced model of obesity. The present study was designed to examine the preventive effect of a grape seed tannin extract (GSE) on the development of obesity-induced by feeding a HFD in hamsters. Feeding hamsters a cholesterol-supplemented diet produces dyslipidemia [15]. This model was also chosen because hamsters have a plasma lipoprotein distribution similar to that of humans. It has been also used by Simon *et al.* [16] and developed obesity traits, and Leung *et al.* [17] showed that the fat-fed Syrian Golden hamster is a good model of nutritionally induced insulin resistance. Elsewhere, GSE is a rich source of (+)-catechin, (+)-epicatechin and procyanidins [18]. Keaney *et al.* [19] reported that obesity is a strong independent predictor of systemic oxidative stress and suggested that obesity is associated with a state of excess oxidative stress. As one of the possible mechanisms of the obesity-related oxidative stress, we also examined the modulation by HFD (with or without GSE) of cardiac production of superoxide anions and NAD(P)H oxidase expression.

2 Materials and methods

2.1 Phenolic extract

The commercial dry phenolic powder extract (GSE) from Chardonnay grape seeds was provided by Partœno (Bordeaux, France).

2.2 Estimation of total phenols

Total polyphenols content was determined by the colorimetric reaction with the Folin Ciocalteau reagent on an aqueous tannin solution at 1 g/L diluted to 1/100 [20].

2.3 Condensed tannins

Condensed tannins (proanthocyanidins) were determined on the phenolic fraction by the colorimetric method described by Bate-Smith [21]. The Bate-Smith reaction is an oxidative depolymerisation of proanthocyanidins that occurs when heating in a mineral acidic medium. It forms anthocyanins, absorbing at 550 nm (red colour). Sample was diluted 1:50 with a 10% ethanol solution to enter the linear range. Must (2 mL) was treated with 6 mL of reaction reagent (HCl/*n*-butanol/Fe_2SO_4) and the colorimetric reaction was carried out at 100°C for 30 min. The spectrophotometric analysis of anthocyanidins was carried out at 550 nm. The quantification was carried out by an external standard calibration curve and results were expressed in mg/L of leucocyanidin equivalents.

2.4 Proanthocyanidic oligomers

Proanthocyanidic oligomers are estimated by the DMACH method according to Nagel and Glories [22] by mixing 5 mL of dimethylaminocinnamaldehyde reagent to 1 mL of aqueous tannin solution (1 g/L). After 10 min the absorbency was measured at 640 nm. The results are expressed as equivalent catechin.

2.5 Standards and HPLC analysis

(+)-Catechin and (+)-epicatechin were obtained from Aldrich (St. Quentin Fallavier, France). Procyanidin dimers B1 and B2 were obtained from grape seeds as previously reported [23]. HPLC analysis with UV detection was performed using a Hewlett-Packard Model 1090 and a Nucleosil 100 C18 column (250×4 mm^2, 5 µm particle size). The solvents used for separation [24] were as follows: solvent A, 50 m mol/L ammonium dihydrogen phosphate adjusted to pH 2.6 with orthophosphoric acid; solvent B, 20% A with 80% ACN; solvent C, 200 m mol/L orthophosphoric acid adjusted with ammonia to pH 1.5. Elution was performed with a gradient previously described [23]. Detection was carried out at 280, 313, 365 and 520 nm.

Table 1. HFD and STD composition

	HFD	STD
% Energy as protein	18.89	19.24
% Energy as carbohydrate	36.75	63.69
% Energy as fat	44.36	17.06
Fibre (g/100 g)	5.90	5.00
Lard (g/100 g)	21.00	0
Soyabean oil (g/100 g)	3.00	8.00
Cholesterol (g/100 g)	0.10	0
% Saturated fat	9.56	1.00
% Monounsaturated fat	10.61	1.81
% Polyunsaturated fat	3.86	0.48
Energy density (kJ/g)	20.85	17.66

2.6 Animals and experimental design

Forty-two male golden Syrian hamsters (Janvier, Le Genest-St-Isle, France) 4 wk old and weighing 70–80 g were randomly divided into groups with approximately equal mean group body weights. The animals were housed in polycarbonate cages in a temperature controlled room (23 ± 1°C) subjected to a 12 h light/dark cycle (lights on at 0700 h) with free access to both food and water. Food intake was daily recorded and body weight every other day. After a 4 day adaptation period, the hamsters were randomly divided into three groups of 14 animals each and fed either a regular diet (standard group, STD) for 12 wk, giving nonpathological controls or a HFD for 12 wk. The diets were prepared in pelleted form by Genestil SA (Royaucourt, France) and contained a standard complement of vitamin and mineral mixes formulated according to AIN-93 guidelines [24] and all essential nutrients. The diet components are listed in Table 1. The hamsters of each group additionally received by daily gavage either tap water (standard group, STD and control group, HFD) or a solution of a grape seed phenolic extract (GSE) in water (experimental group, HFD + GSE). The volume of solutions force feed was adjusted daily to the weight of hamsters: it was established by extrapolating 500 mL/d average beverage consumption for a 70 kg human to the equivalent for the daily weight of hamsters. This represents a volume of 7.14 mL/(kg body wt. day). The amount of GSE given (Chardonnay grape seed granulated tannin) corresponded to 2.5 g/day for a 70 kg human. Hamsters were handled according to the guidelines of the Committee on Animal Care at the University of Montpellier and NIH guidelines [25]. At the end of the experimental period, the hamsters were deprived of food overnight and blood samples were collected under anaesthesia (Pentobarbital) by cardiac puncture. Plasma was prepared by centrifugation at 2 000 × g for 10 min at 4°C, then stored at –80°C until analysis. Plasma glucose and TG were measured by means of an enzymatic technique (KonePro, Konelab, Evry-Les-Lys, France) using reagents from Thermo Electron Corporation (Cergy Pontoise, France). Insulin, adiponectin and leptin were assessed by ELISA using commercial kits (Linco, St. Charles, MO, USA). The homeostatic model assessment for insulin resistance (HOMA-IR) was used because it is a valuable method that shows a strong relationship with euglycemic–hyperinsulinemic clamp [26]. It was calculated from insulin and glucose values using the formula of Matthews *et al.* [27]:

HOMA-IR = Fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5

Adipose tissue from abdominal region was dissected and weighed. The liver was perfused with 0.15 mol/L KCl to remove residual blood, rapidly excised, rinsed in ice cold saline, blotted dry, weighed, sectioned for analyses and stored in liquid nitrogen. Liver was homogenised in 4 volumes of ice cold 0.1 mol/L potassium phosphate buffer (pH 7.4) and the homogenate was spun at 13 000 × g for 15 min at 4°C. The supernatant was then stored at –80°C for subsequent assay of glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) activity on an automat Pentra 400 (HORIBA ABX, Montpellier, France). GSHPx activity was measured by the method of Randox (Randox Laboratories LTD, Crumlin, UK) using a commercial kit (Ransel, no. RS505). SOD activity was determined using a Randox kit (Ransod, no. SD 125).

2.7 Determination of superoxide anion production

Briefly, the left ventricle [28] was washed three times in Krebs buffer and immediately homogenised and centrifuged at 4 000 rpm for 20 min. The supernatant was used to study NAD(P)H-dependent superoxide production. Lucigenin (10 µM)-enhanced chemiluminescence was used to measure superoxide production with NADPH (100 µM). The intensity of luminescence was recorded on a luminometer (Perkin Elmer Wallac, Victor, Turku, Finland). Results were expressed as count/mg of protein.

2.8 Immunoblotting

Proteins were extracted as previously described [28] from the frozen left ventricle. Samples were homogenised using an ultra turrax T25 basic (Irka-Werke) in an ice cold extraction buffer containing 120 mM NaCl, 25 mM KCl, 2 mM $CaCl_2$, 15 mM Tris-Cl pH 7.5, 0.5% Triton, 1 mM PMSF, 0.1 mM DTT, 10 M leupeptin and 1 M pepstatin. Protein concentration in samples was determined by BioRad Dc protein assay using BSA as a standard. Proteins (50 µg) were separated with 12% SDS-PAGE and then transferred to a nitrocellulose membrane (45 min, 100 V). Membranes were blocked overnight and incubated for 1 h with primary antibody against $p22^{phox}$ (1/200, Santa Cruz Biotechnology, Santa Cruz, CA) in blocking buffer. After three washes (6°C, 5 min) in TBS/Tween under gentle agitation, blots were incubated for 1 h with horseradish peroxidase-labelled antibody (1/5000). After further washes, blots were treated

with enhanced chemiluminescence detection reagents (ECL, Amersham) and areas (mm^2) measured using the BIO-Profil 1D software (Fisher Bioblock). Normalisation to β-actin (antiactin, 1:5000, Chemicon International, Temecula, CA) was used to verify the uniformity of protein load and transfer efficiency across the tested tissues (data not shown).

2.9 Statistical analyses

Data are shown as the means ± SEM of 14 measurements *per* group. Data were subjected to logarithmic transformation when necessary to achieve homogeneity of variances. Statistical analysis of data was performed by one-way ANOVA followed by Fisher's Protected Least Significant Difference posthoc procedure using a Stat View IV software (Abacus Concepts, Berkeley, CA). Differences were considered to be significant at $p < 0.05$.

3 Results

3.1 Composition of the grape seed extract

Phenolics levels in the dry powder extract GSE are given for information in Table 2. GSE is a rich source of polyphenols, especially flavanols, which represents approximately 69% dry weight of the extract. Catehins are the predominant flavanols and are mainly comprised of flavanol monomers (as (+)-catechin and (+)-epicatechin). Procyanidin dimers B1 and B2 were also present in the extract that contained 653 mg/g phenolic compounds expressed as gallic acid equivalents.

3.2 Nutritional and blood parameters

The effects of high-fat feeding and GSE treatment on hamster weight, plasma insulin, TG, and glucose are shown in Table 3. Hamsters fed a HFD gained more weight than standard hamsters. Treatment of fat-fed hamsters with GSE decreased their body weight compared to fat-fed hamsters. No difference in food and energy intake was found between control group and group receiving phenolics. Feeding the HFD (control group, HFD) increased body fat accumulation and adipose tissue weight from abdominal region was significantly greater (49%) that in standard hamsters. The consumption of GSE at the same time that the HFD reduced this effect (29%). In spite of fat intake being significantly increased in HFD and HFD + GSE groups, the total energy intake of the three groups of hamsters were comparable, indicating that body weight gain in the HFD-fed group may be related to fat intake but not to higher energy intake. HFD feeding led to significantly greater fasting plasma concentrations of glucose and insulin associated with a three-fold increase in TG (Table 3). Giving GSE did not totally prevent these effects, although they induced a slight improvement of glycemia and triglyceridemia. Hamsters fed the HFD also exhibited higher leptin level and lesser adiponectin level than those fed the standard diet (STD) (Table 3). Giving GSE by gavage to hamsters fed the HFD significantly improved the adipokine pattern thereby totally preventing the leptin increase and significantly increasing adiponectine level by 61% in comparison with obese controls (HFD) (Table 3). Although the slight decrease in plasma insulin (16.5%) did not reach significance, GSE-induced a significant lowering of glycemia and HOMA-IR value (Table 3) without any effect on TG.

Table 2. Phenolics levels in Chardonnay GSE

Phenolic	mg/g
Total phenols content[a)]	653.0
Polymeric tannins[b)]	448.0
Proanthocyanidic oligomers[c)]	127.2
Catechin[d)]	41.4
Epicatechin[d)]	43.1
Dimer B1[d)]	40.7
Dimer B2[d)]	36.1
Sum of catechins[e)] (monomers and dimers)	161.3

a) Expressed as gallic acid equivalent.
b) Expressed as leucocyanidin equivalent.
c) Expressed as catechin equivalent.
d) Obtained by HPLC-UV.
e) Procyanidins dimers B1, B2 + catechin + epicatechin.

3.3 Antioxidant status

In left cardiac ventricle, GSE improved the antioxidant status and decreased superoxide anion production ($O_2^{\bullet -}$) by preventing in part NAD(P)H oxidase expression. As shown in Table 4, superoxide anion production and expression of $p22^{phox}$, a membrane subunit of NAD(P)H oxidase, decreased by 74% and 30%, respectively, in hamsters receiving GSE in comparison with the obese controls (HFD). Liver antioxidant enzymes (Table 5) activity such as SOD and GSHPx were decreased by 30 and 73%, respectively, in hamsters fed GSE in comparison to obese hamsters and stayed higher than (SOD) or did not differ from (GSHPx) those in standard hamsters.

4 Discussion

The high-fat-fed Syrian Golden Hamster is known as a good model of nutritionally induced-insulin resistance, with significant reduction in whole body glucose disposal and insulin suppressibility of endogenous glucose production [29]. Here, the hamster dose of polyphenols is adjusted to a human dealcoholised wine consumption of about two glasses *per* meal. This study shows for the first time that supplementation with grape seed tannins reduced the HFD-induced body weight gain as well as fat accumulation in

Table 3. Effects of a GSE consumption on body weight, food and energy intakes, and plasma glucose, TG, insulin, leptin and adiponectin of hamsters fed a STD, a HFD or a HFD plus a GSE (HFD + GSE) for 12 wk

	STD	HFD	HFD + GSE
Initial body weight (g)	77 ± 1[a]	76 ± 4[a]	74 ± 3[a]
Final body weight (g)	91 ± 2[a]	115 ± 10[b]	100 ± 6[c]
Body weight gain (g)	14 ± 3[c]	38 ± 2[a]	25 ± 2[b]
Abdominal white fat (g)	1.08 ± 0.06[c]	2.45 ± 0.12[a]	1.71 ± 0.04[b]
Abdominal white fat (% BW)	1.19 ± 0.11[c]	2.20 ± 0.12[a]	1.55 ± 0.03[b]
Food intake (g/d)	3.7 ± 0.3[a]	3.0 ± 0.1[b]	2.9 ± 0.1[b]
Fat intake (kJ/d)	11.3 ± 0.7[b]	27.9 ± 1.5[a]	26.7 ± 1.1[a]
Energy intake (kJ/d)	66.2 ± 7.6[a]	64.4 ± 3.5[a]	59.9 ± 2.7[a]
Triglycerides (mmol/L)	0.97 ± 0.09[b]	3.14 ± 0.33[a]	2.76 ± 0.19[a]
Glucose (mmol/L)	4.76 ± 0.41[c]	6.85 ± 0.44[a]	5.57 ± 0.37[b]
Insulin (ng/mL)	0.27 ± 0.02[b]	0.66 ± 0.07[a]	0.55 ± 0.07[a]
HOMA-IR	37.79 ± 3.02[c]	132.95 ± 11.24[a]	90.06 ± 8.66[b]
Leptin (ng/mL)	1.52 ± 0.09[b]	2.63 ± 0.22[a]	1.45 ± 0.23[b]
Adiponectin (ng/mL)	3.11 ± 0.12[a]	1.43 ± 0.15[c]	2.26 ± 0.20[b]

Plasma glucose, TG, insulin, leptin and adiponectin were measured at the fasted state at the end of the 12-week treatment period. Values are means ± SEM ($n = 14$). Means in a row with superscripts without a common letter differ, $p < 0.05$.

Table 4. Cardiac superoxide anion production ($O_2^{\bullet-}$) and expression of NADP(H) oxidase $p22^{phox}$ subunit in hamsters fed a STD or a high-fat diet without (HFD) or with a GSE (HFD + GSE) for 12 wk[a)]

	STD	HFD	HFD + GSE
$O_2^{\bullet-}$ (counts/mg protein)	520 ± 30[A]	896 ± 140[B]	229 ± 45[C]
NADP(H) oxidase expression[b)]	5.5 ± 0.7[A]	20.4 ± 1.8[B]	14.3 ± 1.3[C]

a) Values are means ± SEM ($n = 14$). Means in a row with superscripts without a common letter differ, $p < 0.05$.
b) Blot intensity expressed as arbitrary units $\times 10^{-3}$.

Table 5. Liver antioxidant enzymes SOD and GSHPx activity in hamsters fed a STD or a HFD diet without or with a GSE (HFD + GSE) for 12 wk[a)]

	STD	HFD	HFD + GSE
SOD (U/mg protein)	227 ± 24[A]	654 ± 31[B]	458 ± 42[C]
GSHPx (U/mg protein)	13 ± 4[A]	72 ± 1[B]	19 ± 2[A]

a) Values are means ± SEM ($n = 14$). Means in a row with superscripts without a common letter differ, $p < 0.05$.

abdominal adipose tissue which appears to be positively related to glucose homeostasis. Moreover, GSE improved glusose homeostasis by decreasing glycemia and HOMA-IR value as previously reported in other animal model with a different phenolic extract [30]. By contrast, GSE did not significantly prevent hypertriglyceridemia. Interestingly, GSE acts on the obesity-related imbalance between leptin and adiponectin. Classically, leptin reduces ectopic fat accumulation in nonadipose tissues, enhancing insulin-mediated stimulation of glucose disposal [31], and leptinemia increased here in HFD group. Moreover, adiponectin promotes fatty acid oxidation in muscle and inhibits liver glucose production [32], and adiponectinemia was reduced here in HFD group. Thus, leptin and adiponectin may work hand in hand to sensitise peripheral tissues to insulin. In this study, GSE favours the effects of these adipocytokines, in agreement with previous *in vitro* observation showing that the monomeric procyanidin catechin is an inducer of adiponectin expression and secretion in the adipocyte cell line 3T3-L1 [33].

It is generally assumed that obesity is a prooxidant state related in part to insulin resistance. In addition, autoxidation of glucose and nonenzymatic glycation of proteins may generate superoxide [34], a radical species implicated in vascular cell-mediated LDL oxidation. Hyperglycemia also induces the enzymatic production of superoxide through activation of NAD(P)H oxidase in vascular cells [35]. Elsewhere, insulin itself promotes hydrogen peroxide generation in fat cells [36] prompting speculation that oxidative stress is a principal mechanism of insulin resistance with chronic hyperinsulinemia. This hypothesis is further supported by Fortuno *et al.* [37] suggesting that hyperinsulinemia may contribute to oxidative stress in metabolic syndrome patients through activation of NAD(P)H oxidase. On

the other hand, it has also been suggested that lipid disorders could enhance ROS formation. The observed overproduction of superoxide anion in neutrophils from hyperlipidemic guinea pig could be linked to an induction of NAD(P)H oxidase subunit in particular gp^{91phox} [38]. It has been reported in humans that systemic oxidative stress is strongly associated with visceral fat accumulation and metabolic syndrome [39]. Our observation of a HFD-induced obesity associated with insulin resistance and lipid disorders enhancing NAD(P)H oxidase expression (+270%) in cardiac tissue extends these observations, suggesting that insulin resistance and dyslipidemia conspire in oxidative stress.

NAD(P)H oxidase overexpression and activity could be also related to adipokine imbalance. In adiponectine –/– mice an overexpression of NAD(P)H oxidase subnunits has been observed in heart [40] and kidneys [41] strongly showing that adiponectine could downregulate superoxide anion production. Elsewhere, it has been recently demonstrated that leptin increases NAD(P)H oxidase protein expression and activity in isolated murine cardiomyocytes and this effect is attenuated by endothelin (ET-1) receptor antagonists [42]. The observation that GSE both improves adiponectin and leptin levels and decreases NAD(P)H oxidase expression and activity strongly suggests a potential link between these events. Whether endothelin plays a pathogenic role in obesity is not clear, but in experimental obesity, there is an increase in gene and protein expression of endothelin in the cardiovascular system, including vasculature and kidney [43]. Because leptin stimulates ET-1 production in cardiomyocytes [44] as well as in endothelial cells [45], these results suggest that leptin may stimulate ROS formation through endothelin and NAD(P)H oxidase-dependent pathway and that phenolics present in GST are efficient in reducing these effects. As recently demonstrated by Corder *et al.* [46], phenolic compounds, principally procyanidins, are able to reduce the production of endothelin-1 by endothelial cells. Inhibition of endothelin-1 overexpression is, therefore, a further potential mechanism for the observed protective effects of grape seed tannins consumption. From this study, we can assume that NAD(P)H oxidase is upregulated by HFD due to adipokine imbalance and viceral fat accumulation (Table 3), and possibly ET-1 overexpression. GSE could prevent all these events. Interestingly, HFD increased liver antioxidant enzymes mainly SOD and GSHPx activity that could in part counteract the increase in $O_2^{\bullet -}$ production; this could be due to an oxidative system-induced regulation [47]. In this context, GSE consumption lowered hepatic SOD and GSHPx activities (Table 5). One explanation for this downregulation is that it is a consequence of the sparing effect of dietary antioxidants being able to scavenge oxygen radicals, and thus reduce the requirement for enzymatic endogenous antioxidant [48–50]. Since GSE totally prevent $O_2^{\bullet -}$ production (Table 4) but only partly decreased SOD and GSHPx activities (Table 5), we can assume that GSE favours the antioxidative balance. Thus, GSE has beneficial effects on preventing diet-induced obesity by improving oxidative stress markers.

All of these results suggest that GSE acted by mechanisms operating-at least in part-inside an antioxidant effect and the possibility that adiponectin might modulate oxidative stress, leading to antiobesity effects. Thus, we provided insights into one mechanism, increased oxidant stress, that probably contributes to the pathological after-effects of obesity and that may have important public health implications, being a target for interventions to decrease the pathology. Therefore, the potential for antioxidant therapy to decrease obesity risks needs to be explored.

The authors have declared no conflict of interest.

5 References

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