Long-time alcohol intake modifies resistin secretion and expression of resistin gene in adipose tissue

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Abstract. Elevated serum resistin is implicated in insulin resistance associated with obesity and type 2 diabetes mellitus. Alcohol consumption interferes with the nutritional status, metabolic and hormonal activity of the drinker. Impact of ethanol intake on resistin level and resistin metabolic effects is unknown. Effect of long-time (28 days) *ad libitum* moderate alcohol (6% ethanol solution) intake on serum resistin and resistin mRNA level in adipose tissue of rats (A) was compared to control (C) and pair-fed (PF) animals. PF rats were fed the same caloric amount as A rats on previous day. Alcohol consumption resulted in reduction of food and energy intake, decreased body mass gain, epididymal fat pads mass and smaller adipocytes (*vs*. C rats). Alcohol intake significantly increased serum resistin and glucose, insulinemia remained unchanged. Systemic insulin resistance was not proved by HOMA, QUICKI and McAuley indexes, but impaired insulin effect on glucose transport in isolated adipocytes was present. Elevated serum resistin was positively correlated with glycemia ($r = 0.88$, $p < 0.01$) and negatively with fat cell size ($r = -0.73$, $p < 0.05$). High resistin level as the consequence of long-time alcohol intake could contribute to smaller adipocytes, higher glycemia, attenuation of insulin-stimulated glucose transport in adipocytes. Diminished resistin gene expression in adipose tissue of A and PF rats was present.

Key words: Resistin — Alcohol — Insulin resistance — Adipocytes — Glucose transport

Abbreviations: HDL, high density lipoprotein; NEFA, non-esterified fatty acids; TG, triglycerides

Introduction

Adipose tissue is an endocrine organ producing biologically active proteins, termed adipokines. The discovery of adipocyte secreted protein – resistin initially provoked great interest in its potential role in the pathogenesis of insulin resistance and type 2 diabetes mellitus (Steppan et al. 2001). The original experiments had shown that intraperitoneally administered resistin elevated blood glucose and insulin concentration and impaired hypoglycemic response to insulin infusion. It was also found that treatment of normal mice with resistin decreased glucose tolerance and insulin sensitivity, while administration of antiresistin antibody improved blood sugar and insulin action in obese mice and increased insulin-stimulated glucose uptake in isolated adipocytes (Steppan et al. 2001).

Resistin is not exclusively secreted from adipocytes. The expression of resistin gene was also found in gastrointestinal tract, adrenal gland, skeletal muscle (Nogueiras et al. 2003), brain and pituitary (Morash et al. 2002), monocytes (Savage et al. 2001) and placenta (Yura et al. 2003).

Relation between nutritional status and serum resistin and its gene expression is still unclear. Increased serum resistin was connected with obesity (Steppan et al. 2001; Li et al. 2002), while low resistin mRNA levels were observed during fasting and returned to normal after refeeding (Kim et al. 2001; Steppan et al. 2001; Li et al. 2002). Opposite results described that insulin resistance and obesity were actually associated with decreased resistin gene expression (Juan et al. 2001; Martinez et al. 2001; Way et al. 2001). Thus, the

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evidence supporting resistin as a mediator of insulin resistance remains highly controversial.

The relationship between serum resistin levels and fat mass is also not unambiguous. In some studies, serum resistin was related to fat mass in young, healthy subjects (Yannakoulia et al. 2003), while Lee et al. (2003) did not find any relations between serum resistin and body mass index (BMI), percent of body fat, or insulin sensitivity. The regulation of resistin gene expression is also controversial. Agonists of peroxisome proliferator-activated receptor γ (PPAR-γ), insulin-sensitizing drugs (Zhao and Wu 2004; Jung et al. 2005) were found to inhibit resistin gene expression both in 3T3-L1 adipocytes (Haugen et al. 2001) and in db/db mice (Moore et al. 2001). Contrary to these observations Way et al. (2001) demonstrated stimulation of resistin gene expression by PPAR-γ agonists in obese mice.

Up to now, we do not have clear and unambiguous results explaining physiological function of resistin. The above discrepancies may result from different experimental settings of particular studies such as various subjects (3T3-L1 adipocytes, animals, human), animal strains (Wistar rats, Sprague-Dawley rats), gender and short time from identification of resistin.

Alcohol consumption interferes with the nutritional status, metabolic and hormonal activity of the drinker. The final effect of alcohol intake on human health depends on many factors such as amount and regularity of consumed alcohol. For occasional drinkers, alcohol does not suppress food intake and may actually increase appetite. Chronic consumption appears to have the opposite effect. Alcohol causes euphoria, which depresses appetite, so heavy drinkers tend to eat poorly and become malnourished. Light-to-moderate alcohol intake is associated with enhancement of insulin sensitivity (Lazarus et al. 1997; Furuya et al. 2003) and may protect against the development of diabetes (Mayer et al. 1993; Kiechl et al. 1996). However, high ethanol consumption induces insulin resistance (Onishi et al. 2003) and decreases insulin-stimulated glucose transport in fat cells due to decreased translocation of glucose transporter GLUT-4 to the adipocyte surface (Wilkes et al. 1996; Rachdaoui et al. 2003). In liver, excessive alcohol intake induces impairment and abnormal triglyceride metabolism typically resulting in hyperlipidemia, fatty liver, alcoholic hepatitis and alcoholic cirrhosis (Lieber and Pignon 1989). On the contrary, moderate alcohol consumption has profitable effects due to increase in HDL (high-density lipoprotein cholesterol) (Gaziano et al. 1993; De Oliveira et al. 2000).

Alcohol consumption affects almost all biological functions and metabolic pathways. One target of alcohol intake is also hormonal system leading to non-physiological increase/decrease of hormone gene expression and plasma concentrations. Hormonal imbalance results either in poor or stronger effects on target tissues with consequences of impaired energy metabolism. Up to now, there is no study explaining how consumption of alcohol influences resistin level and its gene expression. The only study demonstrated elevated resistin mRNA and protein in rats after prenatal ethanol exposure (Chen and Nyomba 2003).

The purpose of this study was to determine the effect of chronic (28 days) moderate ethanol intake on i) resistin serum level and resistin gene expression in adipose tissue; ii) food intake, body mass, adipocytes size, serum concentrations of glucose, insulin, cholesterol, triglycerides (TG), non-esterified fatty acids (NEFA), and amount of glycogen, lipids, cholesterol and TG in the liver; iii) *in vitro* basal and insulin-stimulated glucose uptake in adipocytes. Based on obtained results we suppose that increased resistin level due to alcohol intake could be partly involved in impaired metabolic homeostasis.

Materials and Methods

Animals and treatment

Adult male Wistar rats (10–14 animals in each group) (ANLAB, Czech Republic) with initial body mass 200–250 g were used. They were housed under controlled temperature (22–24°C), humidity (~65%) and constant 12-h light/dark cycle and fed with standard laboratory pelleted diet (Diet for small laboratory animals SPF M1), food and water were available *ad libitum* (control group, C). The alcohol-fed group (A) had *ad libitum* access to 6% ethanol solution in tap water (the model adapted according to Macho et al. 2003) as the only drinking fluid for 28 days (Wilkes et al. 1996; Poirier et al. 2001; Sebastian and Nagy 2005). Pair-fed (PF) animals had the same caloric intake in the form of pelleted diet as A rats had consumed during the preceding 24 h. PF animals drank tap water. The amount of consumed food and alcohol solution/water volume was registered daily. At the end of experiment, a half group of animals was allowed to fast overnight for determination of fasted glucose, insulin and TG. Animals were sacrificed by decapitation. Blood was collected for serum separation, liver and part of epididymal fat pads were stored (–80°C) until analysis. A portion of fat tissue was used for fat cell isolation. The Ethic Committee of the Institute of Experimental Endocrinology SAS (Bratislava, Slovakia) and The National Veterinary Institute (Bratislava, Slovakia) approved all presented experiments.

Isolation of fat cells

Adipocytes were isolated from epididymal fat pads by the method of collagenase digestion (E.C. 3.4.24.3. from *Clostridium histolyticum*, type II, Sigma, USA) (Rodbell 1964). Isolated adipocytes were used for following analysis.

Determination of adipocyte size

Fat cell size (diameter) was measured under light microscope (Reichert, Austria) after cells staining in crystal violet. Total fat cells lipids were determined gravimetrically (Folch et al. 1951). Adipocyte volume expressed as the content of total lipids *per* one fat cell was used for calculation of adipocytes number *per* 1 mg of epididymal adipose tissue.

Glucose transport

Glucose transport assay in isolated adipocytes was performed as described by Cherqui et al. (1989). Stimulatory effect of insulin concentrations (10^{-11}–10^{-6} mol/l) was used. All data were corrected for extracellular trapping and passive diffusion by measuring glucose transport in the presence of 25×10^{-6} mol/l cytochalasin B.

Extraction of liver lipids

Liver lipids were extracted according to Folch et al. (1951), dried and measured gravimetrically. Lipid extracts were used for analysis of TG (Randox, UK), total cholesterol (DiaSys GmbH, Germany), the values were expressed *per* 100 mg of liver proteins.

Determination of liver proteins and liver glycogen

Total liver proteins were determined by the method of Bradford (1976). Liver glycogen was determined by the method of Szkudelski et al. (2004) with using amyloglucosidase (EC 3.2.1.3. from *Aspergillus niger*, Sigma, USA). Finally, glucose obtained after glycogen breakdown was determined as glucose in serum.

Biochemical analysis

Serum alcohol concentration was measured by UV method (Randox, UK). Serum glucose was determined photometrically by glucooxidation reaction system (Super GL, Germany), serum insulin by radioimmunoassay kit (Linco, USA) and serum resistin by ELISA immunoassay (BioVendor, Czech Republic). Serum was analysed for cholesterol (DiaSys GmbH, Germany), TG (Randox, UK) and NEFA (Randox, UK). Fast serum levels of glucose, insulin and TG were used for determination of insulin sensitivity/resistance by homeostasis model assessment (HOMA) index (Matthews et al. 1985), quantitative insulin-sensitivity check index (QUICKI) (Katz et al. 2000) and McAuley index (McAuley et al. 2001). The osmolarity of serum was measured cryoscopically (Osmomat 030; Gonotec, Germany) and expressed as mOsmol/l.

Resistin mRNA expression

Total RNA was extracted from approximately 150 mg adipose tissue by the RNeasy Lipid Tissue Mini Kit (Qiagen, Germany). The first-strand cDNA for resistin was synthesized from 2 μg of total RNA, using Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences, UK). The reverse transcription products were amplified by PCR, using DyNAzyme II DNA polymerase (Finnzymes, Finland) and specific primers for resistin (sense: 5'-ACTTCAGCTCCCTACTGCCA-3'; antisense: 5'-GCTCAGTTCTCAATCAACCGTCC-3') (Merck, Slovakia). The amplification conditions were 94°C for 50 s, 58°C for 45 s and 72°C for 1 min (38 cycles). RT-PCR products were electrophoresed in a 1.5% agarose gel, stained with ethidium bromide and quantified with a software PCBAS 2.08e (Raytest, Deutchland). Data were expressed as ratio of resistin gene transcription level to β-actin mRNA level used as an internal control.

Statistical analysis

Results are shown as mean ± SEM of 5–7 rats *per* group. For statistical analysis of differences between two groups, unpaired Student's *t*-test was used. p values <0.05 are considered to be statistically significant. Correlations between serum resistin *vs*. glycemia and resistin *vs*. fat cell size were examined by Pearson's coefficient of correlation.

Results

Blood alcohol, food, liquid and energy intake

Average daily ethanol intake was 0.45 g of pure ethanol/100 g body mass. At the end of experiment, ethanol concentration in blood was 202 ± 33 mg/dl. In comparison with control group, ethanol intake resulted in notably lower pelleted diet consumption from the thirteenth day of treatment (data not shown). Net food intake was reduced to 80% compared with control rats (Tab. 1). Reduced food intake together with calories from alcohol resulted in significant lower total energy intake in A (pelleted diet + actual alcohol consumption) as compared with C group. PF animals consumed food in the same caloric amount as A group on previous day. Liquid intake was reduced in A rats, while there was no difference between PF and C group.

Serum osmolarity

Despite of lower liquid intake in A group, there were no significant differences in serum osmolarity between all experimental groups (Tab. 1).

Table 1. Food, liquid and energy intake, osmolarity, body mass gain, adipose tissue mass and cellularity

	C	A	PF
Solid food intake (g/day)	26.6 ± 0.50	22.0 ± 0.33 $^{***+++}$	24.4 ± 0.39 **
Caloric intake (kJ/day)	374 ± 10.1	339 ± 5.97 ***	339 ± 5.99 ***
Water intake (ml/day)	44.7 ± 0.56	38.0 ± 0.54 $^{***++}$	40.9 ± 0.65 ***
Osmolarity (mOsmol/l)	293 ± 2.70	294 ± 2.47	300 ± 0.91
Body mass gain (g)	88.8 ± 6.46	65.8 ± 3.27 **	71.0 ± 3.17 *
Total mass of EAT (g)	7.16 ± 0.49	5.67 ± 0.18 *	6.10 ± 0.23 *
Relative mass of EAT (g/100 g BW)	0.90 ± 0.05	0.72 ± 0.04 *	0.86 ± 0.07
Fat cells size (diameter, μm)	64.2 ± 1.04	55.5 ± 0.78 ***	53.4 ± 1.04 ***
Fat cells amount/1 mg EAT (×1000)	266 ± 42.9	352 ± 98.5	533 ± 113 *

BW, body weight; EAT, epididymal adipose tissue; C, control group; A, alcohol group; PF, pair-fed group. The data is given as mean ± SEM. Statistical significance is * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* C, $^{++}$ $p < 0.01$, $^{+++}$ $p < 0.001$ *vs.* PF; $n = 5–7$.

Body mass gain, epididymal fat pads mass and fat cell size

A and PF animals with lower energy intake are characterised by reduced body mass gain, lower epididymal adipose tissue mass, decreased relative mass of adipose tissue calculated *per* 100 g of body mass and smaller adipocytes (Tab. 1).

Glucose and insulin in serum

Alcohol consumption during 28 days resulted in a significant elevation of glycemia compared with serum glucose of both C and PF animals. However, insulin concentration in serum was not affected in alcohol group. PF animals had the same glucose and insulin levels as control rats. The HOMA, QUICKI and McAuley indexes (Tab. 2) determine presence/absence of insulin resistance. None of these indexes had confirmed the disturbance in systemic insulin resistance due to alcohol intake or caloric restriction.

Glucose transport

The size of fat cells between experimental groups was considerably different, thus all the transport data were adjusted to the same cell surface area. Basal, unstimulated glucose transport into isolated adipocytes (Fig. 1A) was similar

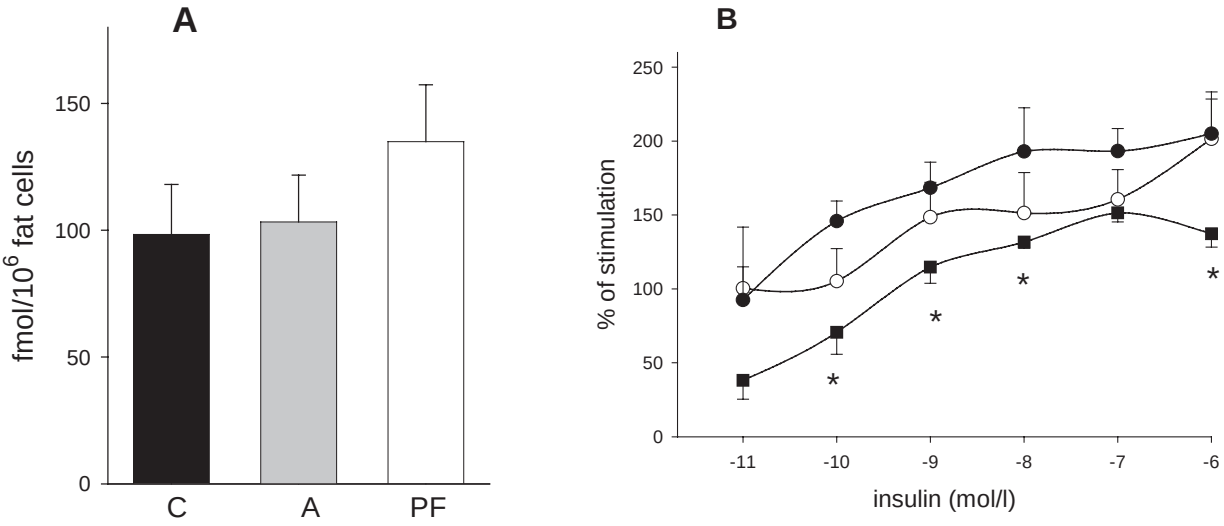

Figure 1. A. Basal, non-stimulated transport of glucose into isolated fat cells. **B.** Dose-response curves of insulin-stimulated glucose transport into isolated adipocytes. Results are expressed as the percent of stimulation over to basal transport. ● control (C), ■ alcohol (A), ○ pair-fed (PF) groups. The data is given as mean ± SEM. Statistical significance is * $p < 0.05$ *vs.* C; $n = 5–7$.

Table 2. Serum and liver parameters and indexes of insulin resistance

	C	A	P
Blood serum			
Alcohol (mg/dl)	–	202.5 ± 33.5	–
Glucose (mmol/l)	7.43 ± 0.09	8.09 ± 0.15 $^{***+}$	7.60 ± 0.09
Insulin (ng/ml)	3.29 ± 0.25	2.98 ± 0.35	3.08 ± 0.44
Cholesterol (mg/dl)	66.8 ± 1.86	57.3 ± 3.79 *	65.7 ± 4.61
Triglycerides (mmol/l)	1.25 ± 0.08	1.57 ± 0.14 $^{*++}$	1.03 ± 0.12
NEFA (mmol/l)	0.24 ± 0.03	0.40 ± 0.04 *	0.42 ± 0.05 *
Insulin resistance indexes			
HOMA	5.33 ± 0.22	4.75 ± 0.23	5.31 ± 0.31
QUICKI	0.30 ± 0.01	0.31 ± 0.01	0.28 ± 0.01
McAuley	6.51 ± 0.33	5.92 ± 0.43	6.17 ± 0.55
Liver			
Total lipids (mg/100mg pt)	34.3 ± 2.28	34.3 ± 2.87	30.4 ± 1.29
Cholesterol (mg/100mg pt)	1.11 ± 0.09	0.85 ± 0.02 *	0.84 ± 0.03 *
Triglycerides (mmol/mg pt)	1.61 ± 0.33	2.79 ± 0.26 $^{*+}$	1.30 ± 0.50
Glycogen (mg/100mg liver)	3.54 ± 0.34	1.36 ± 0.33 **	2.46 ± 0.29

NEFA, nonesterified fatty acids; pt, proteins; C, control group; A, alcohol group; PF, pair-fed group. The data is given as mean ± SEM. Statistical significance is * $p < 0.05$, *** $p < 0.001$ *vs.* C, $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ *vs.* PF; $n = 5–7$.

in all experimental groups. Dose- response curves for insulin-stimulated glucose transport (% of stimulation over basal levels) displayed decreased response at high hormone concentration in adipocytes of A group in comparison with C adipocytes (Fig. 1B). The extent of insulin-stimulated glucose transport of PF group did not differ either from C or A group.

Serum lipid values

Long-lasting ethanol intake significantly increased serum TG in comparison with C and PF rats and reduced serum cholesterol *vs.* C rats only. Serum NEFA were elevated in both A and PF animals. Food deprivation of PF rats did not influence TG and cholesterol (*vs.* C rats) (Tab. 2).

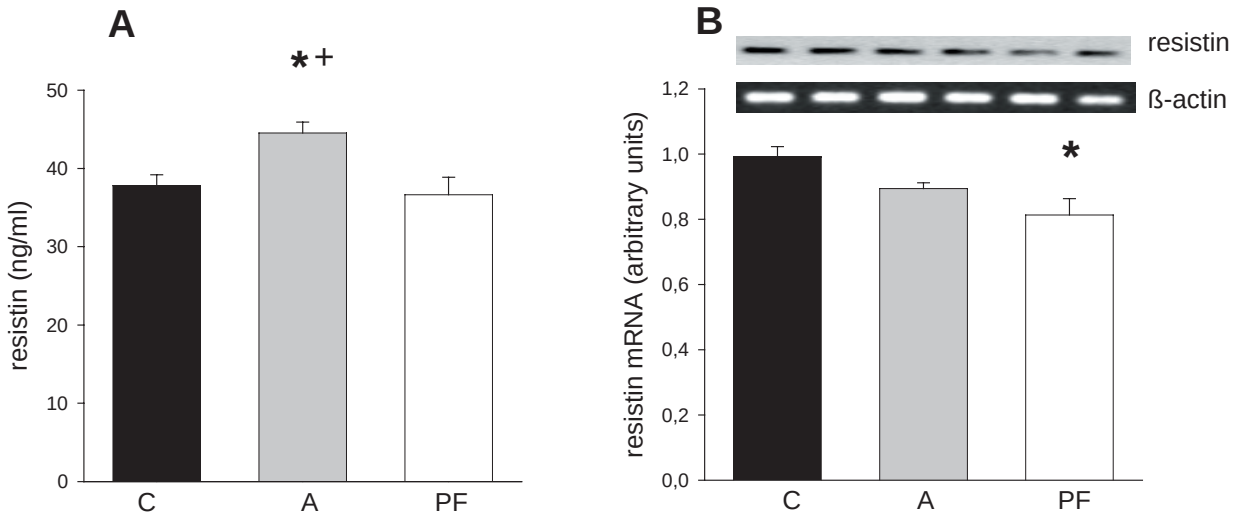

Figure 2. A. Serum resistin. **B.** Resistin mRNA expression in epididymal fat tissue. C, control group; A, alcohol group; PF, pair-fed group. The data is given as mean ± SEM. Statistical significance is * $p < 0.05$ *vs.* C, $^{+}$ $p < 0.05$ *vs.* PF; $n = 5–7$.

Liver lipids and glycogen

Total lipid content in liver (calculated *per* 100 mg of liver proteins) was similar in all experimental groups. Total cholesterol (*per* 100 mg of liver proteins) was lower in liver of both A and PF animals as compared with C animals. Moreover, alcohol consumption resulted in increased TG levels compared to C and PF groups. TG in liver of PF and control rats were similar. Liver glycogen was significantly reduced in A group *vs.* both C and PF groups (Tab. 2).

Serum level and expression of resistin

The concentration of serum resistin (Fig. 2A) was moderately, but significantly elevated after long-term ethanol intake as compared to controls and PF animals. Serum resistin was positively correlated with glycemia both in A ($r = 0.88$, $p < 0.01$, $n = 7$) and C rats ($r = 0.94$, $p < 0.01$, $n = 7$) and negatively with fat cell size in A rats ($r = -0.73$, $p < 0.05$, $n = 7$) only. Level of resistin mRNA in epididymal adipose tissue (Fig. 2B) was slightly reduced in alcohol-treated animals, while in PF group reduced resistin gene expression was significant in comparison to C group only.

Discussion

Generally, alcohol intake is connected with metabolic disturbances depending on duration of intake, amount of consumed alcohol and nutritional status of the consumers.

In our study, rats consuming alcohol had serum alcohol concentration 202 ± 33 mg/dl. This value is considered as the intake of a medium dose of alcohol (Yamakami et al. 1995; Miki et al. 2000). Long-term alcohol consumption resulted in decreased food intake and lower total energy intake followed by reduced body mass gain. Although A and PF rats had the same daily caloric intake, reduced body mass gain was more profound in A (*vs.* C) than in PF rats due to "empty" calories from ethanol (Lieber 1991). Thus the phenomena of reduced food/energy intake could be assigned to the effect of chronic alcohol consumption at least in rats (Fujita et al. 2003; Macho et al. 2003).

Only in alcohol-treated rats reduced body weight gain goes along with diminished relative epididymal adipose tissue mass containing smaller adipocytes. Smaller fat cell size after alcohol treatment was previously described and explained by reduced activities of lipogenic enzymes (Wilson et al. 1988; Shih and Taberner 2001). Resistin increases the storage of TG in muscle and liver instead of adipose tissue (Kim et al. 2001) and *in vitro* participates in induction of lipolysis and reduction of lipid droplet size in adipocytes (Ort et al. 2005). Here we demonstrated significant negative correlation between serum resistin and fat cell size. This finding supports the role of resistin in regulation of fat cell size. We also assume that enhanced lipolysis (proved by the presence of high serum NEFA levels and smaller adipocytes) is the compensatory mechanism for dietary energy deficiency.

We have found that ethanol consumption significantly increased glycemia. Previous observations of high glycemia due to alcohol intake (Forman 1988; Avogaro and Tiengo 1993; Kojima et al. 2005) attributed this metabolic condition to impaired glucose utilization (Poirier et al. 2001), glucose intolerance and/or insulin resistance (Shelmet et al. 1988). We have observed similar insulinemia in all experimental groups likewise in some other reports studying relationship between alcohol intake and insulin level in rats (Štrbák et al. 1998; Macho et al. 2003). Recently, it was shown that physiological function of resistin during fasting is the maintenance of blood glucose by i) impairing the ability of insulin to suppress hepatic glucose production (Rajala et al. 2003; Banerjee et al. 2004), ii) activating expression of gluconeogenic enzymes in the liver (Banerjee et al. 2004) and iii) inhibiting glucose uptake in skeletal muscle (Satoh et al. 2004). Increased serum resistin after alcohol consumption could be involved in mechanism(s) of increased glycemia in our A rats. We have found positive correlation between serum resistin and glycemia both in fed A and fed C rats. This relation confirmed for non-starving animals could indicate a general relation between resistin and glycemia (Kim et al. 2001) independently of metabolic state.

Moreover, increased glycemia and unchanged insulinemia in our A rats indicate the presence of disturbances in glucose metabolism, insulin action and impairment of insulin sensitivity. However, HOMA index, QUICKI index and McAuley index calculated for the expression of insulin resistance/sensitivity did not confirm alteration in insulin sensitivity *in vivo*.

The involvement of resistin and its potential role in the pathogenesis of insulin resistance and type 2 diabetes is well documented (Kim et al. 2001; Steppan et al. 2001). Specifically, resistin has antagonistic effects to those of insulin on glucose metabolism in adipocytes and skeletal muscle (Kim et al. 2001; Steppan et al. 2001; Li et al. 2002).

We have investigated *in vitro* glucose transport in isolated fat cells. While basal glucose transport was similar in all experimental groups, insulin-stimulatory effect was attenuated in adipocytes from alcohol-treated rats compared to C rats. This finding is not due to decreased fat cell size *per se*, as the amount of glucose transported across the membrane was calculated *per* square surface unit. In this respect our observation is in agreement with recent studies describing impaired insulin-stimulated glucose uptake in rat adipocytes after 4-week alcohol intake (Wilkes et al. 1996; Poirier et al. 2001; Sebastian and Nagy 2005). Reduced glucose transport could be the consequence of i) reduced amount of glucose transporter GLUT-4 or diminished insulin-stimulated

GLUT-4 recruitment (Wilkes et al. 1996; Poirier et al. 2001), ii) disturbances in insulin-activated intracellular events (Bhavani et al. 1995; Xu et al. 1995) or iii) disruption of Cbl/TC10 intracellular activation/inactivation cycle mediated by insulin receptor (Sebastian and Nagy 2005).

Other investigators have reported that resistin could inhibit insulin-stimulated glucose transport in cultured 3T3-L1 adipocytes (Fu et al. 2006) and in isolated skeletal muscle cells (Pravenec et al. 2003; Carvalho et al. 2005). Here we demonstrate attenuation of glucose transport stimulated by insulin in isolated adipocytes of alcohol-treated rats. As these animals have elevated resistin levels, it is possible that augmented adipokine interferes with the intrinsic activity of cell surface glucose transporters and/or insulin-signalling pathways mediating glucose uptake (Moon et al. 2003).

Liver is the major site of ethanol metabolism. Chronic ethanol feeding to rats induces hyperlipidemia coupled with elevated plasma cholesterol, TG and free fatty acid concentrations (Balasubramaniyan and Nalini 2003). Altered lipid profile of our alcohol-treated rats, e.g. elevated serum NEFA, increased serum and liver TG are in favour to general and well-known effect of alcohol intake on liver metabolism (Crouse and Grundy 1984; Gandhi and Raina 1984; Buydens-Branchey et al. 1988). Moreover, reduced glycogen content in liver underlines the presence of typical destructive lesions in alcoholic liver (Kubota et al. 1992).

Animal studies have highlighted the ability of resistin to induce hepatic insulin resistance (Banerjee et al. 2004; Muse et al. 2004; Rangwala et al. 2004). Resistin primarily exerts its glucoregulatory effect directly *via* hepatic effects on increased glycogenolysis and gluconeogenesis (Rajala et al. 2003; Muse et al. 2004) and indirectly through hypothalamus (Muse et al. 2007). As regard the presence of elevated serum resistin and lower glycogen content in liver of A rats it seems that this adipokine could be involved in enhanced glycogenolysis and thus contribute to impaired glycemia.

The tendency (in A group) of decreased (in PF group) resistin gene expression in epididymal adipose tissue was induced rather by limited energy intake than alcohol consumption. The similar observations have been described for starving (Kim et al. 2001; Steppan et al. 2001; Li et al. 2002). Despite lower resistin mRNA levels in adipose tissue of A and PF rats, serum resistin was higher (in A group) or similar (in PF group) compared to C animals. This discrepancy could originate from a) the observation that resistin gene expression and secretion occur, besides adipose also in other tissues, and b) disturbed metabolic/degradation processes of resistin.

In conclusion, in our study we demonstrated that long-time consumption of 6% ethanol solution significantly elevated serum resistin level, since resistin gene expression in adipose tissue was not affected. High serum resistin concentration could be involved in changed metabolic processes manifested by smaller adipocyte size, increased glycemia, attenuation of insulin effect on glucose transport in isolated adipocytes. We suppose that alcohol intake *via* increased serum resistin level impairs insulin action in fat cells and liver metabolism with subsequent alterations of glucose and lipid metabolism. Alcohol consumption for period of 28 days did not induce systemic insulin resistance.

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