Adipobiology
ISSN 1313-3705

RESEARCH ARTICLE

EFFECT OF GRAPE POLYPHENOL CONCENTRATE "ENOANT" ON ADIPOKINES SECRETION IN EXPERIMENTAL METABOLIC SYNDROME

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Abstract

Recent studies demonstrate that adipokine secretion is altered in cardiometabolic diseases including atherosclerosis, hypertension, obesity, type 2 diabetes and the metabolic syndrome (MS). Polyphenols are the most important phytochemicals in grape because they possess many biological activities and health-promoting effects. The aim of the present study was to investigate the effect of grape polyphenol concentrate "Enoant" on secretion of adipokines in MS in golden hamster (*Mesocricetus auratus*). We found that the development of experimental MS is accompanied by a significant increase in plasma levels of visfatin and resistin (pro-inflammatory adipokines), whereas the level of adiponectin (anti-inflammatory and anti-diabetic adipokine) was decreased. However, the introduction of grape polyphenol concentrate "Enoant" to animal diet in MS led to a decrease in resistin and visfatin plasma levels, whereas the adiponectin level was significantly increased. To test whether the observed changes may be related to the direct action of grape polyphenols on adipocyte secretion, we used primary cultures of adipocytes isolated from adipose tissue of control animals and from animals with MS. It was found that incubation of cells for 48 h in the presence of grape polyphenol concentrate is accompanied by a significant decrease in the visfatin concentration in the incubation medium, compared to control. In cultured adipocytes isolated from the adipose tissue of animals with experimental MS, polyphenol concentrate induced a significant increase in adiponectin and decrease in visfatin secretion. These findings suggest salutogenic action of grape polyphenol concentrate "Enoant" in experimental MS. Further studies are required to evaluate whether "Enoant" might be beneficial for cardiometabolic health via the secretion of other metabotrophic adipokines (e.g., IL-10, NGF, BDNF).

Adipobiology 2012; 4: 85-90

Key words: adipose tissue, adiponectin, cultured adipocytes, grape polyphenols, visfatin, resistin

Received 5 August 2012, revised 29 August 2012, accepted 30 August 2012.
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Introduction

The metabolic syndrome (MS) is a relatively new definition, designed to help the health care practitioner to easily identify people at risk for the development of cardiometabolic diseases. According to the National Cholesterol Education Program – Adult Treatment Panel III (NCEP-ATP III) (*JAMA* 2001; 285: 2486-2497), a person, to be defined as having the MS, must have any three of five characteristics: abdominal obesity, blood pressure higher than 130/80 mmHg, plasma triglycerides higher than 150 mg/dL, HDL-cholesterol lower than 40 mg/dL (men) or 50 mg/dL (women), and blood glucose higher than 100 mg/dL.

White adipose tissue is a dynamic endocrine and paracrine organ secreting multiple adipokines (reviewed in 1,2) involved in the pathogenesis of obesity and its related diseases (3-9). As described by these authors, adiponectin is a multifunctional, salutogenic adipokine, which circulatory and/or local levels are significantly decreased in cardiometabolic diseases. Visfatin is nicotinamide phosphoribosyltransferase that is the rate-limiting enzyme catalyzing the first step in the

biosynthesis of nicotinamide adenine dinucleotide from nicotinamide. This protein was originally cloned as a putative pre-B cell colony-enhancing factor and recently, in the "era of adipobiology", found to be a visceral fat-secreted adipokine, hence visfatin. It is involved in the control of glucose and inflammation homeostasis. Resistin is an adipokine exerting negative effects on insulin action and on inflammation and thus implicated in the pathogenesis of obesity and diabetes.

A grape is a fruiting berry of the deciduous woody vines of the botanical genus described as *Vitis vinifera*. Polyphenols are the most important phytochemicals in grape because they possess many biological activities and health-promoting benefits (10–12). The phenolic compounds mainly include anthocyanins, flavanols, stilbenes (resveratrol) and phenolic acids (13–15). The use of grape seed polyphenol extract at low doses protects against fat accumulation and improves the plasma lipid profile in hamsters (16). Grape seed polyphenols suppressed high-fat-diet-induced obesity, hyperlipidemia and non-alcoholic fatty liver disease in mice (17). Grape seed polyphenol extract reduces the expression of IL-6 and MCP-1 and enhances the production of the anti-inflammatory adipokine adiponectin suggesting that may have a beneficial effect on low-grade inflammatory diseases such atherosclerosis, obesity, type 2 diabetes and MS (18). Chronic consumption of procyanidin extract from Chardonnay grape seed is shown to reduce obesity and related metabolic pathways including adipokine secretion and oxidative stress (19). Grape seed extract ameliorates the defective insulin and adiponectin signaling pathways in the skeletal muscle, resulting in improved insulin resistance in fructose-fed rats (20).

The dietary concentrate "Enoant" (hereafter to be written Enoant) contains high levels of grape polyphenols: flavonoid monomers and nonflavonoid polyphenols and their derivatives, including malvidin, cyanidin, delphinidin, peonidin, petunidin, quercetin, rutin, (+) catechin, (−) epicatechin, (−) epicatechin-gallate, gallic acid, syringic acid, caffeic acid, protocatechic acid, chlorogenic acid and trans-resveratrol. *In vivo* data from experiments in rats indicates stress-protective, hepatoprotective and antiatherogenic effects of Enoant. However, whether these effects could be mediated via Enoant action on the secretion of adipokines have been less studied.

In the present work we studied the effect of grape polyphenol concentrate Enoant on the secretion of the adipokines adiponectin, resistin and visfatin in golden hamsters under experimental MS.

Material and Methods

4 month-old male Golden hamsters (approximately 90 g of body weight) were housed two per cage in a room with controlled temperature (22–24°C), humidity and an inverse alternating light and dark cycle (12:12 hour light:dark cycle, lights on at 7 am and off at 7 pm). Animals were divided into: group 1 - controls (n=15) given standard diet and water; group 2 – animals with MS (n=15) kept for 4 weeks to a diet containing 29% vegetable oils and animal fats and fructose (2 g/100 g b.w./day); group 3 – MS + Enoant (n=15), animals with MS receiving Enoant (0,05 ml/100 g b.w., *per os*) contained approx. 20 g polyphenols per 1 L for the last 14 days; group 4 - Enoant group (n=17) received Enoant (0,05 ml/100 g b.w., per os) containing approximately 20 g polyphenols.

Enoant was developed in Institute of grape and wine Magarach (Yalta, Ukraine) and developed in Small Private Enterprise „Ressfud" (Yalta, Ukraine).

Adipocytes were isolated as described previously (21). Visceral adipose tissue was removed always between 10.00 and 12.00 h to avoid chronobiological variations in adipokine profiles, and chopped with scissors into 2μml Krebs–Ringer–HEPES (KRH) buffer (131.5 μmM NaCl, 4.7 μmM KCl, 2.5 μmM $CaCl_2$, 1.25μmM $MgSO_4$, 2.5 μmM NaH_2PO_4, 10.0 μmM HEPES), supplemented with 1% BSA. Tissues were digested with collagenase type II (1 μmg/ml) for 1 h at 37 °C in a shaker. After 1 h of digestion, supernatant was removed and adipocytes were washed with fresh KRH buffer; his method of isolation effectively removed macrophages. The number of adipocytes was counted and diluted to 1×10^6 cells/ml with 10% fetal calf serum medium. Our experiments were performed using primary cultures of hamster adipocytes.

Cells were seeded at a concentration of 2×10^6 cells/well in a plate and incubated 48 hours at 37°C in the presence of "Enoant" aliquote, contained approx. 50 μM polyphenols.

Adipokines were measured in (*i*) blood plasma of fasting animals, and (*ii*) the incubation medium of cultured adipocytes. Adiponectin was measured using hamster adiponectin ELISA kit Species Hamster (Gentaur, Belgium); visfatin - mouse visfatin (VF) ELISA Kit, (Biocompare, USA); resistin – a kit from MyBioSource (LLC, USA), according to manufacturer's protocol. Insulin level was measured using ELISA Kit (Biocompare, USA).

Lipids were extracted with chloroform and methanol (1:2 v/v) twice, as described by Bligh *et al* (22), and the supernatant was collected for determination of triacylglycerols (TG) and free fatty acids (FFA); these were determined by enzymatic colorimetric methods with commercial kits (Zhongsheng, Beijing, China). Plasma glucose was measured by a glucose oxidase method with commercial kits (AmplexR Red Glucose/Glucose Oxidase Assay Kit, Molecular Probes, USA).

All data were analyzed for statistical significance with SPSS

13.0 software. Data were presented as means ± standard deviation. Statistical analysis used one-way ANOVA. P<0.05 was considered to be statistically significant.

Results

We found that the development of experimental MS is accompanied by alterations in TG, FFA and glucose levels (Table 1). There was also an increase in insulin level as well as that of the adipokines visfatin and resistin, whereas the adiponectin content was significantly decreased (Table 2). Conversely, the introduction of grape polyphenol concentrate Enoant to the diet of animals with experimental MS led to a decrease insulin, resistin and visfatin levels in blood, whereas the adiponectin level was significantly increased (Table 2).

To test whether the observed changes may be related to the direct action of grape polyphenols on adipocyte secretion, we used primary cultures of adipocytes isolated from adipose tissue of control animals and from animals with MS. It was found that incubation of cells for 48 h in the presence of grape polyphenol concentrate was accompanied by a significant decrease in the visfatin concentration in the incubation medium, compared to control (Fig. 1). The concentrations of adiponectin and resistin in these conditions were not significantly changed.

Upon incubation of adipocytes isolated from adipose tissue of animals with experimental MS, there was a significant increase in the concentration of adiponectin and decreased visfatin in the cells incubation medium (Fig. 2). The resistin release in these conditions was not significantly changed.

Discussion

At present, the MS is considered the major cardiometabolic risk factor, playing a leading role in the development of type 2 diabetes and atherosclerosis. It is known that MS is accompanied by (or, resulted from) low-grade inflammation and oxidative stress leading to changes in lipid and carbohydrate metabolism (25-28).

Effects of different plant polyphenols on metabolism in adipose tissue have been reported (23,24). The changes documented in the present study may be related to the direct action of grape polyphenols on the adipocytes and/or other adipose tissue cells. Plant polyphenols possess great antioxidant activity, which may also contribute to preventing the development of experimental MS (11,17,20). The increase in plasma FFA level may be one of the causes of the visfatin secretion impairment and subsequent insulin resistance (30). Our results are consistent with the literature data on the effects of plant polyphenols (quercetin

Table 1. The increase of glucose, triacylglycerols (TG) and free fatty acids (FFA) level in the blood of experimental animals with the metabolic syndrome (n = 6).

Group	FFA, mmol/l	TG, g/l	Glucose, mmol/ml
Control	0,85±0,04	0.97±0.03	4.65±1.67
MS	1,20±0,14*	2.14±0.06*	12.7±2.6*

The data presented as mean±SD, * –p 0.05 *versus* control animals.

Table 2. Effect of grape polyphenol concentrate Enoant on the insulin, adiponectin, resistin and visfatin level in the blood plasma of gold hamsters with experimental metabolic syndrome (n = 6).

Molecules	MS	MS + Enoant	Control	Enoant
Insulin, ng/ml	1.46±0.20*	0.94±0.15**	0.85±0.22	0.87±0.13
Adiponectin, ng/ml	0.87±0.09*	1.33±0,49**	1.75±0.38	1.85±0.44
Visfatin, ng/ml	35,5±5,4*	22,9±4,2**	19,9±1,4	19,7±2,2
Resistin, ng/ml	7,38±0,64*	5,01±0,46**	4,01±0,47	3,98±0,67

The data presented as mean ± SD; * –p 0.05 *versus* control animals, ** –p 0.05 *versus* MS animals.

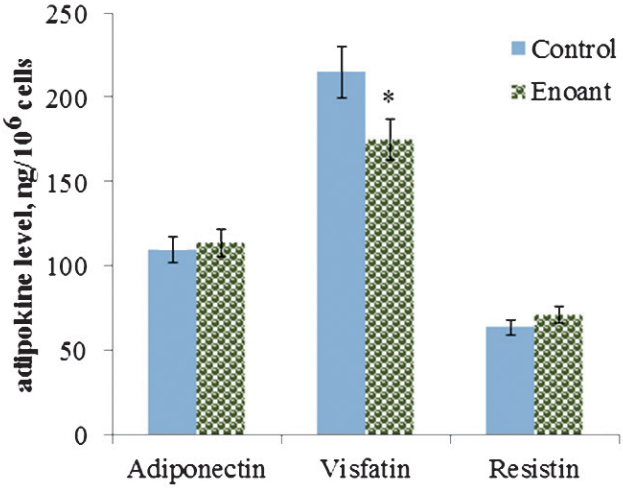

Figure 1. Effect of grape polyphenol concentrate Enoant treatment (48 hours) on adipokine secretion in cultured adipocytes isolated from control animals. Adipokine levels were measured in cell incubation medium. The data presented as mean ± SD; * – p0.05 *versus* control.

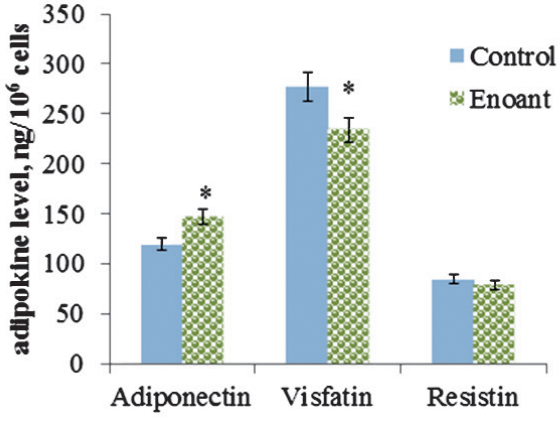

Figure 2. Effect of grape polyphenol concentrate Enoant treatment (48 hours) on adipokine secretion in cultured adipocytes isolated from animals with metabolic syndrome. Adipokine levels were measured in cell incubation medium. The data presented as mean ± SD; * –p 0.05 *versus* control.

and resveratrol) on the adipokine secretion in human Simpson Golabi Behmel Syndrome (SGBS) cells (31, also see the state-of-the-science review of Renes in this volume of *Adipobiolgy*). Grape polyphenols are involved in the regulation of signaling pathways which involve Akt/PKB system (32,33). We suppose that this may also be the case with the effects of Enoant's polyphenols.

In conclusion, the present study demonstrates that the grape polyphenol concentrate Enoant affects the secretion of adipokines both *in vivo* and *in vitro*. The administration of Enoant results in a normalizing the level of insulin, adiponectin, resistin, and visfatin in golden hamsters with experimental MS. Altogether, our results suggest a salutary action of grape polyphenol concentrate Enoant in MS. Further studies are required to evaluate whether Enoant (*i*) may also be beneficial for cardiometabolic health in humans, (*ii*) may up-regulate the secretion of other (along adiopnectin) metabotrophic adipokines, such as NGF, BDNF and IL-10 (34-36), and (*iii*) may like other natural polyphenols (e.g., resveratrol) mimic salutogenic effects of calorie restriction (37,38).

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