Alcohol and Alcoholism Vol. 46, No. 1, pp. 10–16, 2011
Advance Access Publication 7 December 2010
doi: 10.1093/alcalc/agq085

PHARMACOLOGY AND CELL METABOLISM

Weekend ethanol consumption and high-sucrose diet: resveratrol effects on energy expenditure, substrate oxidation, lipid profile, oxidative stress and hepatic energy metabolism

Katiucha Karolina Honório Ribeiro Rocha[1], Gisele Aparecida Souza[2], Fábio Rodrigues Ferreira Seiva[2], Geovana Xavier Ebaid[2] and Ethel Lourenzi Barbosa Novelli[2,*]

[1]Department of Chemistry and Biochemistry, Institute of Biological Sciences, São Paulo State University, UNESP, 18618-000 Botucatu, São Paulo, Brazil and [2]Department of Clinical and Cardiology, School of Medicine, São Paulo State University, UNESP, 18618-000 Botucatu, São Paulo, Brazil

*Corresponding author: Tel: +55-14-38116255; Fax: +55-14-38153744; E-mail: drno@uol.com.br

(*Received* 14 *June* 2010; *in revised form* 25 *September* 2010; *accepted* 4 *November* 2010)

Abstract — Aims: The present study analyzed the association between weekend ethanol and high-sucrose diet on oxygen consumption, lipid profile, oxidative stress and hepatic energy metabolism. Because resveratrol (RS, 3,5,4'-trans-trihydroxystilbene) has been implicated as a modulator of alcohol-independent cardiovascular protection attributed to red wine, we also determined whether RS could change the damage done by this lifestyle. Methods: Male *Wistar* 24 rats receiving standard chow were divided into four groups ($n = 6$/group): (C) water throughout the experimental period; (E) 30% ethanol 3 days/week, water 4 days/week; (ES) a mixture of 30% ethanol and 30% sucrose 3 days/week, drinking 30% sucrose 4 days/week; (ESR) 30% ethanol and 30% sucrose containing 6 mg/l RS 3 days/week, drinking 30% sucrose 4 days/week. Results: After 70 days the body weight was highest in ESR rats. E rats had higher energy expenditure (resting metabolic rate), oxygen consumption (VO_2), fat oxidation, serum triacylglycerol (TG) and very low-density lipoprotein (VLDL) than C. ES rats normalized calorimetric parameters and enhanced carbohydrate oxidation. ESR ameliorated calorimetric parameters, reduced TG, VLDL and lipid hydroperoxide/total antioxidant substances, as well enhanced high-density lipoprotein (HDL) and HDL/TG ratio. Hepatic hydroxyacyl coenzyme-A dehydrogenase (OHADH)/citrate synthase ratio was lower in E and ES rats than in C. OHADH was highest in ESR rats. Conclusions: The present study brought new insights on weekend alcohol consumption, demonstrating for the first time, that this pattern of ethanol exposure induced dyslipidemic profile, calorimetric and hepatic metabolic changes which resemble that of the alcoholism. No synergistic effects were found with weekend ethanol and high-sucrose intake. RS was advantageous in weekend drinking and high-sucrose intake condition ameliorating hepatic metabolism and improving risk factors for cardiovascular damage.

INTRODUCTION

Weekend alcohol (ethanol) consumption is widely provided as snacks, meal accompaniments and aperitif just prior to a high-sucrose diet (Mattes, 2006), but there are several concerns about this lifestyle that should be addressed.

Sucrose-rich diet has been blamed as causally related to the incidence of obesity in populations, changing oxygen consumption, energy expenditure (Novelli *et al*., 2010), inducing dyslipidemia and increasing the risks for cardiovascular damage (Seiva *et al*., 2010). The links between obesity and health adverse effects were derived from the evidence that during energy metabolism, the mitochondrial respiratory chain represents a major intracellular source of reactive oxygen species (ROS), and the use of dietary sucrose for energy generation may result in higher ROS, thus inducing oxidative stress, an imbalance between oxidants and antioxidants in favor of the former. Oxidative stress plays a crucial role in atherosclerosis and hepatic metabolic changes (Novelli, 2005).

On the other hand, dose-dependent atherogenic and antiatherogenic properties may constitute a main pathophysiological link between alcohol consumption and cardiovascular diseases, but the mechanisms that are triggered for these actions are yet poorly understood. Recently we have reported that alcoholism-induced adverse effects were related to enhanced energy expenditure and calorimetric changes, which were reflected on lipid profile and hepatic oxidative stress (Seiva *et al*., 2009a, b), but results are conflicting as to whether drinking pattern modifies lipid levels (Murray *et al*., 2002; Tolstrup *et al*., 2006; Trevisan *et al*., 2001). Therefore, the question is if the balance between beneficial and harmful effects of ethanol is affected by drinking pattern.

Although alcohol consumption and high-sucrose diet have been frequently associated with a cluster of metabolic disorders (Novelli *et al*., 2009, 2010; Seiva *et al*., 2009a, b), neither the precise mechanism of, nor whether weekend ethanol consumption may aggravate high-sucrose diet-related adverse effects have been recognized.

Resveratrol (RS; *trans*-3,4,5-trihydroxystilbene) a polyphenol from grape, *Morus* species, has been shown to be responsible for the cardiovascular benefits associated with moderate red wine consumption (Turner *et al*., 1999). Experimental studies have pointed beneficial effects of RS such as, improvement of lipid profile and lipoprotein metabolism (Milde *et al*., 2007; Wang *et al*., 2005), enhanced insulin sensitivity (Baur and Sinclair, 2006; Park *et al*., 2007), antioxidant activity (Oak *et al*., 2005) and prolonged survival (Baur *et al*., 2006). However, some reports point adverse effects of resveratrol administration (Bandele *et al*., 2008; Seve *et al*., 2005; Szkudelski, 2006). Recent research in our laboratory demonstrated that continuously long-term RS intake was disadvantageous in standard-fed condition, since it enhanced hepatic oxidative stress (Rocha *et al*., 2009). Interestingly, there is still a lack of information linking RS, weekend ethanol consumption and high-sucrose intake conditions.

Therefore, a question must be addressed, what are the effects of weekend ethanol and RS consumption in high-sucrose intake condition?

Thus, the aim of this study was to analyze the association between weekend alcohol and RS consumption with high-sucrose diet intake on energy expenditure, oxygen consumption, lipid profile, oxidative stress and hepatic energy metabolism. We used a rat model, with three weekdays alcohol and RS consumption, to study an unresolved issue in

the field of alcoholism and risk factors for cardiovascular damage that is the effect of weekend-alcohol consumption and its association with RS intake, in high-sucrose diet condition.

EXPERIMENTAL PROCEDURES

Animals

All experiments and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health, and approved by the Ethical Committee of the Institute of Biological Sciences, São Paulo State University, UNESP, Brazil (authorization number 76/07). Male *Wistar* 24 rats, 75 days old, were individually housed in polypropylene cages in an environmentally controlled, clean-air room with a temperature of 22 ± 3°C, 12-h light:dark cycle, a relative humidity of 60 ± 5% and received a standard chow (Purina Ltd., Campinas, SP, Brazil) containing 26.5% protein, 40.0% carbohydrate, 3.8% fat, 4.5% fiber, by weight and 3.0 kcal/g of metabolizable energy. Taking into account the hormonal influences in female, we used only male rats.

The animals were randomly divided into four groups of six rats each. The control group (C) received water throughout the experimental period. To simulate a free living situation in which ethanol consumption was drinking during the weekend (Ferreira and Willoughby, 2008), the (E) group was allowed to drink 30% ethanol aqueous solution during 3 days/week and drink water during the other 4 days of the week. The (ES) group was allowed to drink a mixture of 30% ethanol and 30% sucrose aqueous solution during 3 days/week and drink 30% sucrose aqueous solution during the other 4 days of the week. The ESR group was allowed to drink 30% ethanol and 30% sucrose solution containing 6 mg/l RS (3,5,4'-trans-trihydroxystilbene) during 3 days/week and drink 30% sucrose during the other 4 days of the week. As physical dependence on ethanol can be produced without nutritional impairment in rodents (Novelli *et al*., 1996, 1997; Seiva *et al*., 2009a), and alcohol-induced hepatic abnormalities can occur without overt nutritional deficiencies (Seiva *et al*., 2009b), the same diet was used for all animals. Food, water, sucrose and ethanol aqueous solutions were provided *ad libitum*. The total experimental period was 70 days. Recently we have reported the effects of RS (Rocha *et al*., 2009) and sucrose (Novelli *et al*., 2007, 2009, 2010) on morphometric and calorimetric parameters, as well on lipid profile, oxidative stress and hepatic energy metabolism in rats. Therefore, to study the interactions between the effects of ethanol, sucrose and RS, only sucrose and RS-treated groups were not included in the experimental design.

The RS dose took into account that the average concentration of RS in wine is 5 mg/l, and that the moderate daily consumption of wine is 250 ml (Dong, 2003). Therefore, the mean daily intake of RS in this condition is approximately 0.02 mg/kg in humans (Juan *et al*., 2002). In accordance with these values, we selected a dose that is about 25 times higher than this estimate, but that was under the maximal tolerated dose (Baur and Sinclair, 2006; Crowell *et al*., 2004). The daily RS intake (mg/day) was calculated from the amount of aqueous solution ingestion, and the time of treatment was based on early experiments (Rocha *et al*., 2009). The Table 1 indicated that ESR rats received 0.247 ± 0.014 mg/day RS, thus an average of 0.75 mg/day/week (7.5 mg RS during the experimental period of 10 weeks). This value corresponded to 0.53 ± 0.03 mg/kg/day, 1.59 mg/kg/week and 15.9 mg/kg during all experimental period. Food intake and drinking solutions were evaluated daily at the same time (9:00–10:00h) as the difference between food and drinking solution given and the leftover. The body weights were determined once a week. The food intake, ethanol consumption, caloric value of rodent chow (3 kcal/g), sucrose (4 kcal/g) and ethanol (7 kcal/g), were used to obtain a total energy intake (kcal/day) = [mean food consumption per day (g) × 3] + [amount of sucrose intake per day (g) × 4] + [amount of ethanol intake per day (g) × 7].

Indirect calorimetry

After 60 days of the experimental period, rats were *fasted overnight* (12 h) and placed into metabolic chambers (airflow = 1.0 l/min) of a computer-controlled indirect calorimeter (CWE, Inc., St. Paul, USA). Respiratory quotient (RQ) and energy expenditure, namely resting metabolic rate (RMR) were measured using a respiratory-based software program (software MMX, CWE, Inc., USA). Average oxygen consumption (VO_2) and average carbon dioxide

Table 1. General characteristics and nutritional parameters of rats

	C	E	ES	ESR
Initial body weight (g)	337.9 ± 33.9	342.3 ± 35.2	327.6 ± 33.0	338.4 ± 27.4
Final body weight (g)	422.8 ± 17.4	444.9 ± 23.3	420.5 ± 27.9	465.8 ± 25.2^{ab*c}
Body weight gain (g)	79.6 ± 17.6	102.6 ± 17.8	96.1 ± 15.5	124.7 ± 18.5^{ac}
Food consumption (g/day)	24.52 ± 1.12	23.24 ± 1.26	11.99 ± 1.56^{ab}	12.39 ± 1.37^{ab}
Energy from chow (kcal/day)	67.4 ± 3.1	63.9 ± 3.5	32.9 ± 3.4^{ab}	34.1 ± 3.7^{ab}
Aqueous solution intake (mL/day)	42.57 ± 1.84	35.46 ± 1.69^{a}	36.84 ± 1.79^{a}	41.22 ± 0.83^{bc}
Average ethanol consumption (g/day)	0.00 ± 0.00	4.56 ± 0,47^{a}	4.74 ± 0.44^{a}	5.29 ± 0.47^{abc}
Energy from ethanol (kcal/day)	0.00 ± 0.00	31.9 ± 3.2^{a}	33.2 ± 3.4^{a}	37.1 ± 4.7^{a}
Sucrose consumption (g/day)	0.00 ± 0.00	0.00 ± 0.00	11.50 ± 1.26^{ab}	12.36 ± 0.80^{ab}
Energy from sucrose (kcal/day)	0.00 ± 0.00	0.00 ± 0.00	46.4 ± 5.1^{ab}	49.5 ± 3.2^{ab}
Total energy intake (kcal/day)	67.40 ± 3.1	95.8 ± 5.2^{a}	112.5 ± 2.8^{ab}	120.7 ± 1.5^{abc}
RS intake (mg/kg/day)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.53 ± 0.03^{abc}

Values are means ± SD of the mean and analyzed by a multifactorial analysis of variance followed by Tukey's test. Groups: C, control group; E, weekend alcohol group; ES, weekend alcohol and high-sucrose diet intake; ESR, weekend alcohol and RS with high sucrose diet intake. Significantly different from the C group, $^{a}P < 0.001$; significantly different from the E group, $^{b}P < 0.001$, $^{b*}P < 0.01$; significantly different from the ES group $^{c}P < 0.001$.

production (VCO_2) were integrated over periods of 15 min. Carbohydrate and fat oxidation were calculated from the no protein oxygen consumption, their relative oxidative proportions and the amount of oxygen consumed per gram of substrate oxidized (Labayen *et al.*, 1999): carbohydrate oxidation = $VO_2 \times (RQ - 0.707)/0.293 \times 0.746$; fat oxidation = $VO_2 \times (1 - RQ)/0.293 \times 0.746$, where VO_2 is measured in liters per minute, 1.00 is the RQ for total carbohydrate oxidation, 0.707 is the RQ for total fat oxidation, 0.293 is the difference between 1.000 and 0.707, 0.746 is the number of liters of oxygen consumed per gram glucose oxidized.

Biochemical determinations

After 70 days of the treatments (4 days after water drinking), rats were fasted overnight (12 h). The animals were anesthetized (0.1 ml i.p. of 1% sodium barbiturate) and sacrificed by decapitation. Blood was placed into a centrifuge tube and allowed to clot to obtain the serum that was separated by centrifugation 1400 g for 10 min. The triacylglycerol (TG), total cholesterol and high-density lipoprotein cholesterol (HDL) were determined in serum by enzymatic method (test Kit CELM, Modern Laboratory Equipment Company, São Paulo, Brazil). The very-low-density lipoprotein cholesterol (VLDL), low-density lipoprotein (LDL) (Friedewald *et al.*, 1972), total protein (Lowry *et al.*, 1951), total antioxidant capacity or total antioxidant substances (TAS, Randox Laboratories Ltd., Crumlin, Co., Antrim, UK) and lipid hydroperoxide (LH, Jiang *et al.*, 1991) were also determined in serum. Alanine transaminase (ALT, E.C.2.6.1.2.) was determined in serum (CELM, Modern Laboratory Equipment Company, São Paulo, Brazil) to link ethanol intake with toxic effects (Novelli *et al.*, 1997; Seiva *et al.*, 2009a, b).

The liver was rapidly removed and samples of 200 mg were homogenized in 5 ml of a cold 0.1 M phosphate buffer pH 7.4. Tissue homogenates were prepared in a motor-driven teflon glass Potter Elvehjem, tissue homogenizer (1 min, 100 g). The homogenate was centrifuged at 10 000 g for 15 min. The supernatant was analyzed for total protein (Lowry *et al.*, 1951), lipid hydroperoxide (LH, Jiang *et al.*, 1991), antioxidant and metabolic enzymes.

GSH peroxidase (E.C.1.11.1.9.) was assayed using 0.15 M phosphate buffer pH 7.0 containing 5 mM EDTA, 0.0084 M NADPH, 4 μg of GSH reductase, 1.125 M sodium aside and 0.15 M GSH (Nakamura *et al.*, 1974). Catalase (E.C.1.11.1.6.) activity was determined in reaction mixture of 50 mM phosphate buffer pH 7.0 and 10 mM hydrogen peroxide at 240 nm (Aebi, 1974). Superoxide dismutase (E.C.1.15.1.1.) activity was measured using superoxide radical (O_2^-)-mediated-nitroblue tetrazolium (NBT) reduction by an aerobic mixture of NADH and PMS. The complete reaction system consisted of 50 mM phosphate buffer pH 7.4, 0.1 mM EDTA, 50 μM NBT, 78 μM NADH and 3.3 μM PMS (Ewing and Janero, 1995).

The hepatic energy metabolism was assessed by β-hydroxyacyl coenzyme-A dehydrogenese (OHADH, E.C.1.1.1.35) and citrate synthase (CS, E.C.4.1.3.7) activities (Bass *et al.*, 1969).

Enzyme activities were performed at 25°C using a microplate reader (μQuant-MQX 200 with Kcjunior software to computer system control, Biotech Instruments, Winooski, Vermont, USA). The spectrophotometric determinations were performed in Pharmacia Biotech spectrophotometer with temperature-controlled cuvette chamber (UV/visible Ultrospec 5000 with Swift II applications software to computer system control, 974212, Cambridge, England, UK). All chemicals and solvents were from Sigma (St. Louis, Missouri, USA).

Statistical analyses

Values are presented as means ± standard deviation (SD). Statistical comparisons were carried out by multifactorial analysis of variance followed by Tukey's test. Statistical significance was set at $P < 0.05$ (Systat Software, USA).

RESULTS

General characteristics of rats and nutritional parameters

The body weight gain and final body weight were comparable in ES, E and C groups. The final body weight and body weight gain were highest in ESR rats. Food consumption and energy from chow were lower in ES and ESR than in E and C rats. Aqueous solution intake was lower in E and ES than in C, but ESR rats had higher aqueous solution intake and ethanol consumption than E and ES. There were no significant changes in ethanol consumption and energy from ethanol comparing E and ES rats, as well in sucrose consumption and energy from sucrose between ES and ESR rats. The total energy intake was higher in E than in C, in ES than in E and in ESR than in ES rats (Table 1).

Indirect calorimetry

E rats had higher RMR, RMR adjusted per body weight, VO_2, VO_2/body weight, fat oxidation, as well lower RQ and carbohydrate oxidation than C. Interaction of weekend ethanol and high-sucrose consumption normalized RMR, RMR/body weight, RQ, VO_2, carbohydrate and fat oxidation. The association of weekend ethanol consumption, RS and sucrose enhanced the RMR, RMR/body weight, VO_2 and fat oxidation, but reduced the RQ and carbohydrate oxidation, comparing with ES and C groups. The carbohydrate oxidation and VO_2/body weight were higher in ESR than in E rats (Table 2).

Biochemical determinations

There were no significant alterations in serum total protein and total cholesterol among the groups, while glucose was lower in E, ES and ESR than in C. E rats had higher TG, VLDL, as well lower HDL/TG ratio than C. ES normalized TG and VLDL, but significantly enhanced HDL and HDL/TG ratio. TG and VLDL were lower in ESR than in C, E and ES rats. ESR rats had the highest HDL/TG ratio. ALT was higher in E, ES and ESR than in C (Table 3). The LH/TAS ratio was higher in serum of E and ES rats than in C. LH/TAS ratio was lower in serum of ESR than in E and ES rats (Fig. 1).

Table 4 shows that hepatic CS activity was higher in ES than in E and C rats. ESR rats had lower CS activity than ES and higher CS activity than E and C rats. The OHADH/CS ratio was lower in ES than in E, and lower in E than in C rats. ESR rats had higher OHADH/CS ratio than ES. The OHADH was highest in ESR rats. There were no significant

Table 2. Calorimetric parameters of rats

	C	E	ES	ESR
RMR (kcal/h)	1.01 ± 0.18	1.39 ± 0.14^{a*}	1.19 ± 0.13^{b*}	1.45 ± 0.16^{a*c*}
RMR/body weight (kcal/h kg)	2.38 ± 0.42	3.12 ± 0.22^{a*}	2.83 ± 0.08^{b*}	3.11 ± 0.09^{a*c*}
RQ	0.99 ± 0.15	0.72 ± 0.15^{a*}	0.87 ± 0.08^{b*}	0.74 ± 0.11^{a*c*}
VO_2 (ml/min)	2.35 ± 0.48	3.36 ± 0.19^{a*}	2.76 ± 0.21^{b*}	3.22 ± 0.10^{a*c*}
VCO_2 (ml/min)	2.28 ± 0.21	2.34 ± 0.01	2.36 ± 0.03	2.31 ± 0.01
VO_2/body weight (ml/min kg)	5.56 ± 0.13	7.55 ± 0.16^{a}	6.56 ± 0.32^{ab}	6.91 ± 0.12^{ab}
Carbohydrate oxidation (mg/kg min)	1.48 ± 0.34	0.11 ± 0.04^{a}	0.90 ± 0.17^{b}	0.27 ± 0.05^{a*b*c*}
Fat oxidation (mg/kg min)	0.86 ± 0.36	2.39 ± 0.97^{a}	1.33 ± 0.15^{b*}	2.13 ± 0.05^{a*c}

Values are means ± SD of the mean and analyzed by a multifactorial analysis of variance followed by Tukey's test. Groups: C, control group; E, weekend alcohol group; ES, weekend alcohol and high-sucrose diet intake; ESR, weekend alcohol and RS with high-sucrose diet intake. Significantly different from the C group, $^{a}P < 0.001$, $^{a*}P < 0.01$; significantly different from the E group, $^{b}P < 0.001$, $^{b*}P < 0.01$; significantly different from the ES group, $^{c}P < 0.001$, $^{c*}P < 0.01$.

Table 3. Serum determinations

	C	E	ES	ESR
Protein (g/dl)	7.25 ± 0.65	7.24 ± 0.78	6.29 ± 0.47	6.75 ± 0.54
Glucose (mg/dl)	180.82 ± 19.34	132.42 ± 13.07^{a}	132.45 ± 13.85^{a}	130.29 ± 14.28^{a}
TG (mmol/l)	1.14 ± 0.07	1.99 ± 0.05^{a}	1.14 ± 0.09^{b}	0.89 ± 0.04^{abc}
Cholesterol (mmol/l)	2.26 ± 0.22	1.97 ± 0.18	2.35 ± 0.23	2.14 ± 0.14^{c}
VLDL (mmol/l)	0.23 ± 0.02	0.39 ± 0.01^{a}	0.23 ± 0.01^{b}	0.18 ± 0.02^{abc}
LDL (mmol/l)	0.61 ± 0.25	0.68 ± 0.15	0.59 ± 0.19	0.62 ± 0.17
HDL (mmol/l)	1.42 ± 0.05	0.63 ± 0.08^{a*}	1.53 ± 0.03^{a*b*}	1.34 ± 0.06^{c*}
HDL/TG	1.24 ± 0.04	0.31 ± 0.04^{a}	1.34 ± 0.03^{ab}	1.51 ± 0.05^{ab}
ALT (mmol/l)	50.11 ± 5.08	82.51 ± 6.33^{a}	74.18 ± 7.59^{a}	90.97 ± 7.00^{ac}

Values are means ± SD of the mean, and analyzed by a multifactorial analysis of variance followed by Tukey's test. Groups: C, control group; E, weekend alcohol group; ES, weekend alcohol and high-sucrose diet intake; ESR, weekend alcohol and RS with high sucrose diet intake. Significantly different from the C group, $^{a}P < 0.001$, $^{a*}P < 0.01$; significantly different from the E group, $^{b}P < 0.001$, $^{b*}P < 0.01$; significantly different from the ES group $^{c}P < 0.001$, $^{c*}P < 0.01$.

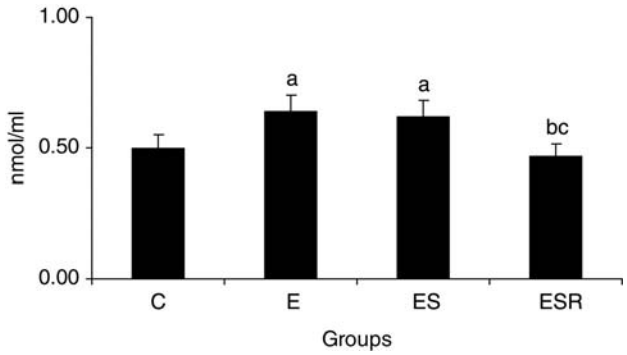

Fig. 1. LH/TAS ratio in serum of rats. Values are means ± SD of the mean and analyzed by a multifactorial analysis of variance followed by Tukey's test. Groups: C, control group; E, weekend alcohol group; ES, weekend alcohol and high-sucrose diet intake; ESR, weekend alcohol and RS with high sucrose diet intake. Significantly different from the C group, $^{a}P < 0.001$; significantly different from the E group, $^{b}P < 0.001$; significantly different from the ES group, $^{c}P < 0.001$.

differences in hepatic GSH peroxidase, between the groups. Hepatic SOD and catalase were highest, while LH was lowest in ESR rats.

DISCUSSION

The majority of the metabolic conditions that accompanied alcohol consumption and sucrose-rich diet remain clinically silent, and only become manifest when health damage is effectively installed, therefore, any strategy to reduce the disorders of these lifestyle conditions may have particular importance. RS has been implicated as a modulator of alcohol-independent cardiovascular protection that is allegedly conferred by drinking red wine (Turner *et al*., 1999). However, the basis for such observation has not been completely established.

To the best of our knowledge this is the first study that evaluated the interaction between three weekdays ethanol and RS consumption with high-sucrose diet intake on calorimetric parameters, some markers of oxidative stress and hepatic energy metabolism, which certainly would bring new insights on high-sucrose diet and ethanol adverse effects control.

Note that weekend drinking rats gained a similar amount of weight than C, despite the fact that the animals of the E group ingested an estimated 38% more calories per day (Table 1). Hence this was the case, then where are the extra calories going?

The alcoholic beverage energy paradox, increased energy intake without weight gain, is most likely attributed to increased energy expenditure (Mattes, 2006). In fact, as previously reported in alcoholism (Seiva *et al*., 2009a, b), RMR was approximately 20% higher in E rats than in C. Therefore, a minor fraction of the available energy was used for ATP synthesis in weekend ethanol consumption (Table 2). Alcohol-induced thermogenesis was higher in healthy non-alcoholic subjects (Suter and Tremblay, 2005). This condition may be in part related to induction of microsomal ethanol oxidizing system for ethanol metabolism,

Table 4. Hepatic energy metabolism and oxidative stress

	C	E	ES	ESR
OHADH (nmol/mg protein)	119.5 ± 12.5	118.8 ± 9.1	130.3 ± 10.8	149.8 ± 11.5^{abc}
CS (nmol/mg protein)	24.5 ± 2.8	27.6 ± 2.3	37.8 ± 2.2^{a*b*}	33.3 ± 3.3^{a*b*c*}
OHADH/CS	4.95 ± 0.20	4.30 ± 0.26^{a*}	3.45 ± 0.29^{ab}	4.52 ± 0.36^{c}
LH (nmol/g tissue)	386.9 ± 5.7	396.9 ± 11.6	399.1 ± 6.9	344.0 ± 13.1^{abc}
Superoxide dismutase (nmol/mg protein)	7.38 ± 0.81	8.85 ± 0.71	8.19 ± 0.46	9.56 ± 0.89^{ab*c}
Catalase (nmol/mg protein)	0.21 ± 0.09	0.15 ± 0.06	0.30 ± 0.08	0.69 ± 0.09^{abc}
GSH peroxidase (nmol/mg protein)	253.9 ± 37.8	263.2 ± 27.7	300.3 ± 29.1	272.4 ± 25.1

Values are means ± SD of the mean and analyzed by a multifactorial analysis of variance followed by Tukey's test. Groups: C, control group; E, weekend alcohol group; ES, weekend alcohol and high-sucrose diet intake; ESR, weekend alcohol and RS with high sucrose diet intake. Significantly different from the C group, $^{a}P < 0.001$, $^{a*}P < 0.01$; Significantly different from E group, $^{b}P < 0.001$, $^{b*}P < 0.01$; Significantly different from the ES group $^{c}P < 0.001$, $^{c*}P < 0.01$.

since part of the body's energy expenditure is accounted for the liver, thus increasing oxygen consumption as showed in both VO_2/body weight and VO_2 consumption. There was also lower RQ and higher fat oxidation that could be other mechanism by which weekend alcohol consumption maintained the body weight gain.

Taking into account the total energy intake (Table 1), and the normalized energy expenditure (Table 2), there was an unexpected maintenance of body weight gain in ES rats. Assuming that the rats had the same amount of activity, this was associated with enhanced carbohydrate oxidation in ES rats, despite the lower fat oxidation in these animals. It was evident that a lower energy intake from chow was compensated by additional calories from the sucrose and ethanol solutions, and that high-sucrose intake in ES rats induced a rise of its own oxidation. Stimulation of carbohydrate oxidation was not surprising, since the change in fuel selection is controlled by carbohydrate intake, and when carbohydrate oxidation rises in response to intake there is a profound counter-regulatory suppression of fat oxidation (Wilson-Fritch *et al*., 2004). The RQ and carbohydrate oxidation indicated that extra sucrose in ES rats was able to replace fat as the main substrate (Table 2).

In contrast, the lower carbohydrate oxidation in ESR rats, as well the higher fat oxidation than ES, indicated reduction of the calorimetric changes due high-sucrose diet. On the other hand, there was higher energy expenditure and oxygen consumption than C, demonstrating analog of alcoholic changes, as found in E rats. Therefore, alcohol effects count more in combination with a high-sucrose diet condition. Curiously, RS had no effects on food consumption, but significantly enhanced the average ethanol palatability, as compared with ES, and this may be *per se* a considerable effect of the association between RS, sucrose and ethanol. Further studies may be considered to clear this open question.

The highest final body weight with ethanol-sucrose-RS combination indicated the efficiency of energy storage. It has been repeatedly demonstrated that overweight and obesity are associated with a cluster of metabolic disorders, including cardiovascular diseases (Novelli *et al*., 2007; Pinheiro-Mulder *et al.*, 2010; Poudyal *et al*., 2010). However, variables other than the traditional body composition and lipid profile may be further improving the discrimination of obesity effects, mainly on cardiovascular system (Novelli *et al*., 2010). Despite this apparent adverse effect of RS, sucrose and ethanol association, RS improved the oxidative stress, since enhanced TAS concentrations and reduced LH/TAS ratio in ESR rats (Fig. 1), and this beneficial effect was reflected on serum lipids. As previously reported in alcoholism (Seiva *et al*., 2009a, b) and high-sucrose intake condition (Novelli *et al*., 2007, 2009, 2010), E and ES rats had higher LH/TAS ratio indicating serum oxidative stress.

Emerging data on the 'antioxidant hypothesis' have indicated that oxidative stress affect cellular signals, damaging cellular DNA, protein and lipids. ROS react with protein thiol moieties to produce a variety of sulfur oxidation states, thus diminishing the cellular uptake of lipids from the blood, inducing dyslipidemia (Brizzi *et al*., 2003)

It has been reported that the hypertriglyceridemia accompanies ethanol consumption in spite of hepatic fat accumulation (Crouse and Grundy, 1984). Therefore, fatty acids synthesized in the liver were not the source of the hypertriglyceridemia, rather than plasma free fatty acids were the major precursors of TG and of VLDL in weekend ethanol consumption condition (Table 3). Delayed lipolysis of VLDL because of the competition for the site of lipoprotein lipase between VLDL from hepatic origin and chylomicrons from intestinal origin could lead to the formation of TG-rich lipoprotein remnants. In these conditions, TG-rich lipoprotein remnants abnormally interact with HDL, changing the clearance of these lipoproteins. Weekend alcohol consumption induced dyslipidemia, decreasing HDL/TG ratio (Table 3). There is an established relationship between the regular light alcohol consumption, 5–10 g/day, and the incidence of cardiovascular disease (Ojeda *et al*., 2008; Puddey *et al*., 1999). Our results showed dyslipidemic profile with 4.56 ± 0.47 g/day ethanol intake during 3 weekdays, demonstrating that this pattern of alcohol consumption had adverse effects, increasing the risk for cardiovascular damage.

There was a protective effect of RS and sucrose intake in weekend ethanol consumption enhancing HDL/TG ratio. RS depressed TG and VLDL in the ESR group, and VLDL is a LDL serum precursor (Novelli, 2005). Several studies have shown effects of RS reducing TG, LDL and VLDL levels (Cho *et al*., 2008; Rivera *et al*., 2009). A wide range of dosage have been tested with various rats and mice models ranging between 1 and 50 mg/kg, or 0.01 and 0.025% diet (Ahan *et al*., 2008; Cho *et al*., 2008; Rocha *et al*., 2009). ESR rats ingested 1.59 mg/kg body weight/week RS, and RS was found to reduce blood cholesterol from 10 mg/kg body weight or more (Rivera *et al*., 2009). Since the biochemical determinations were obtained after 10 weeks of treatment, the effects of various concentrations of RS remain to be determined.

A notable finding of this study was the RS effect on hepatic tissue. The enhanced hepatic OHADH clearly

indicated that ESR rats had higher fatty acid degradation than E, ES and C. Despite the lower CS activity, RS in ESR rats normalized the OHADH/CS ratio (Table 4), indicating adequate fatty acid oxidation relative to flux of metabolites through tricarboxylic acid cycle. The increased fat oxidation in ESR rats than in ES could be associated with RS modulation of AMPK (AMP-activated protein kinase), a sensor of cellular energy status, being activated by increased AMP/ATP ratio. Stimulation of AMPK inhibits the expression of fatty acid synthase and lipid synthesis induced by high carbohydrate intake (Hou *et al*., 2009). Under these conditions, there is decreased activity of acetyl coenzyme-A carboxylase, reducing the malonyl coenzyme-A. As a consequence, decreased production of malonyl coenzyme-A, results in upregulation of lipid oxidation (Novelli, 2005), thus a decrease in fatty acid synthesis and an increase in its oxidation.

OHADH is rate-limiting enzyme in catalyzing fatty acid breakdown in hepatic tissue and thus plays an important role in liver energy metabolism. Since alteration of hepatic OHADH activities may contribute to hepatic dysfunction, uncovering physiopathological mechanisms that lead to the alteration of OHADH activity may be of prime importance to understand alcohol disorders. CS is the key enzyme for the control of the flux of metabolites through tricarboxylic acid cycle. Note that weekend ethanol consumption significantly reduced the OHADH/CS ratio (Table 4), indicating depressed fatty acid oxidation pathway relative to aerobic metabolism in hepatic tissue of E animals. Therefore, despite the whole higher fat oxidation observed from calorimetric parameters (Table 2), there was an inadequate hepatic oxidation of fatty acids. Excess of fatty acids that are not oxidized from tricarboxylic acid are sequestered for TG synthesis and hepatic accumulation.

The calorimetric changes due the weekend ethanol and sucrose intake were found in hepatic tissue of ES rats. The higher hepatic CS activity as well lower OHADH/CS ratio in ES than in E rats indicated depressed fatty acid degradation and enhanced hepatic glucose oxidation, giving substrate for tricarboxylic acid cycle in hepatic tissues of these animals (Table 4).

The metabolic changes found in hepatic tissue may be, at least in part, antioxidant mediated. The biological antioxidant defense system is an integrated array of enzymes and antioxidants. These include SOD that catalysis the destruction of O_2^- by dismutation and hydrogen peroxide formation, catalase and GSH peroxidase that catalyze the conversion of hydrogen peroxide to water (Novelli, 2005). Note (Table 4) that no significant changes were found in LH and antioxidant enzymes in E and ES rats. As SOD and catalase enhanced in ESR rats, despite the maintenance of GSH peroxidase, we can affirm that O_2^- generated by SOD activity was scavenged by catalase, thus reducing the hepatic LH (Table 4).

There is no evidence of a direct role for RS on superoxide dismutase activity. However, RS might enhance the ability of copper ions to linkage SOD active site, even if no dietary copper had been added. There are two forms of SOD. Cytoplasmic SOD has a Cu–Zn active site, and increased Cu availability may, in principle, enhance SOD activity. RS was reported to enhance the ability of copper ions to linkage DNA (Burkitt and Duncan, 2000).

Curiously, E, ES and ESR rats had lower serum glucose than C, indicating that weekend ethanol consumption was able to induce analog hepatic effects of alcoholism (Novelli, 2005). The decreased serum glucose levels found in all experimental groups was associated with alcohol intoxication efficacy of gluconeogenesis that is decreased by ethanol inhibition of hepatic glucose-phosphatase activity. The effects of weekend alcohol consumption were evidenced in all experimental groups, by higher serum ALT, reflecting the ethanol toxicity on liver cells (Novelli *et al*., 1997; Seiva *et al*., 2009a). Hence, the liver is regarded as one of the central metabolic organs, regulating and maintaining homeostasis, small changes may have long-term important effects on health. However, despite the highest ALT level in ESR rats, RS significantly enhanced antioxidant defences and depressed hepatic oxidative stress and this may have long-term beneficial effects on hepatic function.

In conclusion, the present study brought new insights on weekend alcohol consumption and RS intake, demonstrating for the first time that this pattern of alcohol exposure induced dyslipidemic profile, enhanced energy expenditure and induced calorimetric and hepatic metabolic changes, which resemble that of the alcoholic condition. The association of weekend alcohol and high-sucrose intake had no synergistic effects on alcohol induced adverse effects. RS intake, despite the effects enhancing the alcohol preference, was advantageous in weekend drinking and high-sucrose intake condition on energy expenditure, hepatic metabolism, oxidative stress and lipid profile, thus improving risk factors for cardiovascular damage.

Funding — This work was supported from FAPESP, Fundação de Amparo a Pesquisa do Estado de São Paulo and of CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico.

REFERENCES

Aebi H. (1974) *Methods of Enzymatic Analysis*, 2nd edn. New York: Verlag Chemie Weinheim.

Ahan J, Cho I, Kim S *et al*. (2008) Dietary resveratrol alters lipid metabolism-related gene expression of mice on an atherogenic diet. *J Hepathol* **49**:1019–28.

Bandele OJ, Clawson SJ, Osheroff N. (2008) Dietary polyphenols as topoisomerase II poisons: B ring and C ring substituents determine the mechanism of enzyme-mediated DNA cleavage enhancement. *Chem Res Toxicol* **21**:1–8.

Bass A, Brdicka PE, Eyer P *et al*. (1969) Metabolic differentiation of distinct muscle types at the level of enzymatic organization. *Eur J Biochem* **10**:198–206.

Baur JA, Sinclair DA. (2006) Therapeutic potential of resveratrol: the in vivo evidence. *Nat Rev Drug Discov* **5**:493–506.

Baur JA, Pearson KJ, Price NL *et al*. (2006) Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**:337–42.

Brizzi P, Tonolo G, Carusillo F *et al*. (2003) Plasma lipid composition and LDL oxidation. *Clin Chem Lab Med* **4**:56–60.

Burkitt MJ, Duncan J. (2000) Effects of trans-resveratrol on copper-dependent hydroxyl-radical formation and DNA damage: evidence for hydroxyl-radical scavenging and a novel, glutathione-sparing mechanism of action. *Arch Biochem Biophys* **381**:253–63.

Cho IJ, Ahan JY, Kim S *et al*. (2008) Resveratrol attenuates the expression of HMG-CoA reductase mRNA in hamsters. *Biochem Biophysical Res Commun* **367**:190–94.

Crouse J, Grundy SM. (1984) Effects of alcohol on plasma lipoproteins and cholesterol and triglyceride metabolism in man. *J Lip Res* **25**:486–96.
Crowell JA, Korytko PJ, Morrissey RL *et al*. (2004) Resveratrol-associated renal toxicity. *Toxicol Sci* **82**:614–19.
Dong Z. (2003) Molecular mechanism of the chemopreventive effect of resveratrol. *Mutation Res* **523**:145–50.
Ewing JF, Janero DR. (1995) Microplate superoxide dismutase assay employing a nonenzymatic superoxide generator. *Anal Biochem* **232**:243–48.
Ferreira MP, Willoughby D. (2008) Alcohol consumption: the good, the bad, and the indifferent. *Appl Physiol Nutr Metab* **33**:12–20.
Friedewald WT, Levy R, Fredrickson DS. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma without preparative ultracentrifuge. *Clin Chem* **18**:499–502.
Hou X, Xu S, Maitland KA *et al*. (2009) SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. *J Biol Chem* **283**:20015–26.
Jiang ZY, Woollard A, Wolff S. (1991) Lipid hydroperoxide measurement by oxidation of Fe^{3+} in the presence of xylenol orange. *Lipids* **26**:853–56.
Juan ME, Buenafuente J, Casals I *et al*. (2002) Plasmatic levels of trans-resveratrol in rats. *Food Res Int* **35**:195–9.
Labayen I, Forga L, Martinez JA. (1999) Nutrient oxidation and metabolic rate as affected by meals containing different proportions of carbohydrate and fat, in healthy young women. *Eur J Nutr* **38**:158–66.
Lowry DH, Rosebrough NJ, Farr AL. (1951) Protein measurement with the folin-phenol reagent. *J Biol Chem* **193**:265–75.
Mattes R. (2006) Fluid calories and energy balance: the good, the bad, and the uncertain. *Physiol Behav* **89**:66–70.
Milde J, Elstner EF, Grabmann J. (2007) Synergistic effects of polyphenols and carotenoids on human low-density lipoprotein oxidation. *Mol Nutr Food Res* **51**:956–61.
Murray RP, Connet JE, Tyn SY *et al*. (2002) Alcohol volume, drinking pattern and cardiovascular disease morbidity and mortality: is there a J-shaped function? *Am J Epidemiol* **155**:212–18.
Nakamura W, Hosoda S, Hayashi K. (1974) Purification and properties of rat liver glutathione preoxidase. *Biochim Biophys Acta* **358**:251–61.
Novelli ELB. (2005) *Nutrição e vida saudável. Estresse Oxidativo e metabolismo energético*. São Paulo: Tecmedd Editora.
Novelli ELB, Rodrigues NL, Ribas BO *et al*. (1996) Toxicity of chronic ethanol ingestion and superoxide radical formation on seminal vesicles of rats. *Food Chem Toxicol* **34**:1003–7.
Novelli ELB, Rodigues NL, Santos CXC *et al*. (1997) Toxic effects of alcohol intake on prostate of rats. *Prostate* **31**:37–42.
Novelli ELB, Diniz YS, Galhardi CM *et al*. (2007) Anthropometrical parameters and markers of obesity in rats. *Lab Anim* **41**:111–19.
Novelli ELB, Santos P, Assalin H *et al*. (2009) N-acetylcysteine in high sucrose induced obesity: energy expenditure, metabolic shifting for cardiac health. *Pharmacol Res* **59**:74–9.
Novelli ELB, Souza GA, Ebaid GMX *et al*. (2010) Energy expenditure and oxygen consumption as novel biomarkers of obesity-induced cardiac disease in rats. *Obesity* **18**:1754–61.
Oak MH, Bedour JE, Schini-Kerth VB. (2005) Antiangiogenic properties of natural polyphenols from red wine and green tea. *J Nutr Biochem* **16**:1–8.
Ojeda ML, Delgado-Villa J, Llopis R *et al*. (2008) Lipid metabolism in ethanol-treated rat pups and adults: effects of folic acid. *Alcohol Alcohol* **43**:544–50.
Park CE, Kim MJ, Lee JH *et al*. (2007) Resveratrol stimulates glucose transport in C2C12 myotubes by activating AMP-activated protein kinase. *Exp Mol Med* **39**:222–9.
Pinheiro-Mulder A, Aquila MB, Bregman R *et al*. (2010) Exercise counters diet-induced obesity, proteinuria and structural kidney alterations in rat. *Pathol Res Pract* **15**:168–73.
Poudyal H, Panchal S, Brown L. (2010) Comparison of purple carrot juice and beta-carotene in a high-carbohydrate, high-fat diet-fed rat model of the metabolic syndrome. *Br J Nutr* **12**:1–11.
Puddey IB, Rakic V, Dimmitt SB *et al*. (1999) Influence of pattern of drinking on cardiovascular risk factors—a review. *Addiction* **94**:649–63.
Rivera L, Morón R, Zarzuelo A *et al*. (2009) Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats. *Biochem Pharmacol* **77**:1053–63.
Rocha KKHR, Souza GA, Ebaid GMX *et al*. (2009) Resveratrol toxicity: effects on risk factors for atherosclerosis and hepatic oxidative stress in standard and high-fat diets. *Food Chem Toxicol* **47**:1362–7.
Seiva FRF, Amauchi JF, Rocha KK *et al*. (2009a) Effects of N-acetylcysteine on alcohol abstinence and alcohol-induced adverse effects in rats. *Alcohol* **43**:127–35.
Seiva FRF, Amauchi JF, Rocha KKHR *et al*. (2009b) Alcoholism and alcohol abstinence: *N*-acetylcysteine to improve energy expenditure, myocardial oxidative stress, and energy metabolism in alcoholic heart disease. *Alcohol* **43**:649–56.
Seiva FRF, Berbert CMS, Souza GA *et al*. (2010) Energy expenditure, lipid profile, oxidative stress and cardiac energy metabolism after growth hormone treatment in obese young rats. *Horm Metab Res* **42**:496–501.
Seve M, Chimienti F, Devergnas S *et al*. (2005) Resveratrol enhances UVA-induced DNA damage in HaCat human keratinocytes. *Med Chem* **1**:629–33.
Suter PM, Tremblay A. (2005) Is alcohol consumption a risk factor for weight gain and obesity?. *Crit Rev Clin Lab Sci* **42**:197–227.
Szkudelski T. (2006) Resveratrol inhibits insulin secretion from rat pancreatic islets. *Eur J Pharmacol* **552**:176–81.
Tolstrup J, Jensen MK, Tjonneland A *et al*. (2006) Prospective study of alcohol drinking patterns and coronary heart disease in women and men. *BMJ* **332**:1244–48.
Trevisan M, Dom J, Falkner R *et al*. (2001) Drinking pattern and risk of non-fatal myocardial infarction: a population base-control study. *Addiction* **99**:313–22.
Turner RT, Evans G, Zhang M *et al*. (1999) Is resveratrol an estrogen agonist in growing rats? *Endocrinology* **140**:50–4.
Wang Z, Zou J, Cao K *et al*. (2005) Dealcoholized red wine containing known amounts of resveratrol suppresses atherosclerosis in hypercholesterolemic rabbits without affecting plasma lipid levels. *Int J Mol Med* **16**:533–40.
Wilson-Fritch L, Nicoloro S, Choulnard M *et al*. (2004) Mitochondrial remodelling in adipose tissue associated with obesity and treatment with rosiglitazone. *J Clin Invest* **114**:1281–89.