JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

# Vineatrol and Cardiovascular Disease: Beneficial Effects of a Vine-Shoot Phenolic Extract in a Hamster Atherosclerosis Model

Cindy Romain,[†] Sylvie Gaillet,[†] Julie Carillon,[†] Joris Vidé,[†] Jeanne Ramos,[‡] Jean-Claude Izard,[§] Jean-Paul Cristol,[†] and Jean-Max Rouanet*,[†]

[†]Nutrition & Métabolisme, UMR 204 NUTRIPASS, Prévention des Malnutritions & des Pathologies Associées, Université Montpellier Sud de France, Place Eugène Bataillon, 34095 Montpellier, France

[‡]Service d'Anatomie Pathologique, Centre Hospitalo-Universitaire Guy de Chauliac, Avenue Augustin Fliche, 34095 Montpellier, France

[§]Actichem S.A., 121 avenue du Danemark, 82000 Montauban, France

**ABSTRACT:** We evaluated the effect of the intake of a grapevine-shoot phenolic extract (Vineatrol 30) on early atherosclerosis in hamsters fed a hyperlipidic diet. Golden Syrian hamsters received for 13 weeks either a standard diet, a high-fat (HF) diet, or the HF diet plus Vineatrol 30 at 0.04, 0.2, or 1.0 mg/(kg body weight/d). We measured plasma lipids and glucose, insulin, leptin and adiponectin, as well as liver TNF-$\alpha$ and IL-6 levels. Oxidative stress was assessed by measuring plasma paraoxonase activity (PON) and liver superoxide anion production ($O_2^{\bullet-}$). The aortic fatty streak area (AFSA) was also determined. In comparison with HF group, we demonstrated that the highest dose of Vineatrol 30 was capable of decreasing AFSA (67%), insulinemia (40%), and leptinemia (8.7%), which were increased by the HF diet. We also showed increased $O_2^{\bullet-}$ production (35%) and a rise in levels of the liver proinflammatory cytokines TNF-$\alpha$ (22%) and IL-6 (21%), accompanied by a fall in PON activity (56%) due to the HF diet versus the standard diet. In contrast, except plasma adiponectin levels that are not changed, Vineatrol 30 treatment lowered AFSA (67%), $O_2^{\bullet-}$ production (36%), insulin resistance (42%), leptinemia (9%), liver TNF-$\alpha$ (18%) and IL-6 (15%), while it rose PON activity (29%). These findings demonstrate the preventive effects of polyphenols present in Vineatrol 30 in managing cardiovascular, metabolic, and inflammatory risk factors.

**KEYWORDS:** *hamster, atherosclerosis, oxidative stress, liver inflammation, Vineatrol 30*

## INTRODUCTION

Cardiovascular diseases (CVDs) are leading causes of morbidity and mortality in industrialized countries. There is ample evidence that atherosclerosis results from a combination of hypercholesterolemia, oxidative stress, and low-grade inflammation.[1,2] Some studies have suggested that nonalcoholic steato-hepatitis (NASH) contributes to chronic inflammation through the systemic release of pro-inflammatory mediators from the liver.[3,4] In addition, since several studies suggest that polymorphic variations in endogenous antioxidants are linked to increased risk for atherosclerosis,[5] the reactive-oxygen-species-induced depletion of antioxidants is a key factor in the initiation of atherosclerosis. The mechanisms linking non-alcoholic fatty liver disease (NAFLD) and atherosclerosis may thus be represented by increased oxidative stress and chronic subclinical inflammation.[6] Serum paraoxonase (PON) is a 43- to 45-kDa glycoprotein synthesized mainly by the liver that circulates in serum in association with high-density lipoprotein (HDL).[7] PON is a calcium-dependent esterase that hydrolyzes a broad spectrum of substrates including organophosphates, arylesters and lactones.[8] PON was also shown to be able to hydrolyze specific oxidized lipids [9] and thus to reduce oxidative stress in atherosclerotic lesions.[10] PON also protects HDL particles from oxidation and increases its ability to induce macrophage cholesterol efflux.[7]

Many lines of evidence support the beneficial health effects of polyphenolic compounds, which are powerful antioxidants, in the prevention of CVDs. In animal models and humans, evidence supports the hypothesis that the consumption of food and beverages rich in polyphenols is associated with a lower incidence of CVDs.[11−14] We have also previously shown that an enzymatic antioxidant from melons prevents early atherosclerosis and liver steatosis in hypercholesterolemic hamsters.[15] Other studies in hypercholesterolemic rabbits have attributed cardioprotective effects to resveratrol, a natural polyphenol abundant in grapes and red wines.[10] More recently, Zghonda et al.[16] have reported that $\varepsilon$-viniferin, a dimer of resveratrol, is more effective than resveratrol in inhibiting vascular smooth cell proliferation and migration. In addition, it has been shown that resveratrol is a potent anti-inflammatory agent, inhibiting TNF-$\alpha$-induced NF-$\kappa$B activation and inflammatory gene expression, and attenuating monocyte adhesion in human coronary artery endothelial cells.[17]

Given the potential benefits of resveratrol and its oligomers in the modulation of oxidative stress and the inflammatory environment, we aimed to investigate for the first time the effects of Vineatrol 30, a grapevine-shoot extract that contains resveratrol and its oligomers, on the cardiovascular and hepatic system in a diet-induced atherosclerosis model in hamsters, in

Received: April 17, 2012
Revised: October 18, 2012
Accepted: October 19, 2012
Published: October 19, 2012

**Table 1. Body Weight, Food Intake, And Plasma Cholesterol, Triglycerides, Glucose and Insulin in Hamsters Fed a Standard Diet (S), a High-Fat Diet (C), or a HF Diet Plus Vineatrol 30 at 0.04 mg/(kg BW/day) (VT1), 0.2 mg/(kg BW/day) (VT2), or 1.0 mg/(kg BW/day) (VT3) for 13 Weeks[a]**

| | S | C | VT1 | VT2 | VT3 |
|---|---|---|---|---|---|
| Body wt gain (g) | 32.0 ± 2.2[a] | 60.6 ± 5.6[b] | 56.2 ± 3.6[b] | 64.7 ± 5.6[b] | 59.2 ± 5.1[b] |
| Food intake (g/day) | 4.75 ± 0.46[a] | 7.82 ± 0.74[b] | 7.08 ± 0.85[b] | 7.69 ± 1.10[b] | 7.89 ± 1.12[b] |
| Plasma | | | | | |
| TC (mmol/L) | 3.2 ± 0.2[a] | 10.6 ± 0.4[b] | 11.0 ± 0.6[b] | 9.7 ± 0.4[b] | 11.2 ± 0.2[b] |
| HDLC (mmol/L) | 1.82 ± 0.06[a] | 2.70 ± 0.17[b] | 2.90 ± 0.14[b] | 2.63 ± 0.27[b] | 2.94 ± 0.12[b] |
| LDLC (mmol/L) | 1.43 ± 0.15[a] | 7.90 ± 0.48[b] | 8.15 ± 0.66[b] | 7.08 ± 0.51[b] | 8.34 ± 0.34[b] |
| TG (mmol/L) | 0.82 ± 0.06[a] | 2.30 ± 0.41[b] | 2.54 ± 0.17[b] | 2.03 ± 0.23[b] | 2.66 ± 0.28[b] |
| Glucose (mmol/L) | 6.36 ± 0.32[a] | 9.40 ± 0.66[b] | 9.15 ± 0.27[b] | 8.22 ± 0.73[b] | 9.18 ± 0.50[b] |
| Insulin (pmol/L) | 140 ± 21[a] | 411 ± 109[b] | 395 ± 98[b] | 206 ± 23[a] | 246 ± 28[a] |
| HOMA-IR | 5.7 ± 0.7[a] | 24.8 ± 1.3[b] | 23.1 ± 2.3[b] | 10.9 ± 1.7[c] | 14.4 ± 1.2[c] |

[a]Values are mean ± SEM ($n$ = 8). For each dietary treatment, means in a row with different letters differ, $P < 0.05$. Plasma total cholesterol (TC), HDL-cholesterol (HDLC), LDL-cholesterol (LDLC), triglycerides (TG), glucose, insulin and homeostatic model assessment for insulin resistance (HOMA-IR) were measured after overnight fasting, at the end of the 13-week treatment period.

order to gain insight into the mechanisms underlying the antioxidant and anti-inflammatory effects ascribed to these phenolics.

## MATERIALS AND METHODS

**Chemicals.** Lucigenin, sodium cacodylate, hematoxylin, eosin, paraoxon, paraformaldehyde, glutaraldehyde and Oil Red O were from Sigma-Aldrich (St. Quentin Fallavier, France).

**Materials.** Vineatrol 30 is a grapevine-shoot extract developed and manufactured by Actichem (Montauban, France) that contains considerable amounts of resveratrol and its oligomers, as follows: 15.2% resveratrol, 13.2% ε-viniferin, 4.4% ampelopsin A, 2.8% hopeaphenol, 2.1% iso-*trans*-ε-viniferin, 1.9% vitisin A, 1.9% vitisin B, 1.8% piceatannol, and 1.6% miyabenol C.[18]

**Animals, Diets, and Experimental Design.** Forty male weanling Golden Syrian hamsters (Janvier, Le Genest-St-Isle, France) weighing ~65 g each were randomly divided into five groups of 8 animals. They were housed at 23 ± 1 °C, subjected to a 12 h light/dark cycle and handled in compliance with European Union rules and according to the guidelines of the NIH[19] and the Committee for Animal Care at the University of Montpellier (France). Four groups were fed with a high-fat atherogenic diet (HF), consisting of 200 g/kg casein, 3 g/kg L-methionine, 393 g/kg corn starch, 33 g/kg maltodextrin 10, 154 g/kg sucrose, 50 g/kg cellulose, 100 g/kg hydrogenated coconut oil (21.2% energy of the diet), 2 g/kg cholesterol, 35 g/kg mineral mix and 10 g/kg vitamin mix, for 13 weeks. The four HF groups were given daily by gavage either tap water (control group, C) or an aqueous solution of Vineatrol 30 at a dose of 0.04 mg/kg body weight (BW)/d (VT1), 0.2 mg/kg BW/d (VT2) or 1 mg/kg BW/d (VT3). The volume of solutions force-fed was 1 mL/(d/hamster). For reference, the fifth group was given a standard diet (S) consisting of 236 g/kg casein, 3.5 g/kg L-methionine, 300 g/kg corn starch, 30 g/kg maltodextrin 10, 290.5 g/kg sucrose, 50 g/kg cellulose, 45 g/kg vegetable oil (10.3% energy of the diet), 35 g/kg mineral mix and 10 g/kg vitamin mix. Vitamin and mineral mixes were formulated according to AIN-93 guidelines.[20] This group was also given tap water daily by gavage.

**Analytical Procedures.** At the end of the experimental period (13 weeks), the hamsters were deprived of food overnight and fasting blood samples were collected by cardiac puncture. Plasma was prepared by centrifugation at 2000*g* for 10 min. Plasma total cholesterol (TC) and HDL cholesterol (HDL-C) were determined using commercially available enzymatic kits (CH 200 and CH 203, respectively, Randox Laboratories Ltd., Crumlin, U.K.). Very low- and low-density lipoprotein cholesterol in the plasma were precipitated using phosphotungstate reagent,[21] and HDL-C was measured in the supernatant. Plasma triglycerides (TG) were measured using a Randox enzymatic kit (TR 1697) and glucose levels by means of an enzymatic technique (KonePro, KoneLab, Evry-Les-Lys, France) using reagents from the Thermo Electron Corporation (Cergy Pontoise, France).

Paraoxonase activity (PON) was determined using paraoxon as a substrate and measured by the increase in absorbance at 412 nm due to the formation of 4-nitrophenol, according to the technique of Jaouad et al.[22]

Plasma insulin concentrations were determined using commercially available ELISA kits (Mercodia AB, Uppsala, Sweden); the homeostatic model assessment for insulin resistance (HOMA-IR) was calculated from insulin and glucose values using the formula of Matthews et al.:[23] HOMA-IR = Fasting glucose (mmol/L) × fasting insulin (mU/L)/22.5.

Plasma adiponectin and leptin concentrations were assessed using immunoassay kits (R&D Systems, Lille, France).

The liver was excised, weighed, and sectioned; some samples were removed for histology, while others were stored at −80 °C until further use. For pathological analysis, liver samples were fixed in 10% neutral buffered formaldehyde and paraffin embedded, and 3 μm-thick serial sections were prepared. Sections were deparaffinized and stained with hematoxylin and eosin.

Unfixed liver samples were homogenized in 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. Liver TNF-α and IL-6 levels were quantified using specific ELISA kits according to the manufacturer's instructions (R&D Systems Europe, Lille, France).

Hepatic superoxide anion ($O_2^{\bullet -}$) production was evaluated by the intensity of lucigenin-enhanced chemiluminescence (10 μM lucigenin), measured with a luminometer (Perkin-Elmer Wallac, Victor, Turku, Finland) as previously described.[24] Results were expressed as relative luminescence units (RLU) per milligram of protein.

Following blood collection and liver removal, the intact aortic arch was first perfused with phosphate buffered saline containing 1 mmol/L $CaCl_2$ and 15 mmol/L glucose for 5 min, and then with 0.1 mmol/L sodium cacodylate buffer, pH 7.4, containing 2.5 mmol/L $CaCl_2$, 2.5% paraformaldehyde and 1.5% glutaraldehyde, in order to fix the vasculature. The aorta was carefully dissected and processed as previously described, and lipids were stained with Oil Red O according to the protocol of Auger et al.[12] An Olympus microscope equipped with an image acquisition and analysis system (Image J, Scion Corporation, Frederick, MD) was used to capture and analyze the total Oil Red O-stained area for each aortic arch. The area covered by foam cells (aortic fatty streak area or AFSA) was expressed as a percentage of the total area surveyed.

**Statistical Analysis.** Data are shown as means ± SEM. Statistical analysis of the data was carried out using StatView IV software (Abacus Concepts, Berkeley, CA), with a one-way ANOVA followed by Fisher's protected least significant difference test. A $p$-value of ≤0.05 was taken to indicate a significant difference.

## RESULTS

**Food Intake and Body Weight.** No significant difference in food intake or body weight gain was found between the control group (C) fed the HF diet alone, and groups VT1−VT3, fed Vineatrol 30 in addition to the HF diet. However, food intake and body weight gain were significantly lower in standard-diet-fed hamsters (S) (Table 1).

**Plasma Analysis.** The HF diet produced a significant increase in plasma lipid and glucose concentrations in group C compared with those in group S (Table 1). Regardless of the dose used, Vineatrol 30 did not induce any improvement in these parameters.

In the plasma, PON is localized in the HDL fraction. The HF diet (group C) strongly reduced the ratio of PON activity to HDL by about 56% ($P < 0.0001$) in comparison to the standard diet (Figure 1). Vineatrol 30 at 1 mg/(kg BW/day) (VT3) was the only dose that was effective at significantly improving PON activity in HF-fed groups, by about 29% ($P = 0.0052$).

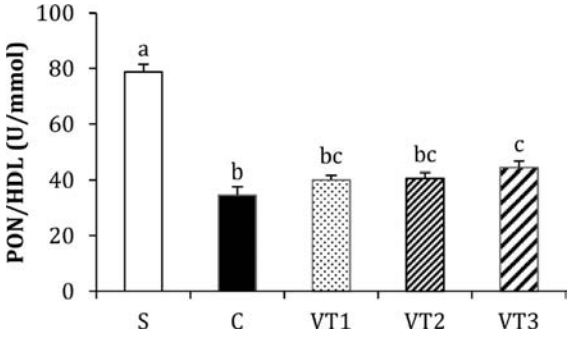


**Figure 1.** Paraoxonase activity ratio (PON/HDL) in hamsters fed a standard diet (S), a high-fat diet (C), or a high-fat diet plus Vineatrol 30 at 0.04 mg/(kg BW/day) (VT1), 0.2 mg/(kg BW/day) (VT2) or 1 mg/(kg BW/day) (VT3), for 13 weeks. Values indicate means ± SEM ($n = 8$). For each dietary treatment, bars with different index letters are significantly different ($P < 0.05$).

**Cardiac and Liver Biomarkers of Oxidative Stress.** In the liver, $O_2^{\bullet-}$ production increased 52% in group C when compared to group S ($P = 0.0066$), but was significantly decreased (by 40% on average) in groups given Vineatrol 30 (VT1−3) when compared to group C (Figure 2).

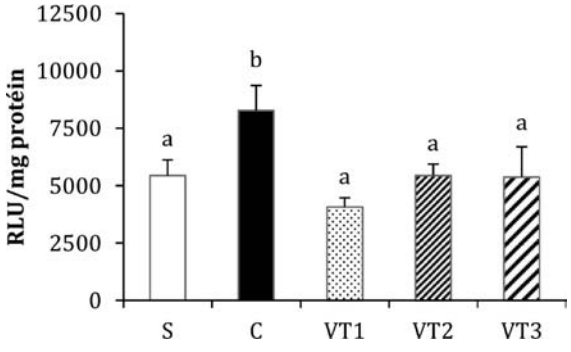


**Figure 2.** Liver NAD(P)H oxidase activity in hamsters fed a standard diet (S), a high-fat diet (C), or a high-fat diet plus Vineatrol 30 at 0.04 mg/(kg BW/day) (VT1), 0.2 mg/(kg BW/day) (VT2) or 1 mg/(kg BW/day) (VT3), for 13 weeks. Values indicate means ± SEM ($n = 8$). For each dietary treatment, bars with different index letters are significantly different ($P < 0.05$).

Average aortic fatty streak area (AFSA), or the area stained by Oil Red O expressed as a percentage of the total area surveyed (Figures 3 and 4), progressively decreased in hamsters

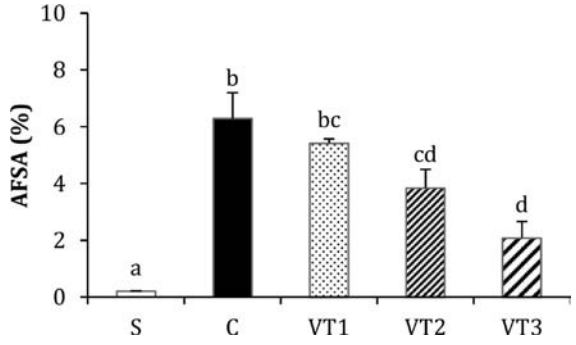


**Figure 3.** Aortic fatty streak area (AFSA) in hamsters fed a standard diet (S), a high-fat diet (C), or a high-fat diet plus Vineatrol 30 at 0.04 mg/(kg BW/day) (VT1), 0.2 mg/(kg BW/day) (VT2) or 1 mg/(kg BW/day) (VT3), for 13 weeks. Values indicate means ± SEM ($n = 8$). For each dietary treatment, bars with different index letters are significantly different ($P < 0.05$).

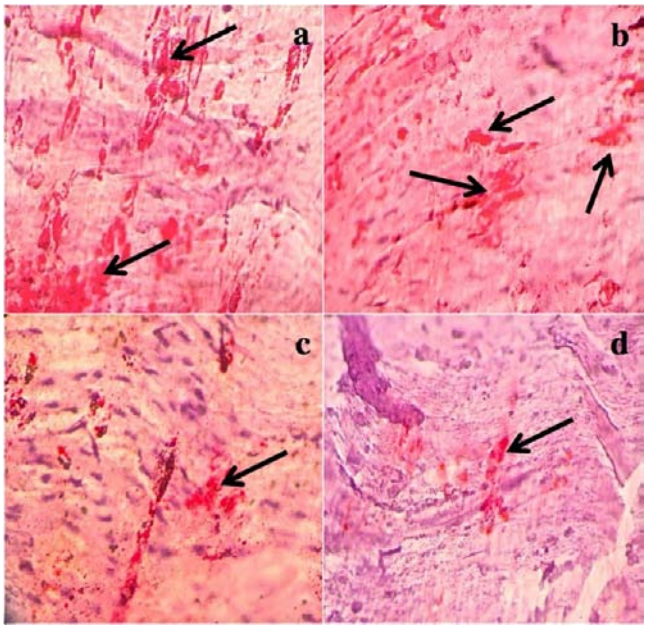


**Figure 4.** Photomicrographs of sections of the surface of the aortic arch stained with Oil Red O, in hamsters after 13 weeks of an atherogenic diet (a) or an atherogenic diet supplemented with Vineatrol 30, i.e., VT1 (b), VT2 (b), VT3 (c). The images show examples of the aortic arch surface covered by lipid inclusions in the intima, colored red by Oil Red O (arrows). The standard group (not shown here) showed only traces of lipid droplets. In contrast, lipid droplets are extensively distributed in the control group (C). The quantification of fatty streaks is summarized in Figure 3. All photomicrographs have the same scale.

given 0.04−1.0 mg/(kg/day) Vineatrol 30 (VT1−3) in comparison with group C. Groups VT1 and VT2 displayed less important AFSA decreases (14%, NS and 39%, $P = 0.0187$, respectively) than VT3, which exhibited a 67% reduction ($P = 0.0004$) in the AFSA when compared to group C. As expected, only traces of lipid deposits were seen in the standard diet-fed group, S (0.2 ± 0.0%).


dx.doi.org/10.1021/jf303549t | J. Agric. Food Chem. 2012, 60, 11029−11036

**Liver Steatosis and Inflammation.** Standard diet-fed hamsters (group S) did not exhibit any histological evidence of hepatic steatosis or fibrosis (Figure 5a). In contrast, the presence of microvesicular steatosis of moderate to severe intensity was noted in group C, accompanied by an inflammatory reaction resulting in the presence of polymorphonuclear cells (Figure 5b). No marked reduction in the degree of steatosis was noted in the livers of HF-fed hamsters treated with any dose of Vineatrol 30 (Figure 5c). However, polymorphonuclear cells could no longer be observed.

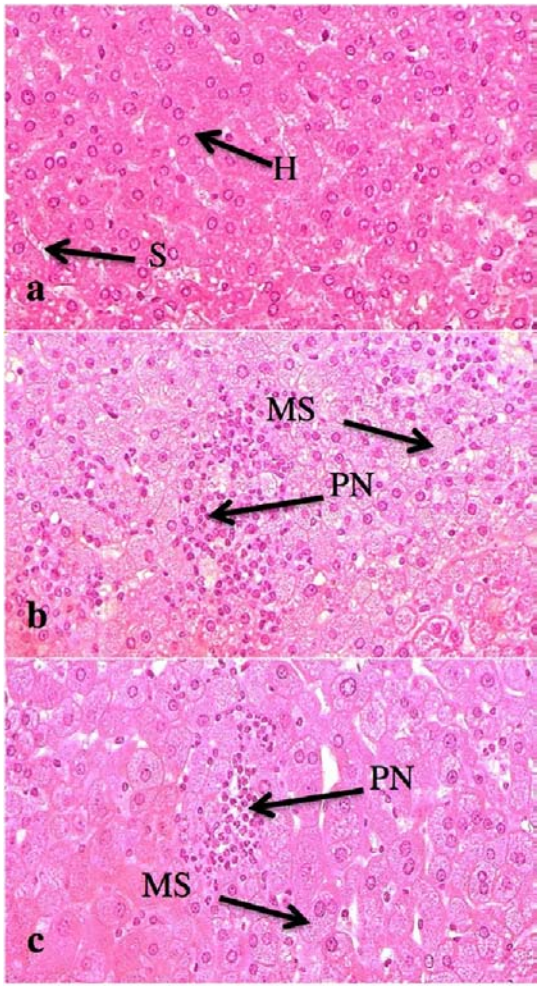


**Figure 5.** Histological evaluation of hepatic steatosis in standard diet-fed (a), high-fat atherogenic diet-fed (b), or Vineatrol 30-treated (1 mg/(kg BW/day); group VT3; (c)) hamsters after 13 weeks. Representative liver sections (40× magnification) are illustrated. Atherogenic hamsters fed with no Vineatrol 30 (b) illustrate the development of moderate to severe hepatic steatosis, along with inflammation indicated by the widespread presence of polynuclear cells, when compared to standard diet-fed animals (a). (c) Hepatic steatosis is not affected in Vineatrol-fed hamsters, although polynuclear cells are reduced compared to control animals (b). Sinusoidal capillary (S); hepatocyte (H); microvascular steatosis (MS); polynuclear cells (PN).

The concentrations of IL-6 and TNF-$\alpha$ in the liver of group C hamsters were significantly increased (by 26%, $P$ = 0.0096 and 28%, $P$ = 0.0103, respectively) in comparison with concentrations in group S hamsters (Figure 6). This low-grade inflammatory state was only reversed significantly by the ingestion of the highest dose of Vineatrol (group VT3), which led to a slight reduction in the concentration of IL-6 ($P$ = 0.0438) and TNF-$\alpha$ ($P$ = 0.0318) by 18% and 28% compared to group C, respectively. No change in inflammatory status was seen in groups VT1 and VT2.

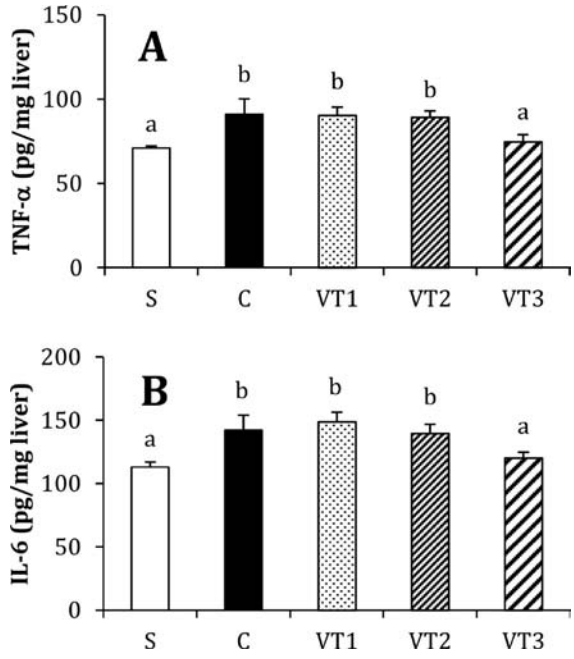


**Figure 6.** Inflammatory state indexed by cytokine concentrations in the liver: TNF-$\alpha$ (A) and IL-6 (B) in hamsters fed a standard diet (S), a high-fat diet (C), or a high-fat diet plus Vineatrol 30 at 0.04 mg/(kg BW/day) (VT1), 0.2 mg/(kg BW/day) (VT2), or 1 mg/(kg BW/day) (VT3), for 13 weeks. Values indicate means ± SEM ($n$ = 8). For each dietary treatment, bars with different index letters are significantly different ($P$ < 0.05).

**Blood Metabolic Markers.** The feeding of hamsters with a HF diet led to a 47% higher fasting plasma concentration of glucose ($P$ = 0.0056) (Table 1) and a 3-fold increase in the concentration of insulin ($P$ = 0.0058) (Table 1) leading to an insulin resistance in group C compared to group S ($P$ < 0.0002). Although Vineatrol 30 did not influence glycemia, it induced a significant decrease in plasma insulin (45% on average compared to group C) at 0.2 mg/(kg BW/day) (VT2) ($P$ = 0.0244) and 1 mg/(kg BW/day) (VT3) ($P$ = 0.0309). Despite this, Vineatrol 30 at 0.2 mg/(kg BW/day) (VT2) and 1 mg/(kg BW/day) (VT3) induced a significant lowering of HOMA-IR value (Table 1) that however remained higher than baseline values.

Hamsters in group C also exhibited slight but significantly higher leptin levels (23%, $P$ = 0.0001) than those in group S (Figure 7), and adiponectin level was not modified. Giving Vineatrol 30 by gavage significantly improved the adipokine pattern by partially preventing the leptin increase ($P$ = 0.0103). However, adiponectin levels were not significantly modified and stood close to baseline values.

## DISCUSSION

This study provides evidence for the first time that dietary supplementation with a grapevine-shoot extract rich in resveratrol and its oligomers protects against diet-induced oxidative stress and atherosclerosis in hypercholesterolemic hamsters. No evidence of toxicity or other unwanted

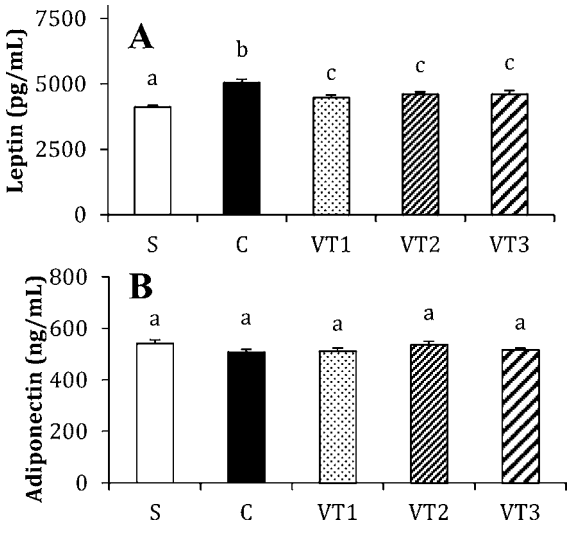


**Figure 7.** Plasma leptin (A) and adiponectin levels (B) in hamsters fed a standard diet (S), a high-fat diet (C), or a high-fat diet plus Vineatrol 30 at 0.04 mg/(kg BW/day) (VT1), 0.2 mg/(kg BW/day) (VT2), or 1 mg/(kg BW/day) (VT3), for 13 weeks. Values indicate means ± SEM ($n$ = 8). For each dietary treatment, bars with different index letters are significantly different ($P < 0.05$).

pharmacological effects of Vineatrol 30 was noted, indicating that at the doses used here, Vineatrol 30 could be a safe nutraceutical supplement.

As previously reported, the HF diet led to an increase in plasma cholesterol levels after 13 weeks, that in turn led to lipid deposition in the aortic arch. Interestingly, this diet-induced hypercholesterolemia was accompanied by a tendency to superoxide anion ($O_2^{\bullet-}$) overproduction, in agreement with previous studies in the hamster model of atherosclerosis.[24] According to the oxidative hypothesis of atherosclerosis, it is postulated that NAD(P)H oxidase works with high LDL cholesterol levels to induce foam cell fatty streak deposition and subsequent atherosclerosis.[25] Here, the reduction of both $O_2^{\bullet-}$ production and AFSA by Vineatrol 30 further supports this hypothesis. Moreover, $O_2^{\bullet-}$ production in the liver is closely dependent on the activity of the NAD(P)H oxidase subunit $p22^{phox}$.[15] In agreement with this, liver production of $O_2^{\bullet-}$ was decreased by 40% on average in animals fed with Vineatrol 30, that is, a return to production levels observed in standard diet-fed animals.

PON activity is a marker of oxidative stress. It has been proposed that PON plays a crucial role in the antioxidant activity of HDL.[26] This protection is most probably related to the ability of PON to hydrolyze oxidized phospholipids [27] and/or lipid peroxidation products.[28] PON is therefore believed to be a protective factor against atherosclerosis, and some studies have shown that PON can reduce oxidative stress in aortic lesions.[29] In the current study, only the highest dose of Vineatrol 30 was effective in increasing PON activity (Figure 1). This is consistent with a reduced AFSA in VT3 animals (Figure 3), even if there is no statistical difference between VT2 and VT3 group. Vineatrol 30 reduced the production of liver $O_2^{\bullet-}$ levels (Figure 2), possibly via the decreased activity of NAD(P)H oxidase and, therefore, the oxidative stress induced by the atherogenic diet. While this effect was independent of dose, it does suggest an antioxidant effect of Vineatrol 30, at least at the systemic level. This is in accordance with previous data from Müller et al.[18] showing that Vineatrol 30 enhances the gene-promoting activities of the antioxidant enzymes glutathione peroxidase and superoxide dismutase in the fibroblast cell line V79 in Chinese hamster, while this effect could not be demonstrated with resveratrol alone. The daily consumption of Vineatrol 30 for 13 weeks resulted in substantially lower fatty streak deposition in the arteries of VT1−3 hamsters compared to controls, with stronger effects at higher doses (0.2 or 1 mg/(kg BW/day)) (Figures 3 and 4). This marked limitation of the onset of atherosclerosis was not associated with any significant change in the plasma cholesterol profile, in keeping with the demonstration by Andrews et al.[30] that absolute cholesterolemia is not pivotal in determining the extent of aortic fatty streak deposition. However, it has been shown in the fibroblast cell line V79 that Vineatrol 30 inhibits lipid peroxidation.[18] These results thus strengthen the hypothesis that the oxidation of LDL is implicated in the pathogenesis of atherosclerosis. This could explain, at least in part, the effects of Vineatrol 30 or its phenolic components (resveratrol and its oligomers and ε-viniferin) on aortic atherosclerosis. Resveratrol and ε-viniferin are the most important components of Vineatrol 30. One of the most well-known benefits of resveratrol is for cardiovascular health.[31] Resveratrol, at nutritional doses, increases the expression in human vascular endothelial cells of eNOS.[32] As well, it decreases the expression of the potent vasoconstrictor endothelin.[32] This involved SIRT1 activation, coupled with a decrease in mitochondrial uncoupling protein 2 and an increase in mitochondrial ATP synthesizing efficiency.[33] Resveratrol has been considered as a potent activator of SIRT1. More recently, it has been shown that ε-viniferin increases mitochondrial Sirtuin 3 (SIRT3) in models of Hungtinton disease, an inherited neurodegenerative disorder caused by an abnormal polyglutamine expansion in the protein Huntingtin (Htt), and it has been suggested that SIRT3 mediates the neuroprotection of viniferin.[34] Elsewhere, we have shown that Vineatrol 30 increases SIRT1 expression in senescent fibroblasts in a dose-dependent manner, that is, 48% and 66% at 0.25 and 1.00 μg/mL medium, respectively.[35] Thus, a possible mechanism by which Vineatrol 30 is eliciting its effect could be SIRT-dependent. However, it should be kept in mind that other complex mechanisms may be involved since resveratrol could exert its cardioprotective effects by inhibition of platelet aggregation and its antioxidant effects on cholesterol metabolism,[31] and since ε-viniferin is more effective than its monomer resveratrol in improving the functions of vascular endothelial cells and the heart.[16] Therefore, the observed effects most likely result of a synergistic action of Vineatrol 30 components.

Kleemann et al.[36] have demonstrated that dietary cholesterol is not only a lipid risk factor but also a trigger for hepatic inflammation and, as such, also involved in the evolution of the inflammatory arm of atherosclerotic disease. Here, dietary cholesterol could result in an inflammatory pro-atherogenic response that enhances early lesion formation. The early presence of polynuclear cells in liver tissue at the time of manifest insulin resistance (HOMA-IR; Table 1) raises the hypothesis that these cells may orchestrate the inflammatory process in liver tissue with the subsequent development of IR. The body weight of hamsters in groups C, VT1, VT2 and VT3 was not different, suggesting that the weight of fat was not either. In addition, in group C, there was a significant leptin/adiponectin imbalance, even if small. Classically, leptin reduces ectopic fat accumulation in nonadipose tissues, enhancing

insulin mediated stimulation of glucose disposal,[37] and leptinemia increased here in C group. Moreover, adiponectin promotes fatty acid oxidation in muscle and inhibits liver glucose production,[38] and adiponectinemia was reduced here in C group. Thus, leptin and adiponectin may work hand in hand to sensitize peripheral tissues to insulin. This is what we showed a previous work on diet induced obesity in hamsters receiving a high fat diet (HFD)[39] leading to high plasma glucose, insulin and greater insulin resistance (HOMA-IR) values. A phenolic grape seed extract rich in proanthocyanidins (GSE) prevented in part these effects, reducing insulinemia and leptinemia, whereas adiponectin level increased compared with obese controls. It lowered glycemia and HOMA-IR. Here, variations of adipocytokines are less pronounced in group C because we did not use a model of obesity. It is also possible that procyanidins from grape seeds are most effective in restoring the imbalance leptin/adiponectin than resveratrol and its oligomers.

This condition of leptin/adiponectin imbalance in group C led to low-grade inflammation (increased liver polynuclear cells, TNF-$\alpha$ and IL-6) and oxidative stress (high $O_2^{\bullet -}$ production probably due to increased NAD(P)H oxidase activity). Moreover, IL-6 has been postulated by Yudkin et al.[40] to play a key role in the development of coronary heart disease, among numerous inflammatory modulators and inflammasome. Vineatrol 30 showed a beneficial effect on all these parameters at the highest dose used (group VT3). This could be attributed at least to resveratrol, since recent data suggest that dietary resveratrol and polyphenolic preparations suppress the oxidative and inflammatory stress response to a high-fat, high-carbohydrate diet hence reducing the severity of local inflammation.[41]

Steatosis-derived radical oxygen species, possibly resulting from the overactivity of NAD(P)H oxidase[42] (as measured here in the control group), increased fatty acid oxidation, and the attendant hepatocyte injury and cytokine release, are likely to perpetuate the liver damage caused by steatosis and add more atherogenic stimuli to the already high oxidative status. The steatosis observed here was a simple fat overload corresponding to nonalcoholic fatty liver disease (NAFLD), according to Figure 4. Schwimmer et al.[43] hypothesized that fatty liver is a risk factor for the early onset of atherosclerosis, independent of other prognostic factors. This suggests a more complex amplification loop involving the intertwined relationships between NAFLD and atherosclerosis. The possible biological mechanisms linking NAFLD and accelerated atherosclerosis are still poorly known. NAFLD in its more advanced forms might act as a trigger for further increasing whole-body insulin resistance and dyslipidemia, leading to accelerated atherosclerosis. Here, the insulinemia and glycemia of group C hamsters match those in a state of insulin resistance, and this can be put in parallel with the close linear relationship between liver fat content and direct measures of hepatic insulin sensitivity.[44] Only the highest doses of Vineatrol 30 (VT2 and VT3) improve the state of IR, but they do not allow to reach the baseline values. Another possible mechanism underlying NAFLD and atherosclerosis could be the link between increased oxidative stress and chronic subclinical inflammation, which are thought to be causal factors in the progression to more advanced forms of NAFLD.[45] To our knowledge, using such a diet-induced atherosclerosis in hamsters, there was never any evidence of inflammation or imbalance of leptin/adiponectin. This is what we have researched and shown here in the group of controls, although the change rates of pro-inflammatory cytokines and adipocytokines are not very important. This suggests that these animals develop early atherosclerosis but that metabolic syndrome has not yet fully installed.

In conclusion, we have demonstrated here for the first time that the grapevine-shoot extract Vineatrol 30 is capable of strongly inhibiting aortic fatty streak deposition in hamsters fed an atherogenic diet, through antioxidant and anti-inflammatory mechanisms. As discussed by others,[18,46] the individual polyphenols present in the extract could act synergistically, eliciting a strong beneficial effect by preventing the progression of the lesions observed in this hamster model of atherosclerosis. Because of the central role of oxidative stress and inflammation in disease pathogenesis and progression, the use of antioxidants as therapeutic agents to counteract cardiac and liver damage supports the potential use of Vineatrol 30 as a dietary supplement.

## AUTHOR INFORMATION

### Corresponding Author

*Address: UMR NUTRIPASS, CC 023, Université Montpellier Sud de France, Place Eugène Bataillon, 34095 Montpellier Cedex 05, France. Phone/Fax: +33 0467143521. E-mail: jm.rouanet@univ-montp2.fr.

### Notes

The authors declare no competing financial interest.

## REFERENCES

(1) Hansson, G. K. Inflammation, atherosclerosis, coronary artery disease. *N. Engl. J. Med.* **2005**, *352*, 1685−1695.

(2) Steinberg, D. Hypercholesterolemia and inflammation in atherogenesis: two sides of the same coin. *Mol. Nutr. Food Res.* **2005**, *49*, 995−998.

(3) Van Gaal, L. F.; Mertens, I. L.; De Block, C. E. Mechanisms linking obesity with cardiovascular disease. *Nature* **2006**, *444*, 875−880.

(4) Yoneda, M.; Mawatari, H.; Fujita, K.; Iida, H.; Yonemitsu, K.; Kato, S.; Takahashi, H.; Kirikoshi, H.; Inamori, M.; Nozaki, Y.; Abe, Y.; Kubota, K.; Saito, S.; Iwasaki, T.; Terauchi, Y.; Togo, S.; Mayema, S.; Nakajima, A. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. *J. Gastroenterol.* **2007**, *42*, 573−582.

(5) Hiroi, S.; Harada, H.; Nishi, H.; Satoh, M.; Nagai, R.; Kimura, A. Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with non-familial idiopathic dilated cardiomyopathy in Japanese. *Biochem. Biophys. Res. Commun.* **1999**, *261*, 332−339.

(6) Adams, L. A.; Angulo, P. Recent concepts in non-alcoholic fatty liver disease. *Diabetic Med.* **2005**, *22*, 1129−1133.

(7) Shamir, R.; Hartman, C.; Karry, R.; Pavlotzky, E.; Eliakim, R.; Lachter, J.; Suissa, A.; Aviram, M. Paraoxonases (PONs) 1, 2 and 3 are expressed in human and mouse gastrointestinal tract and in Caco-2 cell line: selective secretion of PON1 and PON2. *Free Radical Biol. Med.* **2005**, *39*, 336−344.

(8) Rosenblat, M.; Karry, R.; Aviram, M. Paraoxonase 1 (PON1) is a more potent antioxidant and stimulant of macrophage cholesterol efflux, when present in HDL than in lipoprotein-deficient serum: relevance to diabetes. *Atherosclerosis* **2006**, *187*, 74−81.

(9) Aviram, M.; Hardak, E.; Vaya, J.; Mahmood, S.; Milo, S.; Hoffman, A.; Billicke, S.; Draganov, D.; Rosenblat, M. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation* **2000**, *101*, 2510−2517.

(10) Mackness, B.; Mackness, M. I.; Durington, P. N.; Arrol, S.; Evans, A. E.; McMaster, D.; Ferrières, J.; Ruidavets, J. B.; Williams, N. R.; Howard, A. N. Paraoxonase activity in two healthy population with

differing rates of coronary heart disease. *Eur. J. Clin. Invest.* **2000**, *30*, 4−10.
(11) Auger, C.; Caporiccio, B.; Landrault, N.; Teissèdre, P. L.; Laurent, C.; Cros, G.; Besançon, P.; Rouanet, J. M. Red wine phenolic compounds reduce plasma lipids and apolipoprotein B, and prevent early aortic atherosclerosis in hypercholesterolemic Golden Syrian hamsters (Mesocricetus auratus). *J. Nutr.* **2002**, *132*, 1207−1213.
(12) Auger, C.; Teissèdre, P. L.; Gérain, P.; Lequeux, N.; Bornet, A.; Serisier, S.; Besançon, P.; Caporiccio, B.; Cristol, J. P.; Rouanet, J. M. Dietary wine phenolics catechin, quercetin and resveratrol efficiently protect hypercholesterolemic hamsters against aortic fatty streak accumulation. *J. Agric. Food Chem.* **2005**, *53*, 2015−2021.
(13) Wang, Z.; Zou, J.; Coa, K.; Hsieh, T. C.; Huang, Y.; Wu, J. M. Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. *Int. J. Mol. Med.* **2005**, *16*, 533−540.
(14) Nadtochiy, S. M.; Redman, E. K. Mediterranean diet and cardioprotection: the role of nitrite, polyunsaturated fatty acids, and polyphenols. *Nutrition* **2011**, *27*, 733−744.
(15) Décordé, K.; Ventura, E.; Lacan, D.; Ramos, J.; Cristol, J. P.; Rouanet, J. M. An SOD rich melon extract Extramel prevents aortic lipids and liver steatosis in diet-induced model of atherosclerosis. *Nutr. Metab. Cardiovasc. Dis.* **2010**, *20*, 301−307.
(16) Zghonda, N.; Yoshida, S.; Araki, M.; Kusunoki, M.; Mliki, A.; Ghorbel, A.; Miyazaki, H. Greater effectiveness of ε-viniferin in red wine than its monomer resveratrol for inhibiting vascular smooth muscle cell proliferation and migration. *Biosci. Biotechnol. Biochem.* **2011**, *75*, 1259−1267.
(17) Csiszar, A.; Smith, K.; Labinskyy, N.; Orosz, Z.; Rivera, A.; Ungvari, Z. Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappaB inhibition. *Am. J. Physiol.: Heart Circ. Physiol.* **2006**, *291*, H1694−H1699.
(18) Müller, C.; Ullman, K.; Wilken, A.; Winterhalter, P.; Toyokuni, S.; Steinberg, P. Potent antioxidative activity of Vineatrol® 30 grapevine-shoot extract. *Biosci. Biotechnol. Biochem.* **2009**, *78*, 1831−1836.
(19) National Research Council. *Guide for the Care and the Use of Laboratory Animals*; National Institutes of Health Publication no.85-123 (rev.); U.S. Government Printing Office: Washington, DC, 1985.
(20) Reeves, P. G.; Nielsen, F. H.; Fahey, G. C., Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76 rodent diet. *J. Nutr.* **1993**, *123*, 1939−1951.
(21) Weingand, K. W.; Daggy, B. P. Quantitation of high-density lipoprotein cholesterol in plasma from hamsters by differential precipitation. *Clin. Chem.* **1990**, *36*, 575−580.
(22) Jaouad, L.; de Guise, C.; Berrougui, H.; Cloutier, M.; Isabelle, M.; Fulop, T.; Payette, H.; Khalil, A. Age-related decrease in high density lipoprotein antioxidant activity is due to an alteration on the PON1's free sulfhydryl groups. *Atherosclerosis* **2006**, *185*, 191−200.
(23) Matthews, D. R.; Hosker, J. P.; Rudenski, A. S.; Naylor, B. A.; Treacher, D. F.; Turner, R. C. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412−419.
(24) Sutra, T.; Décordé, K.; Riss, J.; Dallas, C.; Cristol, J. P.; Rouanet, J. M. A commercial extract of fruits and vegetables, Oxxynea, acts as a powerful antiatherosclerotic supplement in an animal model by reducing cholesterolemia, oxidative stress, and NADPH oxidase expression. *J. Agric. Food Chem.* **2007**, *55*, 4258−4263.
(25) Heinloth, A.; Heermeier, K.; Raff, U.; Wanner, C.; Galle, J. Stimulation of NADPH oxidase by oxidized low-density lipoprotein induces proliferation of human vascular endothelial cells. *J. Am. Soc. Nephrol.* **2000**, *11*, 1819−1825.
(26) Durrington, P. N.; Mackness, B.; Mackness, M. I. Paraoxonase and atherosclerosis. *Arterioscler., Thromb., Vasc. Dis.* **2001**, *21*, 473−480.
(27) Watson, A. D.; Berliner, J. A.; Hama, S. Y.; La Du, B. N.; Faull, H. K.; Fogelman, A. M.; Navab, M. Protective effect of high density lipoprotein associated paraoxonase—Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J. Clin. Invest.* **1995**, *96*, 2882−2891.
(28) Aviram, M.; Billecke, S.; Sorenson, R.; Bisgaier, C.; Newton, R.; Rosenblat, M.; Erogul, J.; Hsu, C.; Dunlop, C.; La Du, B. N. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities - Selective action of human paraoxonase allozymes Q and R. *Arterioscler., Thromb., Vasc. Biol.* **1998**, *18*, 1617−1624.
(29) Mackness, B.; Mackness, M. I.; Durington, P. N.; Arrol, S.; Evans, A. E.; McMaster, D.; Ferrieres, J.; Ruidavets, J. B.; Williams, N. R.; Howard, A. N. Paraoxonase activity in two healthy populations with differing rates of coronary heart disease. *Eur. J. Clin. Invest.* **2000**, *30*, 4−10.
(30) Andrews, B.; Burnan, K.; Paganga, G.; Browse, N.; Rice-Evans, C.; Sommerville, K.; Leake, D.; Taub, N. Oxidisability of low density lipoproteins in patients with carotid or femoral artery atherosclerosis. *Atherosclerosis* **1995**, *112*, 77−84.
(31) Markus, M. A.; Morris, B. J. Resveratrol in prevention and treatment of common clinical conditions of aging. *Clin. Interventions Aging* **2008**, *3*, 331−339.
(32) Nicholson, S. K.; Tucker, J. A.; Brameld, J. M. Effects of dietary polyphenols on gene expression in human vascular endothelial cells. *Proc. Nutr. Soc.* **2008**, *67*, 42−47.
(33) Della-Morte, D.; Dave, K. R.; Defazio, R. A.; Bao, Y. C.; Raval, A. P.; Perez-Pinzon, M. A. Resveratrol pretreatment protects rat brain from cerebral ischemic damage via a sirtuin 1-uncoupling protein 2 pathway. *Neuroscience* **2009**, *159*, 993−1002.
(34) Fu, J.; Jin, J.; Cichewicz, R. H.; Hageman, S. A.; Ellis, T. K.; Xiang, L.; Peng, Q.; Jiang, M.; Arbez, N.; Hotaling, K.; Ross, C. A.; Duan, W. trans-(-)-{epsilon}-viniferin increases mitochondrial Sirtuin 3 (SIRT3), activates AMP-activated protein kinase (AMPK), and protects cells in models of Huntington disease. *J. Biol. Chem.* **2012**, *287*, 24460−24472.
(35) Izard, J. C. Grapevine resveratrol derivatives: health and beauty benefits. First International Conference of Resveratrol and Health, Copenhagen, September 13−15, 2010.
(36) Kleemann, R.; Verschuren, L.; van Erk, M. J.; Nikolsky, Y.; Cnubben, N. H. P.; Verheij, E. R.; Smilde, A. K.; Hendriks, H. F. J.; Zadelaar, S.; Smith, G. J.; Kaznacheev, V.; Nikolskaya, T.; Melnikov, A.; Hurt-Camejo, Z.; van der Greef, J.; van Ommen, B.; Kooistra, T. Atherosclerosis and liver inflammation induced by increased dietary cholesterol intake: a combined transcriptomics and metabolomics analysis. *Genome Biol.* **2007**, *8*, R200.
(37) Yaspelkis, B. B.; Singh, M. K.; Krisan, A. D.; Collins, D. E.; Kwong, C.; Bernard, J. R.; Crain, A. M. Chronic leptin treatment enhances insulinstimulated glucose disposal in skeletal muscle of high-fat-fed rodents. *Life Sci.* **2004**, *74*, 1801−1816.
(38) Pajvani, U. B.; Scherer, P. E. Adiponectin: Systemic contributor to insulin sensitivity. *Curr. Diabetes Rep.* **2003**, *3*, 207−213.
(39) Décordé, K.; Teissèdre, P. L.; Sutra, T.; Ventura, E.; Cristol, J. P.; Rouanet, J. M. Chardonnay grape seed procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers. *Mol. Nutr. Food Res.* **2009**, *53*, 659−666.
(40) Yudkin, J. S.; Kumari, M.; Humphries, S. E.; Mohamed-Ali, V. Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? *Atherosclerosis* **2000**, *148*, 209−214.
(41) Ghanim, H.; Sia, C. L.; Korzeniwski, K.; Lohano, T.; Abuaysheh, S.; Marumganti, A.; Chaudhuri, A.; Dandona, P. A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 1409−1414.
(42) De Minicis, S.; Brenner, D. A. Oxidative stress in alcoholic liver disease: role of NADPH oxidase complex. *J. Gastroenterol. Hepatol.* **2008**, *23* (S1), S98−S103.
(43) Schwimmer, J. B.; Deutsch, R.; Behling, C.; Lavine, J. E. Fatty liver as a determinant of atherosclerosis. *Hepatology* **2005**, *42* (S1), 610A [abstract].

(44) Yki-Jarvinen, H.; Westerbacka, J. The fatty liver and insulin resistance. *Curr. Mol. Med.* **2005**, *5*, 287−295.
(45) Adams, L. A.; Angulo, P. Recent concepts in non-alcoholic fatty liver disease. *Diabetic Med.* **2005**, *22*, 1129−1133.
(46) Billard, C.; Izard, J. C.; Roman, V.; Kern, C.; Mathiot, C.; Mentz, F.; Kolh, J. P. Comparative antiproliferative and apoptotic effects of resveratrol, epsilon-viniferin and vine-shots derived polyphenols (vineatrols) on chronic B lymphocytic leukemia cells and normal human lymphocytes. *Leuk. Lymphoma* **2002**, *43*, 1991−2002.