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Preventive Effect of a Melon Extract Rich in Superoxide Scavenging Activity on Abdominal and Liver Fat and Adipokine Imbalance in High-Fat-Fed Hamsters

Kelly Décordé,[†] Anta Agne,[†] Dominique Lacan,[§] Jeanne Ramos,[#] Gilles Fouret,[⊥] Emilie Ventura,[†] Christine Feillet-Coudray,[⊥] Jean-Paul Cristol,[†] and Jean-Max Rouanet*,[†]

[†]UMR 204 Prévention des Malnutritions et des Pathologies Associées, Université Montpellier Sud de France, France, [§]Bionov Sarl, Site Agroparc, Avignon, France, [#]Service d'Anatomie Pathologique, Centre Hospitalo-Universitaire Gui de Chauliac, Montpellier, France, and [⊥]UMR 866 Différenciation Cellulaire & Croissance, INRA, Montpellier, France

Studies showed that dietary antioxidants could be a therapy against obesity that is associated with a state of oxidative stress. Thus, this paper investigates whether a dietary ingredient, a melon juice extract rich in superoxide dismutase, would prevent the development of such obesity in hamsters. Five groups received a standard diet or a high-fat diet (HF) plus a daily gavage with water (control) or extract at 0.7, 2.8, or 5.6 mg/day. After 84 days, the higher dose lowered triglyceridemia (68%), production of liver superoxide anion (12%), mitochondrial cytochrome *c* oxidase activity (40%), lipid and protein oxidation products (35 and 35%, respectively), and leptinemia (99%) and increased adiponectinemia (29%), leading to a concomitant reduction in insulinemia (39%), insulin resistance (41%), and abdominal lipids (25%). The extract triggered a remarkable decrease of liver lipids (73%) and fully prevented the steatohepatitis induced by the HF diet. Chronic consumption of this melon extract may represent a new alternative to reduce obesity induced by a high-fat diet.

KEYWORDS: Obesity; melon extract; hepatic ballooning degeneration; hamsters; antioxidants

INTRODUCTION

High-fat diets and sedentary lifestyles are important risk factors for obesity, which is a key feature of metabolic syndrome and which greatly predisposes individuals to liver diseases, cardiovascular disease, type 2 diabetes, dyslipidemia, hypertension, and numerous cancers and is associated with markedly diminished life expectancy (*1*). We previously investigated a hamster model of diet-induced obesity and insulin resistance that exhibits oxidative stress and some correlates to human obesity (*2*). A close correlation has been found between increased oxidative stress in accumulated fat and the pathogenic mechanism of obesity and obesity-associated metabolic syndrome, and Keaney et al. (*3*) reported that obesity is a strong independent predictor of systemic oxidative stress, suggesting that obesity is associated with a state of excess oxidative stress. This may be the source of several metabolic dysfunctions such as inflammation, hypertension, and impaired glucose intake in muscle and fat, which are highly related to obesity.

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 defenses and/or a strong free radicals production. Of the many potential cellular sources of chronic ROS production, mitochondria and nonphagocytic NAD(P)H oxidase are the major sources under physiological conditions (*4*, *5*). Increased mitochondrial ROS generation and dysfunction are associated with cardiovascular and many other diseases (*6*).

Elsewhere, adipocytes synthesize and secrete adipocytokines. Dysregulated production of these adipocytokines participates in the pathogenesis of obesity-associated metabolic syndrome. Among them, adiponectin exerts insulin-sensitizing (*7*) and antiatherogenic effects (*8*) and, hence, a decrease in plasma adiponectin is causative for insulin resistance and atherosclerosis in obesity. However, obesity has been shown to be one of the conditions that decrease antioxidant capacity (*10*), and it seems to accomplish this by lowering the levels of antioxidant enzymes (catalase, glutathione peroxidase, and glutathione reductase) (*9*). In animal (*10*) and human studies (*11*), obesity is associated with a decrease in tissue or plasma antioxidant capacity. In obese mice, treatment with NADPH oxidase inhibitor reduced ROS production in adipose tissue, attenuated the dysregulation of adipocytokines, and improved (*12*) diabetes, hyperlipidemia, and hepatic

*Address correspondence to this author at Équipe d'Accueil 4188, Nutrition Humaine, Biodisponibilité et Athérogénèse, CC 023, Université Montpellier 2, Place E. Bataillon, 34095 Montpellier Cedex 05, France [telephone/fax (33) 04 67 14 35 21; e-mail jm.rouanet@univ-montp2.fr].

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steatosis. Because obesity may induce systemic oxidative stress, increased oxidative stress should be an important target for the development of new therapies.

A diet low in antioxidants can contribute to the occurrence of oxidative stress (*13*), and the importance of antioxidants in human health has become increasingly clear. Generally, dietary antioxidants are vitamins, fibers, and phytochemicals supplied by fruits and vegetables. Recently, much attention has been focused on new approaches of antioxidant therapy by providing antioxidant enzymes. Thus, a melon juice concentrate containing high levels of superoxide dismutase (SOD) (*14*) and other antioxidant enzymes has been developed, and its antioxidant and anti-inflammatory properties have been demonstrated (*15*). This melon concentrate combined with wheat gliadin polymer, called Glisodin, was given for 4 weeks to mice, reducing the levels of thiobarbituric acid reactive substances, a marker of lipid peroxidation that correlates with oxidative stress, and leading to a 4-fold increase of circulating and hepatic tissue antioxidant enzyme activity (SOD and catalase) (*16*).

In this context, the present study was designed to quantitatively examine the properties of Extramel microgranules, a melon juice extract coated with palm oil and rich in antioxidants and particularly SOD, on the development of obesity in our golden Syrian hamster model of diet-induced obesity. To do that, plasma adipocytokines, hepatic and mitochondrial markers for oxidative stress, and liver lipids were measured, and a comparison was made with nonpathologic hamsters fed a standard diet.

MATERIALS AND METHODS

Extramel is a freeze-dried melon juice extract obtained by physical treatment (crushing the melon, recovery of the pulp, centrifugation, filtration, freeze-drying) of a specific variety of melon (not GMO, Clipper variety) (Patent FR 94 02459), which contains enzymic antioxidants, mainly SOD (90 IU/mg), measured according to the method of Oberley and Spitz (*17*), and, at a lower extent, catalase (10 IU/mg), which was determined according to the method of Clairbone (*18*). The powdered melon juice, coated with palm oil, that contains 14 IU SOD/mg of powder is called Extramel microgranules and manufactured by Bionov Co. (France).

Animals and Experimental Design. Male golden Syrian hamsters (Janvier, Le Genest-St-Isle, France) weighing 85−95 g were used. They were housed at 23 ± 1 °C, subjected to a 12 h light/dark cycle with free access to both food and water, and handled according to the guidelines of the Committee on Animal Care at the University of Montpellier and NIH guidelines (*19*). After a 4-day adaptation period, they were randomly divided into four groups of 12 animals each and fed a high-fat (HF) diet for 12 weeks. Additionally, they received daily by gavage either tap water (HF) or an aqueous solution of Extramel at 0.7 (E1), 2.8 (E2), or 5.6 (E3) mg/day in aqueous solution, corresponding to 10, 40, and 80 IU SOD/day, respectively. The diets (**Table** 1) were prepared in pelleted form by SSNIFF (Spezialdiäten GmbH, Soest, Germany) and contained a standard complement of vitamin and mineral mixes formulated according to AIN-93 guidelines (*20*) and all essential nutrients. For reference, an additional group of 12 hamsters was fed a regular diet (standard group, SD) plus a daily gavage of tap water, corresponding to nonpathological controls. Food intake and body weight were daily recorded.

Sampling. At the end of the experimental period, the hamsters were deprived of food overnight and were anesthetized with an intraperitoneal injection of pentobarbital (60 mg/mL at a dosage of 60 mg/kg of body weight). Then blood samples were collected by cardiac puncture. Plasma and erythrocytes were prepared by centrifugation at 2000*g* for 10 min and stored at −80 °C until analysis. Adipose tissue from the abdominal region was dissected and weighed. The liver was perfused with 0.15 mol/L KCl to remove residual blood, rapidly excised, weighed, sectioned for analyses, and stored in liquid nitrogen. To determine liver markers of oxidative stress, a sample of liver was homogenized in ice-cold 0.1 mol/L potassium phosphate buffer (pH 7.4), the homogenate was spun at 13000*g* for 15 min at 4 °C, and the supernatant was then stored at −80 °C. The remaining liver tissue was also stored at −80 °C.

Table 1. High-Fat Diet (HF) and Standard Diet (SD) Composition

	HF	SD
% energy as protein	18.89	19.24
% energy as carbohydrate	36.75	63.69
% energy as fat	44.36	17.06
fiber (g/100 g)	5.90	5.00
lard (g/100 g)	21.00	0
soybean 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

Plasma Analysis. Plasma glucose and triglycerides (TG) were measured by means of an enzymatic technique (KonePro, Konelab, Evry-Les-Lys, France) using reagents from Thermo Electron Corp. (Cergy Pontoise, France). Insulin, adiponectin, and leptin were assessed by ELISA using commercial kits (Linco, St. Charles, MO). The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated from insulin and glucose values using the formula of Matthews et al.(*21*):

$$\text{HOMA-IR} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin (mU/L)}/22.5$$

Liver Histology. Before the liver was frozen until enzymic analysis, tissue samples from each group of animals were removed and fixed in 10% neutral buffered formaldehyde for pathologic analysis. Formalin-fixed and paraffin-embedded livers were processed routinely for hemetoxylin and eosin staining. The histological examinations under a light microscope were performed twice by the same liver pathologist unaware of the treatments.

Total Hepatic Lipids. Lipids were extracted from fresh liver (100 mg of wet tissue) according to the method of Folch et al. (*22*).

Liver Markers for Oxidative Stress. The activity of two antioxidant enzymes, glutathione peroxidase (GSHPx) and SOD, was measured on an automat Pentra 400 (HORIBA ABX, Montpellier, France). GSHPx activity was measured using a commercial kit (Ransel, no. RS505, Randox Laboratories LTD, Crumlin, U.K.). SOD activity was determined using a Randox kit (Ransod, no. SD 125). Manganese superoxide dismutase activity (Mn-SOD) was also measured according to the method of Marklund (*23*). Protein oxidation was assessed by measurement of advanced oxidation protein product (AOPP) using technical recommendations described by Witko-Sarsat et al. (*24*). AOPP concentrations were expressed as micromoles per liter of chloramine-T equivalents. Lipid peroxidation levels or thiobarbituric acid-reactive substances (TBARS) were measured according to the method of Sunderman et al. (*25*). Briefly, liver supernatant was incubated with thiobarbituric acid (TBA) for 1 h at 100 °C in KCl solution with 1% H_3PO_4 (pH 2.0). TBARS were extracted with butanol and then detected by colorimetry (532, 556, and 508 nm). Corrected absorbance was $A = A_{532} - (A_{508} + A_{556})/2$. 1,1,3,3-Tetraethoxypropane was used as standard. TBARS concentration was expressed as nanograms per milligram of protein. Protein content was determined according to the method of Smith et al. (*26*).

Determination of Liver NAD(P)H-Dependent Superoxide Anion Production. As previously described (*27*), the liver was washed three times in Krebs buffer and immediately homogenized and centrifuged at 4000 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 counts per milligram of protein.

Determination of Liver Mitochondrial Respiratory Complex Activities. Complex I (CI) activity was measured according to the method of Janssen et al. (*28*): the method is based on measuring spectrophotometrically 2,6-dichloroindophenol reduction by electrons accepted from decylubiquinol, reduced after oxidation of NADH by complex I. Complex II (CII) and complex II+III (CII + CIII) activities were measured

Table 2. Body Weight, Food and Energy Intakes, Plasma Glucose, Triglycerides, Insulin, Leptin, and Adiponectin in Hamsters Fed a Standard Diet (SD), a High-Fat Diet (HF), or a HF Diet plus Extramel at 0.7 (E1), 2.8 (E2), or 5.6 mg/Day (E3) on Blood Lipids for 12 Weeks[a]

	SD	HF	E1	E2	E3
initial body wt (g)	86.3 ± 3.2a	87.6 ± 1.5a	89.3 ± 2.3a	92.1 ± 2.1a	96.7 ± 1.8b
final body wt (g)	115.4 ± 1.3a	132.4 ± 3.5b	121.4 ± 4.1c	123.8 ± 3.4c	128.5 ± 3.4b
body wt gain (g)	30.1 ± 1.0a	44.8 ± 2.0b	32.1 ± 1.8a	31.6 ± 2.3a	31.7 ± 1.6a
food intake (g/day)	5.12 ± 0.80a	5.88 ± 1.60a	5.21 ± 0.60a	5.16 ± 0.30a	5.29 ± 0.55a
fat intake (kJ/day)	15.4 ± 2.4a	53.9 ± 14.1b	47.0 ± 5.2b	46.5 ± 2.6b	47.7 ± 4.9b
energy intake (kJ/day)	105.3 ± 8.1a	122.4 ± 33.0a	108.5 ± 12.5a	107.4 ± 6.2a	110.1 ± 11.4a
abdominal fat (g)	1.26 ± 0.08a	1.78 ± 0.06b	1.48 ± 0.02c	1.41 ± 0.07c	1.29 ± 0.04 a
abdominal fat (% BW)	1.10 ± 0.07a	1.34 ± 0.04b	1.22 ± 0.01c	1.14 ± 0.05a	1.00 ± 0.03a
plasma					
triglycerides (mmol/L)	0.87 ± 0.08a	6.29 ± 0.53b	6.71 ± 0.18b	1.90 ± 0.03c	2.05 ± 0.07c
glucose (mmol/L)	5.43 ± 0.42a	6.34 ± 0.53b	5.75 ± 0.47ab	6.55 ± 0.51b	6.20 ± 0.35b
insulin (ng/mL)	0.33 ± 0.08a	0.43 ± 0.00b	0.34 ± 0.07a	0.33 ± 0.04a	0.26 ± 0.02a
HOMA-IR	52.7 ± 2.0a	80.2 ± 1.5b	57.5 ± 1.0c	63.5 ± 1.1d	47.4 ± 0.4e
leptin (ng/mL)	0.22 ± 0.02a	0.40 ± 0.05b	0.35 ± 0.04bc	0.29 ± 0.04ac	0.28 ± 0.04ac
adiponectin (ng/mL)	7.30 ± 1.70a	4.98 ± 0.25b	5.65 ± 0.40bc	6.10 ± 0.68bc	6.40 ± 0.36ac

[a] *Values are means± SEM (n=12). For each dietary treatment, means in a row with different letters differ, P< 0.05. Plasma glucose, triglycerides, insulin, leptin, and adiponectin were measured at the fasted state at the end of the 12-week treatment period. HOMA-IR, homeostatic model assessment for insulin resistance.*

according to the method of Rustin et al. (*29*): succinate−ubiquinone reductase and succinate−cytochrome *c* reductase activities were respectively determined spectrophotometrically. Cytochrome *c* oxidase (COX) activity was measured according to the method of Wharton and Tzagoloff (*30*), whereby the oxidation of reduced cytochrome *c* is followed spectrophotometrically. Citrate synthase (CS) activity was measured according to the method of Srere (*31*): the activity of the enzyme is measured by following the color of 5-thio-2-nitrobenzoic acid, which is generated from 5,5′-dithiobis-2-nitrobenzoic acid present in the reaction of citrate synthesis and caused by the deacetylation of Acetyl-CoA.

Statistical Analyses. Results were expressed as the mean ± standard error of the mean (SEM) from 12 measurements. Data were analyzed by one-way analysis of variance (ANOVA). A probability of < 0.05 was considered to be significant.

RESULTS

Study Design and Measurements Performed. At the end of a 12-week experimental period, insulin, plasma adipocytokines, hepatic and mitochondrial markers for oxidative stress, and liver histology were measured in hamsters fed a high-fat diet supplemented or not with Extramel, and a comparison was made with nonpathologic hamsters fed a standard diet.

Characteristics of the Hamsters. As summarized in **Table** 2, hamsters fed the HF diet gained more weight than standard hamsters (≈33%). On average, treatment of fat-fed hamsters with Extramel induced a body weight increase 29% lower by comparison to HF-fed group, and no significant difference appeared in body weight gain among the three groups receiving Extramel. However, due to identical food intakes, fat intake and energy intake were not modified, indicating that body weight gain in the HF-fed group may be related to fat intake but not to higher energy intake. Feeding the high-fat diet (control group, HF) increased body fat accumulation, and abdominal adipose tissue weight was greater (18%) that in standard hamsters. The consumption of Extramel along with the high-fat diet reduced this effect, particularly in E2 (2.8 mg/day) and E3 (5.6 mg/day) groups.

Nutritional Parameters. High-fat feeding of hamsters led to greater fasting plasma concentrations of glucose and insulin associated with a huge 6-fold increase in triglycerides and an increase in HOMA-IR (**Table** 2). Giving Extramel strongly improved these effects (68% on average for triglycerides in E2 and E3 groups), although no betterment of glycemia appeared. Hamsters fed the HF diet also exhibited a higher leptin level (82%) and a lower adiponectin level (32%) than those fed the standard diet (**Table** 2). Supplying hamsters fed the high-fat diet with Extramel significantly improved the adipokine pattern, thereby totally preventing the leptin increase (2.8 and 5.6 mg/day) and significantly increasing adiponectine level by 13−29% in comparison with HF. Thus, although Extramel did not influence glycemia, it induced a significant decrease of plasma insulin and HOMA-IR values at the same time as improving triglyceridemia.

Histological Analysis of Liver. We evaluated liver sections histologically to assess the extent to which Extramel attenuated the hepatic modifications. Lean hamsters (SD) exhibited no histologic evidence of hepatic steatosis and fibrosis, showing apparent sinusoidal capillaries, endothelial cells, and hepatocytes (**Figure** 1**a**). In contrast, hepatic steatosis characterized by a remarkable fat accumulation as a grainy fatty cytoplasm and a ballooning degeneration of hepatocytes characterized by cell swelling with empty intracellular content and impairment of sinusoidal capillary indicating cell necrosis (**Figure** 1**b**,**c**) were observed in hamsters fed the HF and E1 (0.7 mg/day) diets for 12 wk. Hepatocellular ballooning degeneration, which is one of the histological hallmarks of steatohepatitis, was improved with Extramel from 2.8 mg/day, which is the threshold of the efficient dose, and disappeared at the highest dose of 5.6 mg/day, returning to similar standard features, that is, again apparent sinusoidal capillaries, endothelial cells, and hepatocytes (**Figure** 1**d**,**e**).

Liver Lipids and Oxidative Stress. Liver lipids were gradually decreased in hamsters given 0.7−5.6 mg/day Extramel by 21−71% in comparison with HF, being close (2.8 and 5.6 mg/day) to that exhibited by the SD group (**Table** 3). Liver antioxidant enzyme (**Table** 3) activities such as superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) were obviously raised by the HF diet, but Extramel did not modify SOD activity, whereas it significantly lowered GSHPx activity at 0.7 and 2.8 mg/day. Manganese SOD (Mn-SOD) was lower in the HF group by comparison to hamsters consuming Extramel. Liver lipid oxidation products assessed by the TBARS assay were markedly lowered by the consumption of Extramel according to a pattern close to that observed for lipids (**Table** 3). Protein oxidation (AOPP measurements) was increased with the HF diet by comparison to the SD group, and a preventive effect against oxidation of proteins was clearly observed with the three doses of Extramel (34.5% on average).

NAD(P)H-Dependent Anion Superoxide Production and Mitochondrial Respiratory Complex Activities. Extramel (2.8 and

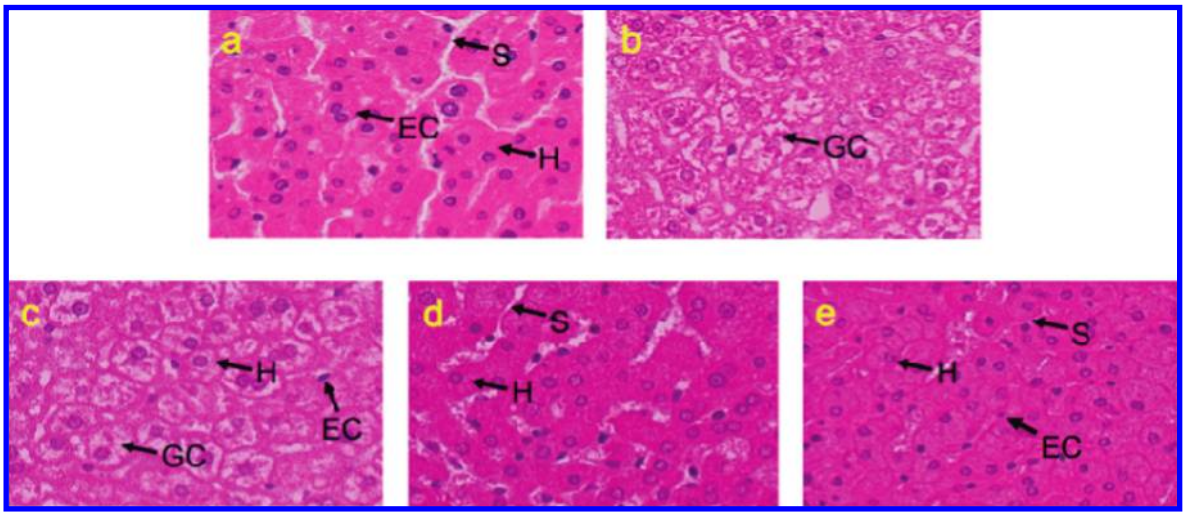

Figure 1. Histologic evaluation of hepatic modifications in obese (**b**) or Extramel-treated hamsters (**c**−**e**) for 12 weeks: (**a**) liver section from a hamster fed the standard diet; (**b**) obese hamster fed no Extramel illustrates the development of hepatic ballooning degeneration; (**c**) no degeneration reduction after treatment with Extramel at 0.7 mg/day; (**d**, **e**) gradual improvement with Extramel at 2.8 and 5.6 mg/day. S, sinusoidal capillary; EC, endothelial cell; H, hepatocyte; GC, grainy fatty cytoplasm. Representative liver sections (original magnification × 200) are illustrated.

Table 3. Liver Lipids and Oxidative Status of Hamsters Fed a Standard Diet (SD), a High-Fat Diet (HF), or a HF Diet plus Extramel at 0.7 (E1), 2.8 (E2), or 5.6 mg/Day (E3) for 12 Weeks[a]

	SD	HF	E1	E2	E3
lipids (mg/g)	6.60 ± 2.5a	21.31 ± 1.6b	12.34 ± 1.90c	6.11 ± 1.12a	5.73 ± 0.61a
GSH-Px[b] (U/mg of protein)	2191 ± 38a	4397 ± 590b	2980 ± 268c	2912 ± 314c	3588 ± 590bc
(Cu−Zn)-SOD (U/mg of protein)	21.9 ± 1.4a	31.6 ± 2.3b	31.0 ± 1.7b	32.6 ± 2.0b	34.6 ± 2.0b
Mn-SOD (U/mg of protein)	3.26 ± 0.02a	2.87 ± 0.24a	3.35 ± 0.17a	3.64 ± 0.17b	3.64 ± 0.12b
TBARS[c] (mmol/mg of protein)	6.19 ± 1.50a	11.30 ± 2.00b	8.96 ± 1.95b	7.53 ± 3.03ab	7.39 ± 1.40ab
AOPP (mmol/mg of protein)	19.9 ± 1.6a	32.7 ± 6.7b	20.4 ± 3.0a	22.8 ± 2.4a	21.0 ± 1.6a

[a] *Values are means ± SEM (n = 12). For each dietary treatment, means in a row with different letters differ, P < 0.05.* [b] *Glutathion peroxidase.* [c] *Thiobarbituric acid reactive substances.*

Table 4. Liver NAD(P)H-Dependent Anion Superoxide Production and Mitochondrial Respiratory Complex Activities in Hamsters Fed a Standard Diet (SD), a High-Fat Diet (HF), or a HF Diet plus Extramel at 0.7 (E1), 2.8 (E2), or 5.6 mg/Day (E 3) for 12 Weeks[a]

	SD	HF	E1	E2	E 3
$O_2^{\bullet -}$ production[b]	1080 ± 143a	1810 ± 435b	1750 ± 404b	1579 ± 63c	1606 ± 260b
CS (mU/mg of protein)	89.6 ± 3.5a	80.0 ± 5.1a	87.5 ± 6.5a	85.2 ± 6.7a	89.0 ± 5.4a
CI/CS	0.25 ± 0.013a	0.15 ± 0.009b	0.18 ± 0.006b	0.18 ± 0.021b	0.16 ± 0.018b
CII/CS	0.93 ± 0.07a	0.79 ± 0.07b	0.51 ± 0.07c	0.72 ± 0.09b	0.65 ± 0.09b
(CII + CIII)/CS	0.016 ± 0.003a	0.014 ± 0.001a	0.010 ± 0.001a	0.014 ± 0.002a	0.012 ± 0.001a
COX/CS	0.445 ± 0.087a	0.598 ± 0.100a	0.519 ± 0.074a	0.401 ± 0.058a	0.331 ± 0.038b

[a] *Values are means ± SEM (n = 12). For each dietary treatment, means in a row with different letters differ, P < 0.05. CS, citrate synthase; COX, cytochrome c oxidase; CI, CII, CIII, complex I, II, and III activity.* [b] *Expressed as counts per mg of protein.*

5.6 mg/day) decreased NAD(P)H-dependent superoxide anion production ($O_2^{\bullet -}$) by 13 and 11%, respectively, in comparison with HF. However, due to high SEM, the 11% reduction in E3 (5.6 mg/day) was not significant (**Table** 4).

Mitochondrial Complex I and Complex II Activities. (CI/CS and CII/CS) were altered in hamsters fed the high-fat diet by comparison to control (40 and 15% decrease, respectively), and the addition of Extramel did not prevent these alterations (**Table** 4). CII + CIII activity (CII + III/CS) was not modified with the HF diet by comparison to controls. COX activity (COX/CS) decreased while increasing Extramel level in hamsters fed the HF diet, and this effect was significant at the higher level (5.6 mg/day). Citrate synthase activity, generally considered to be an index of mitochondrial density, was not modified whatever the diet. The results suggested a modification of mitochondrial maximal respiratory complex activities by HF diet and an effect of Extramel on maximal COX activity.

DISCUSSION

As expected, feeding the high-fat diet (HF) induced a disease state as compared with the standard group (SD) (**Tables** 2 and 3; **Figure** 1).

The major novel finding in our experimental conditions, and in comparison to HF, is that Extramel prevented obesity in HF-fed hamsters by decreasing body weight, abdominal fat, triglyceridemia, insulinemia, insulin resistance, liver lipids, and nonalcoholic

steatohepatitis (NASH) and preventing adipokine imbalance. The gradual increase in plasma adiponectin concentration after Extramel feeding is strongly associated with a gradual decrease in liver fat content and may play an important role in reversing abnormality in liver histological features, fat concentration, and insulin resistance in hamsters.

Nonalcoholic fatty liver disease encompasses a wide spectrum of liver damage ranging from simple fatty overload to NASH and then to cirrhosis (*32*). Liver injury in NASH is usually characterized by fat accumulation, infiltration of inflammatory cells, and a varying extent of ballooning degeneration of hepatocytes (*33*). Hepatocyte "ballooning" is an often used term to designate a special form of liver cell degeneration associated with cell swelling and enlargement found particularly in steatohepatitis. Ballooning degeneration is one of the histological hallmarks of steatohepatitis (*34*). This key feature is emphasized here in HF-fed hamsters (**Figure** 1**b**) along with accumulation of liver lipids (**Table** 3), and consumption of Extramel (5.6 mg/day) strongly improved these symptoms. Our histochemical study was undertaken to evaluate the presence of steatohepatitis with ballooned hepatocytes after feeding of the HF diet. Our study showed marked loss of ballooned hepatocytes associated with steatohepatitis from the threshold of efficient dose at 2.8 mg/day and the recovery of a healthy state using Extramel at 5.6 mg/day.

In this study we clearly observed an increased oxidation of lipids and proteins in livers of hamsters fed the HF diet and a preventive effect of Extramel on liver oxidative stress; oxidative stress is implicated in NASH. Mitochondrial dysfunction (*35*) and the NAD(P)H oxidase system (*27*) are two main sources of ROS production and oxidative stress. Increased hepatic NAD(P)H-dependent superoxide anion production and altered mitochondrial electron transport chain activity were observed in hamsters fed the HF diet. Antioxidant enzymic activities are high in the HF diet compared with SD and could be due to an induction mechanism for SOD and GSHPx (*36*). Such an increase is not seen using a diet deficient in antioxidant micronutrients (*27*). In the present work, Extramel prevents an increase in liver MDA and AOPP levels and has no effect on NAD(P)H oxidase ($O_2^{\bullet -}$ production) or on already induced SOD activity that cannot thus increase. The observed alteration of the maximal activity of the mitochondrial respiratory chain in the liver of hamsters fed the HF diet was coherent with the observation of steatosis, as a dysregulation of mitochondrial function might be implicated in fat accumulation in the liver (*37*). In fact, according to Chavin et al. (*38*), increased liver expression of uncoupling protein-2 (UCP-2) occurs with obesity-induced steatosis; this increases H^+ leakage across the inner mitochondrial membrane, dissipates the membrane potential, and decreases ATP synthesis. Moreover according to our results, decreased respiratory complex activity was found in the livers from HF-diet-fed rats (*35*), in the livers of *ob*/*ob* mice (*39*), and in liver biopsies from patients with NASH compared to normal liver (*40*). Although these studies clearly point to a defect in mitochondrial bioenergetics, additional studies are required to identify and better understand the molecular mechanisms and targets responsible for mitochondrial dysfunction in obesity-induced fatty liver disease (*37*). Extramel at the highest dose lowered liver production of superoxide anion (12%) and mitochondrial cytochrome *c* oxidase (COX) maximal activity (40%).

Adipose tissue plays an important role in lipid homeostasis by storing excess energy in the form of triglyceride. Insulin promotes lipid storage in adipose tissue by suppressing lipolysis, thus regulating the release of free fatty acids. Impairment of adipose tissue storage function, as seen in obesity, is associated with the accumulation of ectopic triglycerides in the liver and skeletal muscles (*41*). In addition to functioning as a storage organ, adipose tissue also secretes adipokines, which play an important role in regulating energy homeostasis and lipid metabolism. Notably, some of them, such as leptin and adiponectin, have the ability to influence lipid metabolism not only locally in the adipose tissue but also in the liver and muscle. Leptin can reduce lipid accumulation in the liver (*42*). Hypoadiponectinemia is closely associated with oxidative stress (*43*), and the increase in plasma adiponectin is strongly associated with a decrease in hepatic fat content and improvements in hepatic and peripheral insulin sensitivity (*44*). Thus, reduced plasma levels of adiponectin have been implicated in the pathogenesis of obesity in rodents and humans (*45*), and some studies (*46*) have provided evidence that increased hepatic fat content is an important determinant of hepatic insulin resistance in type 2 diabetic patients. These findings are supported here because fatty liver is evidenced in HF-fed hamsters and improved by feeding Extramel; the HOMA-IR index followed the same pattern. These results of the present study strongly suggest that the plasma adiponectin concentration may be the crucial link between hepatic fat content and insulin sensitivity and strengthen the suggestion that weight loss induces an increase in adiponectin levels in obesity (*47*) as elicited by Extramel. The mechanisms responsible for the increase in hepatic fat content are unclear. However, as the abdominal fat exhibited by HF-fed hamsters is reduced according to the dose of Extramel, it could be hypothesized according to Wahrenberg et al. (*48*) that fatty liver results from accelerated fatty acid mobilization from expanded visceral fat stores and their deposition in the liver as well as decreased hepatic fatty acid oxidation, which behaved as targets for Extramel.

Multiple factors have been associated with hepatic steatosis, including obesity, type 2 diabetes, and hyperlipidemia, with insulin resistance postulated as an essential factor. Moreover, hyperglycemia and hypertriglyceridemia that arose in HF-fed hamsters have the ability to induce oxidative stress arising largely in part via mitochondrial defects. It has been proposed that once steatosis is present, the liver becomes more susceptible to the oxidative stress, which is thought to be one of several stimuli for the progression from simple fatty liver to NASH (*49*). In fact, oxidative stress may especially contribute to the pathogenesis of NASH, a condition in which decrease and loss of the intermediate filament cytoskeleton in liver cells have been demonstrated (*50*) and damaged intermediate filament cytoskeleton has been shown in response to oxidative stress in a variety of human liver diseases and in mouse models of steatohepatitis (*51*).

To summarize, Extramel prevented obesity in HF-fed hamsters by decreasing body abdominal and liver fat and by preventing adipokine imbalance. Furthermore, oxidative stress was reduced (decreased levels of lipids and protein oxidation products), and the main sources of ROS production (NADPH and mitochondria) were modified as NADPH-dependent $O_2^{\bullet -}$ production and mitochondrial maximal activity of COX decreased with Extramel. The mechanisms through which Extramel works need to be investigated.

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