

Resveratrol Reduce Metabolic Disorders in Obese Rats

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ABSTRACT

Extensive research within the past two decades has revealed that obesity, a major risk factor for type 2 diabetes, atherosclerosis, cancer, and other chronic diseases, is a proinflammatory disease. Several spices have been shown to exhibit activity against obesity through antioxidant and anti-inflammatory mechanisms. Among them, resveratrol, has been investigated most extensively as a treatment for obesity and obesity-related metabolic disorders.

The aim of our present study was therefore to evaluate the ability of a chronic oral administration of resveratrol to correct metabolic disorders induced by a cafeteria diet in the rat. Male Wistar rats weighing 185-220g were used they were subdivided into four groups of homogeneous weights, Classical biochemical parameters were determined Plasma glucose, HDL (High Density Lipoprotein) cholesterol, total cholesterol, triglycerides, glucose, insuline and phospholipids were determined using a Konelab automatic plasma analyser. And the Parameters of the oxidative stress AOPP détermination and TBARS were determined, Systolic Blood Pressure and the evolution of the weight gain percentage of all the groups along the period of treatment were assessed.

The result showed resveratrol-treatment restored significantly insulin in cafeteria diet rats, IR index of HOMA, was higher in cafeteria than in control and restored to control levels in cafeteria-treated animals. At the end of the 5-week, cafeteria-fed and untreated rats had significantly higher weight gain ($32.51 \pm 5.76\%$ vs $23.24 \pm 2.32\%$) than the control group,

Plasma glucose was higher in cafeteria-fed rats and beta cell function index (HOMA BCF) respectively, between the groups. The result showed also the Administration of resveratrol restored the mean systolic blood pressure in cafeteria fed rats to a level not significantly different from that of control animals.

KEY WORDS: AOPP, HOMA, Cafeteria diet, Resveratrol, Metabolic disorders, Obesity

INTRODUCTION

Resveratrol has anti-obesity properties by exerting its effects directly on the fat cells," Fischer-Posovszky said. "Thus, resveratrol might help to prevent development of obesity or might be suited to treating obesity." It has been known for some time that moderate and regular consumption of polyphenol rich beverage can reduce the risk of cardiovascular diseases. This concept is known as "the French Paradox"(1). Such effects are due to various compounds of polyphenol, particularly resveratrol

These natural constituents, found in most of fruit and vegetables (tea, apple, grapes...) are particularly known for their antioxidant and free radical-scavenging properties leading to a decrease in LDL oxidation (2;3) and platelet aggregation. They possess a lot of other biological activities, such as anti-inflammatory, anti-cholesterolemic, vaso-active or anti-carcinogenic effects. Yet their mechanism of action still remains uncertain.

Obesity has been positively correlated with insulin resistance in humans (4;5) and in genetically defective animals such as fatty Zucker rats (6) and predisposes to hypertension, atheroma and dyslipidaemia, in particular hypertriglyceridaemia. Furthermore the association between obesity, hypertension, insulin-resistance and dyslipipidaemia has been described as the metabolic syndrome or syndrome X (7;8;9).

For a long time, cafeteria diet has been used as an experimental model to study obesity. It represents a model of human obesity induced by "Western" diet.

Most of the time the overweight induced by the cafeteria diet is associated with hyperinsulinemia and insulin resistance (10) and with other metabolic abnormalities such as hypertriglyceridemia or hypercholesterolemia. Furthermore, an oxidative stress is associated with the high fat diet-induced obesity (11). The amount of oxidative stress can be measured by the increased indices of free radical mediated damage to lipids proteins, and DNA in plasma and urine and these markers are associated with other several pathophysiological status like aging, atherosclerosis, insulinresistance and diabetes, (12). This oxidative stress has been related to hyperglycemia, elevated fatty acids and hyperinsulinemia which is responsible for accelerated cardio-vascular diseases, and is a pathogenetic factor in the development of diabetic complications and other diseases.

The aim of our present study was therefore to evaluate the ability of a chronic oral administration of resveratrol to correct metabolic disorders induced by a cafeteria diet in the rat, and consequently to understand better their mechanism of action. Effects on classics biochemical parameters and on two plasmatic markers of oxidative stress, AOPP (Advanced Oxidation Protein Products) and TBARS (Thiobarbituric acid reactive substances) also called MDA (Malonic dialdehyde acid) were determined.

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Their actions on hemodynamic parameters were also evaluated by measure of blood pressure.

The main finding of the present study is that resveratrol treatment restores lipids abnormalities, hyperinsulinemia, and hypertension induced by the cafeteria diet. Resveratrol, a natural polyphenolic compound, was shown to protect rodents against high-fat-diet induced diabetes by boosting energy metabolism this broad spectrum of effects is enlarged by new data demonstrating a great potency of this compound in relation to obesity and diabetes (23).

MATERIALS AND METHODS

Animals and treatment

Male Wistar rats weighing 185-220g (Iffa-Credo, Arbresle, France) were used and maintained on a 12 hour light / dark (6:00 am – 6: 00 pm), temperature and humidity controlled environment. After an adaptation period of one week, they were subdivided into four groups of homogeneous weights with two dietary conditions for 6 weeks: an untreated control group (C), a treated control group (C+R), an untreated cafeteria group (Caf), and a treated cafeteria group (Caf+R), 8 animals in each group, one animal per cage. The two control groups received A04 standard rat chow commercialised by UAR (Villemoisson-sur-Orge, France) with a composition described in detail in Table 1.

Table 1: Composition of diet “A 04”

Diet Ingredients	Standard diet
Constituants	g/Kg
Protein ¹	165
Fat Material	30
Mineral Ashes mix ²	52
Cellulose	40
Other components	
Energy	about 3000 Kcal/Kg
Added Compouds	
Vitamin A (UI/Kg)	6500
Vitamin D3 (UI/Kg)	800
Vitamin E (mg/Kg)	30
Copper (mg/Kg)	25

Cafeteria groups were daily offered cookies, liver pate (as a “sandwich” with cookies), bacon, standard chow, water and whole milk supplemented with 10% sucrose. All the food items were weighed and daily presented in excess.

Selective food consumption and the weight of all animals were measured every day.

Table 2: Composition of cafeteria diet

Diet Ingredients	Mean % supplied by the manufacturer and Energy
Cookies	
Carbohydrates	69%
Protein	6%
Lipid	21%
Other components	
Energy	4888 Kcal/Kg
Liver pate	
Protein	17.9%
Lipid	24.8%
Other components	
Energy	3520 Kcal/Kg
Bacon	
Energy	6590 Kcal/Kg
Sweeted whole milk	
Whole milk	4.7%
-Carbohydrate	3.2%
-Protein	3.6%
-Lipid	10%
Added sucrose	
Energy	612Kcal/Kg
-Whole milk	1012 Kcal/Kg
-Sweeted whole milk	

C+R and Caf+R groups were daily treated by oral gavage with a resveratrol solubilized in distilled water (200mg/kg of body weight, 10ml/kg). The dose was chosen as the optimal dose able to reduce hyperglycemia in streptozotocin (STZ)-induced diabetic rats during an oral glucose tolerance test (OGTT). The same dose was shown to lower hyperglycemia in STZ rats under chronic condition of treatment (Al-Awwadi et al., 2004). Untreated groups received water in the same conditions. At the end of the 5-week study, animals were killed by decapitation and blood collected on heparinized tube. Plasma was stored at -20°C until further use.

Classical biochemical parameters

Plasma glucose, HDL (High Density Lipoprotein) cholesterol, total cholesterol, triglycerides, and phospholipids were determined using a Konelab automatic plasma analyser.

Determination of cholesterol level

The principle consists in the hydrolysis of the esterified cholesterol by cholesterol esterase in free cholesterol and fatty acids. Then cholesterol oxydase oxidizes cholesterol in cholestene-4, one-3 et en hydroperoxide, which combines using a peroxydase with amino-4-phénazone and phenol to give water and a colored compound absorbing at 500nm, monoiminoparabenzquinone-4-phenazone.

Determination of triglycerid level

Triglycerids' hydrolysis by lipase gives glycerol and free acids. Glycerol, by the action of glycerokinase in presence

¹ Protein mixture : oat, wheat, corn, bran, soya, fish protein.

² Mineral mixture contained principally: Ca₂PO₄, CaCO₃

of ATP, gives glycerol-3-phosphate and ADP. Glycerol-3-phosphate undergoes the action of glycerol-3-phosphate oxydase to give dihydroacetone. Hydroperoxyde, using a peroxydase, combines with amino-4-antipyrine and parachlorophenol to give water and a colored compound absorbing at 500nm, quinonéimine.

Determination of phospholipid level

Phospholipids are hydrolysed in presence of water by phospholipase D in choline which is dosed by the Trinder reaction, phosphatidic acid, lysophosphatidic acid and N-acylsphingosyl phosphate. Choline is oxidized by choline oxydase in betaïne and hydroperoxyde, which combines using a peroxydase with amino-4 antipyrine and parachlorophenol to give water and quinoneimine, a compound absorbing at 500nm.

It must be noted that the technique does not allow the dosage of all of the phospholipids: glycerophospholipids as phosphatidylcholine, phosphatidylethanolamine or phosphatididylserine, and ceramids as sphingomyeline. Only cholin phospholipids (phosphatidylcholin or lecithin, lysolecithin and sphingomyelin) are evaluated.

Assessment of the plasma insulin level

It was determined by the radio immunological method (14) using rat insulin as standard, ¹²⁵I, and an pig anti-serum . Dosage was made in a final volume of 500µl of borate buffer 0.025M with 0.5% BSA. After two days of incubation at 4°C, separation of the free insulin was achieved using activated coal 5% (Prolabo, ref.26008.296) in borate buffer 0.1M after addition of 100µl of horse serum (Biomeda) by sample. Samples were then diluted in 2ml of borate buffer 0.1M and centrifuged at 3000rpm during 5 min and residue-containing insulin unbound and complexed with coal was counted with a scintillation gamma counter (LKB-Wallac).

Calculation of HOMA (The homeostatic model assessment) parameters .

In each subject, the degree of insulin resistance was estimated at the baseline by HOMA according to the method described by Matthews et al. (32). In particular, an insulin resistance score HOMA-IR was computed with the formula: **plasma glucose (mmol/l) times serum insulin (mU/l) divided by 22.5**. Beta cell function was assessed by the HOMA-BCF score : **20 times serum insulin (mU/l) divided by (plasma glucose (mmol/l) – 3.5)**.

Parameters of the oxidative stress

AOPP determination (Advance oxidative protein products).

AOPP were evaluated by spectral analysis following the experimental method of Witko-Sarsat et al. (15).

The principle consists in the determination of the absorbance at 340nm of the plasma in acidic conditions using a microplate reader.

Plasma samples were diluted in phosphate buffer pH 7.4. The protocol was calibrated with chloramine-T solutions

which in the presence of potassium iodide absorb at 340nm.

AOPP concentrations were expressed in µmol/L of chloramines-T equivalents.

Evaluation of lipid peroxidation by TBARS (Thio-parpitaric acid reactive substance).

Plasma TBARS were determined by the fluorimetric method of Yagi (16).

Briefly, plasma samples were deproteinized with N/12 sulfuric acid and 10% phosphotungstic acid which precipitates proteins and lipoproteins, and then heated with TBA in NaOH (0.67 %) at 95°C for 1h. The fluorescent pigment resulting from the reaction of lipoperoxids and TBA was extracted by butanol. Fluorimetric measurements (excitation at 515nm and emission at 553 nm) were performed on supernatants. Results were expressed as µmol/L plasma.

Systolic Blood Pressure

Blood pressure was assessed with the tail-cuff method with a Letica Scientific Instrument electrosphyngomanometer composed by a thermoregulated room containing six restrainers and a microprocessor, after the rats were warmed at 35°C for 10 minutes. Blood pressure was measured under conscious conditions during the 6th week of the study. The average of 3 pressure readings was recorded for each measurement.

Treatment group	EI (Kcal)	% WG
C	2193.11 ± 61.43a	22.99 ± 2.12a
C+R	2113.21 ± 68.77a	27.01 ± 3.58ab
Caf	4472.56 ± 186.38b	33.16 ± 4.69b
Caf+R	4304.69 ± 130.66b	31.37 ± 3.11ab

Statistical analysis

Data are shown as the mean ± SEM Statistical comparisons were performed with the Stat graphics software (Unware, Paris, France) using a multiple range test after ANOVA analysis. When a significant difference was obtained (p ≤ 0.05), a least significance difference (LSD) test was used to compare each pair of means.

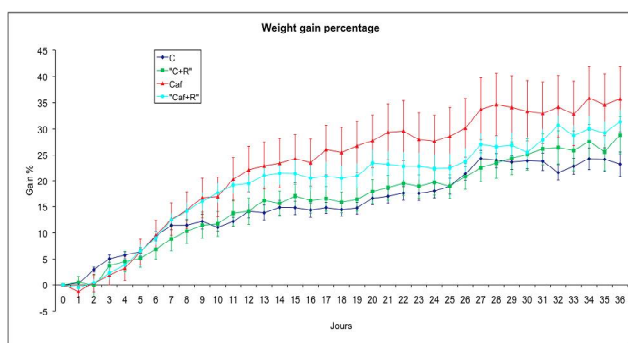
RESULTS

General features of the animal

Tables 1 indicates, the average of energy intake (EI) and weight gain percentage (WG) of the rats at sacrifice at the of the 5 weeks of the study. No mortality was observed in any treatment-group.

Figure 1: Progression of the weight gain percentage of rats in control (C), control-resveratrol (C+R), cafeteria (Caf) and cafeteria-resveratrol (CAF+R) treated groups (mean±SEM) (n=8) during the 5 week- study. For each treatment-group, means in a column with different superscripts differ (p<0.05).

Figure 1 represents the evolution of the weight gain percentage of all the groups along the period of treatment.



The amount of each food items consumed was weighed and converted into their energetic value so as to determine the daily energy intake. Throughout the experiment, cafeteria-fed rats (Caf and Caf+R) groups reduced their consumption of standard diet in favour of the cafeteria diet, and their energy intake at the end of the experiment was twice that of control rats (C and C+R). No differences were noted between treated and untreated control groups (C vs C+R) but the differences were found between cafeteria non treated group (Caf) vs. control group (C).

Fluid intake was calculated by the sum of water and milk consumption. This parameter was significantly higher in the cafeteria-fed rats due to the milk absorption. (Data not shown).

Differences in the body weights were evaluated by the percentage of weight gain. From the 11th day, cafeteria rats started getting fatter than the control rats. At the end of the 5-week, cafeteria-fed and untreated rats had significantly higher weight gain ($32.51 \pm 5.76\%$ vs $23.24 \pm 2.32\%$) than the control group, except one animal, which didn't respond to the diet although his metabolic parameters were significantly altered (see later). No differences were seen for the other groups.

Table 1: General characteristics of rats, Energy intake (EI) and weight gain (WG) percentage in control (C), control-resveratrol (C+R), cafeteria (Caf) and cafeteria-resveratrol (Caf+R) treated groups (mean \pm SEM) (n=8). For each treatment-group, means in a column with different superscripts differ (p<0.05).

Treatment group	GLY (mmol/L)	Ins (mU)	HOMA (IR)	HOMA (BCF)
C	5.34 \pm 0.18a	40.22 \pm 6.76ab	10.64 \pm 1.34a b	480.53 \pm 52.30a
C+R	5.39 \pm 0.21a	45.58 \pm 6.55ab	10.81 \pm 2.48a b	391.97 \pm 106.63a
Caf	6.07 \pm 0.28a	67.42 \pm 13.68a	18.44 \pm 4.77a	548.51 \pm 102.64a
Caf+R	5.67 \pm 0.21a	40.49 \pm 6.17b	9.89 \pm 1.43b	410.53 \pm 53.95a

Metabolic data

The mean plasma glucose, insulin and index of insulin-resistance (HOMA: ir) and insulin-secretion or beta cell function (HOMA: bcf) in control and experimental animals are shown in table 2.

Table 2: Mean (\pm SEM) plasma glucose (GLY), insulin (Ins) and index of insulin-resistance (HOMA: ir) and

insulin-secretion or beta cell function (HOMA: bcf) of rats in control (C), control-resveratrol (C+R), cafeteria (Caf) and cafeteria-resveratrol (Caf+R) treated groups (n=8). For each treatment-group, means in a column with different superscripts differ (p<0.05).

Treatment group	EI (Kcal)	% WG
C	2131.72 \pm 60.41a	24.05 \pm 2.51a
C+R	2117.22 \pm 58.76a	28.72 \pm 3.49ab
Caf	4398.89 \pm 161.51b	32.66 \pm 4.67b
Caf+R	4201.93 \pm 121.14b	30.97 \pm 3.41ab

Plasma insulin was higher in cafeteria-fed rats but not significantly different from those of control groups. In the cafeteria treated group (Caf+R) resveratrol-treatment restored significantly insulin to a level vs cafeteria group (C), even lower than that of control. Similarly insulin-resistance evaluates using the IR index of HOMA, was higher in cafeteria than in control and restored to control levels in cafeteria-treated animals.

Plasma glucose was higher in cafeteria-fed rats but no differences were observed in plasma glucose and beta cell function index (HOMA BCF) respectively, between groups

Resveratrol improve beta cell function in cafeteria (Caf+R, C+R) and control treated rats even lower than that of control non-treated rats.

Table 3 gives the mean plasma levels of plasma total cholesterol (TC), triglycerides (TG), phospholipids (PL), HDL cholesterol (HDL-C), in control and experimental animals.

Table 3: Mean (\pm SEM) plasma triglycerides (TG), phospholipids (PL), HDL cholesterol (HDL-C), of rats in control (C), control-resveratrol(C+R), cafeteria (Caf) and cafeteria-resveratrol (Caf+R) treated groups (n=8).

For each treatment-group, means in a column with different superscripts differ (p<0.05).

Treatment group	TC (g/L)	HDL (g/L)	TG (g/L)	PL (g/L)
C	0.61 \pm 0.03a	0.52 \pm 0.04a	0.55 \pm 0.06a	1.18 \pm 0.04a
C+R	0.65 \pm 0.02ab	0.53 \pm 0.02a	0.72 \pm 0.05a	1.26 \pm 0.02a
Caf	0.71 \pm 0.02b	0.48 \pm 0.06a	1.73 \pm 0.03b	1.59 \pm 0.05b
Caf+R	0.67 \pm 0.03ab	0.51 \pm 0.03a	1.01 \pm 0.12a	1.29 \pm 0.03a

Plasma triglycerides, and phospholipids were significantly higher in cafeteria-fed rats than in control and cafeteria resveratrol treated groups and total cholesterol was significantly higher in cafeteria-fed rats than in untreated control group. No significant difference was seen in HDL cholesterol.

Resveratrol- treatment of cafeteria-fed rats restored total cholesterol, triglycerides and phospholipids to a level not significantly different from that of control animals.

Markers of oxidative stress

As depicted in table 4, which presents parameters of oxidative stress, TBARS and AOPP, respectively, neither

cafeteria-diet nor resveratrol-treatment induce any significant change in plasma TBARS and AOPP at the end of the study when compared to the control group.

Table 4: Mean (\pm SEM) of thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP) in plasma of rats in control (C), control-resveratrol (C+R), cafeteria (Caf) and cafeteria-resveratrol (Caf+R) treated groups (n=8). For each treatment-group, means in a column with different superscripts differ ($p < 0.05$).

Treatment group	TBARS	AOPP
C	0.40 \pm 0.03a	10.4 \pm 1.7a
C+R	0.46 \pm 0.02a	11.5 \pm 1.5a
Caf	0.49 \pm 0.04a	11.6 \pm 0.7a
Caf+R	0.44 \pm 0.03a	12.5 \pm 1.6a

Blood pressure

As shown in the Table 5, which represents the mean systolic blood pressure during the 5-week of the study, this parameter was higher in cafeteria-fed rats but not significantly different from those of control groups. Administration of resveratrol restored this parameter to a level not significantly different from that of control animals.

Table 5: Mean (\pm SEM) systolic blood pressure (mmHg) of rats in control (C), control-resveratrol (C+R), cafeteria (Caf) and cafeteria-resveratrol (Caf+R) treated groups (n=8). For each treatment-group, means in a column with different superscripts differ ($p < 0.05$).

Treatment group	BP (mmHg)
C	119.16 \pm 2.31ab
C+R	116.12 \pm 1.84a
Caf	131.63 \pm 1.97b
Caf+R	119.94 \pm 2.15a

DISCUSSION

The present study investigated in Wistar rats the effects of a cafeteria diet, on growth, blood pressure, metabolic parameters and oxidative stress, and determined the ability of resveratrol administrated by chronic oral administration to prevent alterations induced by this model. Such a diet has been extensively used as an experimental model to study obesity and energy balance expenditure.

Offering several energy dense human foods to the rats induced voluntary hyperphagia leading to an increase in energy intake, in agreement with previous reports (17;18), as cafeteria diet rats consumed about twice more calories than control rats, and this induced overweight. These findings are consistent with those of other authors who study the cafeteria model (10;17;19;20;21) or the high fat diet model (11;22). But sometimes, several studies particularly with the high fat diet model, reveal that diet exhibits a bimodal pattern in body weight (10;22), and authors subdivide the animals into obesity prone and

obesity resistant groups. We can't tell that our cafeteria rats were obese but they had a significant overweight associated with early alterations in metabolic parameters. Even in the animal that did not gain excessive weight, the highly palatable diet significantly increased metabolic (lipids, insulin and blood pressure) parameters. We know that these parameters play important roles in the development of cardio-vascular disease in obesity.

If we had continued the study the difference observed between the groups would have increase. Generally most of the studies with a cafeteria or high fat diet are done for a long period (10 weeks or more).

Resveratrol treatment was reduce the hyperphagia and the following increases in energy intake and weight gain in agreement with the reports of Swindell WR Mech Ageing Dev 2008. Which in recent works have demonstrated that resveratrol supplementation mimics caloric restriction in mice, and he suggested that resveratrol could be a good candidate for the development of obesity therapies, also in agreement with (37), (38) They found that resveratrol inhibited the pre-fat cells from increasing and prevented them from converting into mature fat cells, and that resveratrol also hindered fat storage. Resveratrol reduced production of certain cytokines (interleukins 6 and 8), substances that may be linked to the development of obesity related disorders, such as diabetes and clogged coronary arteries. Resveratrol also stimulated formation of a protein called adiponectin that is reduced in obesity. Adiponectin is known to decrease the risk of heart attack.

Concerning the metabolic parameters overweight was associated with dyslipidaemia, as total cholesterol, triglycerides and phospholipids underwent a significantly increase.

In accord with previous studies, this cafeteria-diet model was associated with an increase in blood pressure, (11;22) and insulin level although not significant. These authors observed an blood pressure augmentation at the end of 10 weeks of a high fat diet Polyphenols also decreased the hyperinsulinemia observed in cafeteria-fed rats.

Resveratrol administration restores these augmentations. These findings correspond to those of (33;34;35;36) who showed that in mice, long-term CR results in a host of behavioral, physiological, and metabolic changes, including increased foraging activity; decreased body temperature, heart rate, blood pressure, blood glucose, insulin, fat mass, and body weight; and increased glucose tolerance and insulin sensitivity, also (24), who showed that the other compounds of polyphenol like the grape tannins, tannic acid and tea catechins are able to decrease cholesterol level in the rat.

This hypocholesterolemic action of resveratrol has been related to an increase in cholesterol transport so as to eliminate it and a decrease in its intestinal absorption.

We know that tannins can bind to several endogenous proteins in the digestive tractus, like digestives enzymes, and inhibit them (25). As a consequence of this property, proteins, lipids and carbohydrates digestion are inhibited. Amylase inhibition and the decrease in carbohydrates hydrolysis can reduce postprandial glycemia (30). Besides, polyphenols can form complex with polysaccharides like

amidon and modify glycemia and insulin level (31). Polyphenol compounds decreased in a significant manner, the hypertension, in accord with previous studies (26;27). The effects of polyphenols on hemodynamic functions has been widely studied and their anti-hypertensive effects has been related to an improve NO vasodilatation.

In the obese state, hyperinsulinemia and insulin resistance have been shown to be associated with hypertension although conflicting data exists on the role played by insulin in the development of hypertension. (28;29).

Hypersinsulinemia is possibly also responsible for the abnormal lipid profil.

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