doi:10.4149/endo_2009_03_117

# ALCOHOL INTAKE MODIFIES LEPTIN, ADIPONECTIN AND RESISTIN SERUM LEVELS AND THEIR MRNA EXPRESSIONS IN ADIPOSE TISSUE OF RATS

PRAVDOVA E, MACHO L, FICKOVA M

*Institute of Experimental Endocrinology, Slovak Academy of Sciences, 833 06 Bratislava, Slovak Republic*
*e-mail: ueenfick@savba.sk*

**Objective.** Alcohol intake is known to interfere with endocrine system functions thus inducing hormonal and metabolic imbalance. The aim was to investigate the impact of chronic intake of mild alcohol concentration on serum leptin, adiponectin and resistin and their gene expression in epididymal adipose tissue (EAT) of rats.

**Methods.** The 28 days study was based on 3 experimental groups of adult male Wistar rats: 1/ *ad libitum* intake of 6 % ethanol solution and pelleted diet (A), 2/ *pair fed* animals (PF) fed pelleted diet in the same caloric amount as A rats on previous day (alcohol+diet), 3/ control rats (C) with unrestricted intake of water and pelleted diet.

RT-PCR method was used for determination of adipokines gene expression in epididymal adipose tissue, serum levels were measured by ELISA kits.

**Results.** The animals of A group were characterized by reduced food and energy intake (-10 % vs C), lower body mass gain, reduced epididymal fat mass with smaller adipocytes. Alcohol consumption significantly increased glycemia, serum insulin was not affected. The raise of NEFA in A and PF rats gives the evidence of intensified lipolysis due to the deficiency of energy intake. Alcohol consumption significantly increased serum leptin and resistin, elevated adiponectin was present in A and PF rats. In parallel with increased serum levels the expression of adiponectin gene in epididymal adipose tissue was elevated in the same A and PF rats. Leptin and resistin mRNA levels were similar as in C regardless of alcohol intake or pair-fed feeding. Increased leptin and resistin levels positively correlated with glycemia and negatively with the size of adipocytes. Elevated serum leptin and resistin together with high adiponectin after chronic moderate alcohol intake could contribute to alteration of energy metabolism either individually or in reciprocal coordination.

**Conclusions.** 28 days consumption of 6 % ethanol solution changed the nutritional status of rats and induced significant elevation of serum leptin and resistin, while elevated gene expression in epididymal adipose tissue was proved for adiponectin only. Elevated serum adipokines could contribute to increased glycemia and altered glucose homeostasis.

**Keywords:** Alcohol – Adiponectin – Leptin – Resistin – Adipose tissue – Gene expression

**Corresponding author:** Maria Fickova, PhD, DSc, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlarska 3, 833 06 Bratislava, Slovak Republic, Phone +421 2 5477 2800, Fax: +421 2 54774247, e-mail: ueenfick@savba.sk

**Abbrevations**

A group = Alcohol-treated group; PF group = pair fed group; C group = control group; GAPDH = Glyceraldehyde-3-phosphate dehydrogenase; NEFA = Nonesterified fatty acids

Adipose tissue appeared highly active metabolic and endocrine organ producing a variety of bioactive peptides and proteins – called adipokines – which, among others, are also implicated in the regulation of food intake, energy expenditure, body weight, insulin action, glucose and lipid metabolism in the liver, muscles and adipose tissue.

Leptin, the first recognized adipokine (Zhang et al. 1994), is considered one of the main peripheral endocrine signals involved in the regulation of food intake, body mass and adiposity by reducing the food intake and stimulating the thermogenesis (Halaas et al. 1995; Campfield et al. 1996). Moreover, leptin is involved in the regulation of metabolic balance by reducing the incorporation of glucose into lipids (Ceddia et al. 1998) and increasing the lipolysis (Wang et al. 1999). The role of leptin in insulin effects on glucose uptake, glycogen synthesis and lipogenesis was also reported (Campfield et al. 1996; Muller et al. 1997). In addition to white adipose tissue, leptin is also expressed in other tissues and organs such as hypothalamus, pituitary or skeletal muscle (Morash et al. 1999; Jin et al. 2000).

Adiponectin, being exclusively expressed and secreted by adipose tissue, plays an important role in the regulation of insulin sensitivity directed to the improvement of glucose tolerance and reduction of insulin resistance (Yamauchi et al. 2001; Chandran et. al. 2003). High adiponectin levels together with high adiponectin mRNA and high insulin sensitivity were found in lean subjects (Kern et al. 2003). Plasma adiponectin level inversely correlated with body mass index (Yang et al. 2002), percent of body fat (Weyer et al. 2001), waist-to-hip ratio (Cnop et al. 2003), muscle lipid content (Weiss et al. 2003) and serum triglycerides, while its positive correlation was observed with high-density lipoprotein cholesterol (Cnop et al. 2003; Weiss et al. 2003).

Resistin is a hormonal signal involved in the induction of insulin resistance by decreasing glucose tolerance and insulin sensitivity (Steppan et al. 2001). It also impairs insulin action in the liver, skeletal muscle and adipocytes (Moon et al. 2003; Rajala et al. 2003). In obesity, increased serum resistin and resistin mRNA were found (Steppan et al. 2001; Li et al. 2002), while low resistin mRNA level in adipose tissue during fasting returned to normal level after refeeding (Kim et al. 2001; Li et al. 2002).

Alcohol consumption was found to affect almost all biological functions including metabolic pathways and hormonal systems resulting in non-physiological increase/decrease of plasma concentrations and gene expression of hormones (Obradovic and Meadows 2002). Hormonal imbalance alters the effects of hormones in target tissues thus resulting in impaired energy metabolism. However, there are various and non-consistent data how the alcohol intake modifies adipokine levels. Discrepancies between individual studies may result from different experimental settings like various subjects, animal strains, gender, food composition, mode and regime of alcohol intake as well as alcohol doses and/or duration of treatment. In human and animal studies increased leptin levels (Nicolas et al. 2001; Obradovic and Meadows 2002; Szkudelski et al. 2004), reduced leptin concentrations (Calissendorff et al. 2004) or unchanged leptin levels after alcohol intake were observed (Strbak et al. 1998; Wannamethee et al. 2007; Beulens et al. 2008). In moderate alcohol consumers elevated circulating adiponectin level was related to increased insulin sensitivity (Sierksma et al. 2004; Beulens et al. 2006; Joosten et al. 2008), but Avogaro et al. (2003) did not find any changes of serum adiponectin in men drinking moderate dose of alcohol. Recently, elevated adiponectin was proved in alcohol-dependent patients (Hillemacher et al. 2009). Currently, limited and inconsistent data of serum resistin in humans consuming alcohol are known, both increased levels of resistin (Hillemacher et al. 2009) and unaffected values (Beulens et al. 2008) being reported.

The present study was aimed to investigate the impact of chronic intake of mild alcohol concentration on serum leptin, adiponectin and resistin level as well as their gene expression in epididymal adipose tissue of rats. Moreover, the alcohol effect was compared not only to control/untreated animals but also to precisely controlled the pair-fed animals with identical energy intake (in the form of standard laboratory chow) as alcohol treated rats. The results obtained are discussed in relation to the impact of altered adipokines on the metabolic status.

## Materials and Methods

**Animals and treatment.** Adult male Wistar rats (**10** animals per group) obtained from ANLAB (Czech Republic) with initial body mass of 200-250 g were used. The animals were housed under controlled temperature (22-24 ºC), humidity (~65 %) and constant 12-hour light/dark cycle. The animals were fed standard pelleted laboratory diet (Bonagro, Czech Republic), solid food and water was available ad libitum for control rats (C). The ethanol group (A group) had free access to 6 % (v/v)

**Table 1**

**Food, energy and liquid intake, body mass gain, total and relative epididymal adipose tissue mass, fat cell size and serum parameters**

| | C | PF | A |
|---|---|---|---|
| Food intake (g/day) | 26.6±0.5 | 24.4±0.4** | **22.0±0.3*****+++** |
| Caloric intake (kJ/day) | 374±10.1 | 339±6.0*** | **339±6.0***** |
| Water/alcohol intake (ml/day) | 44.7±0.6 | 40.9±0.6*** | **30.00±0.5*****+++** |
| Body mass gain (g) | 88.8±6.5 | 71.0±3.2* | **65.8±3.3**** |
| Total epididymal fat (g) | 7.16±0.5 | 6.10±0.2* | **5.67±0.2*** |
| Relative epididymal fat (g/100g BW) | 0.90±0.05 | 0.89±0.07 | **0.72±0.04*** |
| Fat cell size (diameter, μm) | 64.2±1.0 | 53.4±1.0*** | **55.5±0.8***** |
| Serum glucose (mmol/l) | 7.4±0.1 | 7.6±0.1 | **8.1±0.1*****+** |
| Serum insulin (ng/ml) | 3.30±0.2 | 3.08±0.4 | **2.98±0.3** |
| **NEFA (mmol/l)** | **0.24±0.03** | **0.42±0.05*** | **0.40±0.04*** |

C-control; PF-*pair-fed*; A-alcohol-drinking (n=10); BW-body weight, NEFA-nonesterified fatty acids. The data are given as mean (±SEM). Differences between groups are indicated by: *p<0.05, **p< 0.01, ***p<0.001 (A, PF vs C), $^{+}$p<0.05, $^{+++}$p<0.001 (A vs PF).

ethanol solution in tap water as the only drinking fluid for 28 days, while the access to solid food was free. Pair-fed animals (PF group) were allowed to consume solid food in the same caloric value as A group had consumed during the preceding 24 hrs, while water intake was free. The amount of consumed food and volume of ethanol solution/water were registered daily. The animals were sacrificed by decapitation between 8.00-10.00 a.m. and blood was collected for serum separation. A portion of epididymal fat removed under sterile condition was stored at -80 °C. Fresh portion of adipose tissue was used immediately for fat cell isolation. All protocols were approved by Animal Care Committee at the Institute of Experimental Endocrinology SAS (Bratislava, Slovakia) and National Veterinary Institute of Slovakia (Bratislava, Slovakia).

**Determination of adipocyte size.** Adipocytes were isolated from epididymal fat pads according to Rodbell (1964) by collagenase digestion (Sigma, USA). Fat cell size (cell diameter) was measured under light microscope (Reichert, Austria) after staining the cells with crystal violet.

**Serum biochemical analyses.** Concentrations of serum glucose were determined by glucooxidation reaction system (Super GL, Germany) and insulin level by radioimmunoassay (Linco, USA). ELISA immunoassays were used for determination of serum leptin (DSLabs, USA), adiponectin (AssayPro, USA) and resistin (BioVendor, Czech Republic). Non-esterified fatty acids (NEFA) were determined by the colorimetric method (RANDOX, UK).

**Gene expression.** Total RNA was extracted from 150 mg frozen epididymal adipose tissue by RNeasy Lipid Tissue Mini Kit (QIAGEN, Germany). Double-stranded cDNA was synthesized by Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences, UK). The reverse transcription products were amplified by PCR and DyNAzyme II DNA polymerase (Finnzymes, Finland) with specific primers for leptin, adiponectin and resistin (Merck, Germany). RT-PCR products were separated in a 1.5 % (w/v) agarose gel, stained with ethidium bromide, visualized with SRS 62201 (Ultra-Lum, USA) and quantified by software PCBAS 2.08e (Raytest, Germany). Data were expressed as the ratio of gene transcription level to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal control.

**Statistical evaluation.** Data were expressed as mean ± SEM (n= 10 rats per group). For statistical evaluation one-way analysis of variance (ANOVA test) followed by Bonferroni correction of differences between groups was used (GraphPad Prism 4, USA). Correlations between adipokine serum concentrations and other variables were examined by Pearson's coefficient of correlation. The values of p<0.05 were considered statistically significant.

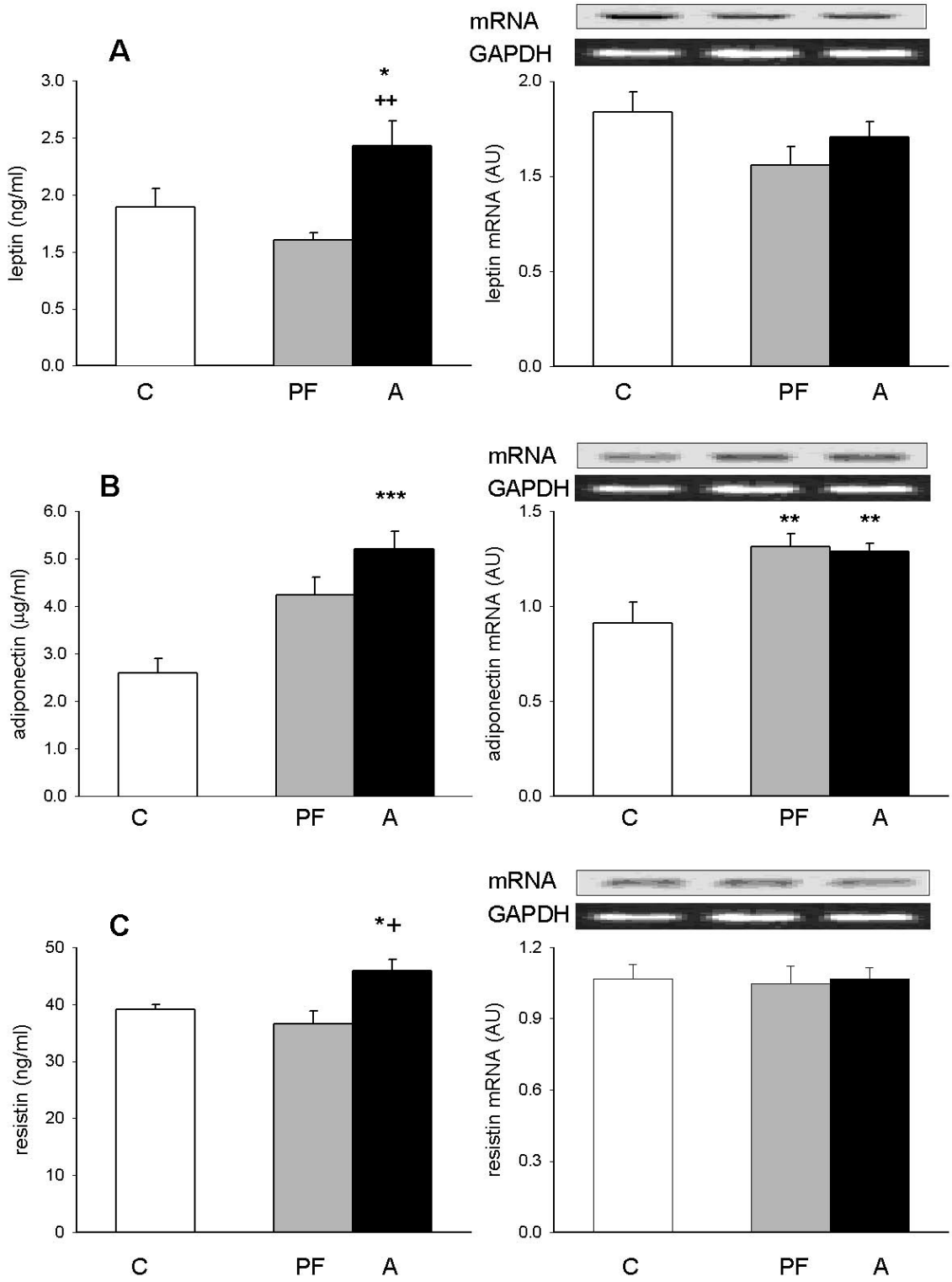


**Fig 1 Serum levels (left panel) and mRNA expression (right panel) in rat epididymal fat tissue of A (leptin), B (adiponectin), C (resistin). Groups of rats (n=10 each): C-control; PF-*pair-fed;* A-alcohol, AU-arbitrary units. Differences between groups are indicated by: *p< 0.05 A, PF vs. C, +p< 0.05 A vs. PF.**
***p<0.05, **<0.01 (A, PF vs C), ***p<0.001 (A vs C), $^{+}$p<0.05, $^{++}$p<0.01 (A vs PF).**

## Results

**Food and energy intake.** Table 1 shows that alcohol treated rats consumed less of solid food as control animals. Total energy intake from solid food and alcohol was significantly lower than that for controls (~ 10 % reduction) and this amount was identical for *pair fed* rats.

**Body mass gain, epididymal adipose tissue mass and fat cell size.** Simultaneously with reduced energy intake in both A and PF animals, body mass gain, adipose tissue mass (absolute and relative) and fat cell size were diminished (A and PF vs. C – Table 1).

**Serum parameters** (Table 1). Alcohol intake (A group) resulted in a significant increase of glycemia compared to that of both C and PF group. However, insulin level in serum was not affected either by alcohol intake or by PF feeding. Increased NEFA level was found in the A group and PF group with restricted energy intake as compared to controls (C group).

**Serum level and mRNA of leptin, adiponectin and resistin** (Fig. 1). Long-time alcohol consumption resulted in the increase of serum leptin and resistin as compared to control and pair-fed groups, while significantly increased serum adiponectin was present not only in alcohol treated rats, but also in *pair fed* group versus controls. Adiponectin gene expression was significantly higher in epididymal adipose tissue of both alcohol fed and *pair fed* rats (versus controls), while the amount of mRNA for leptin and resistin was similar in all groups.

Serum leptin of A group positively correlated with glycemia ($r = 0.67$, $p<0.05$) and negatively with fat cell size ($r = -0.63$, $p<0.05$). Serum adiponectin positively correlated with adiponectin mRNA in A ($r = 0.63$, $p<0.05$) and in PF rats ($r =0.80$, $p<0.01$). Serum resistin of A group positively correlated with glycemia ($r = 0.88$, $p<0.01$) and negatively with adipocytes size ($r = -0.61$, $p<0.05$).

## Discussion

Chronic alcoholism represents an important socio-economic problem due to associated serious health disorders. The disturbances of almost all biological functions comprise also endocrine system with impaired hormone gene expression (Corella 2007) and plasma hormone concentrations (Obradovic and Meadows 2002; Szkudelski et al. 2004). Hormonal imbalance contributes to the impairment of overall metabolism and energy equilibrium (Strbak et al. 1998; Szkudelski et al. 2004). The extent of changes depends on the amount of alcohol and duration of the intake. Several studies have confirmed that excessive alcohol consumption is associated with glucose intolerance and insulin resistance (Onishi et al. 2003), while moderate alcohol intake is considered beneficial for enhancing insulin sensitivity (Davies et al. 2002; Sierksma et al. 2004; Beulens et al. 2008*)* and decreasing the prevalence of metabolic syndrome (Djousse et al. 2004).

The aim of this study was to determine the effect of chronic intake of moderate alcohol concentration on the expression and secretion of adipokines involved in hormonally controlled energy metabolism. To precisely characterize the effect of alcohol consumption, the strict control of energy intake was established, e.g. solid food and alcohol ingested were measured daily. To discriminate the effect of alcohol itself, we included an additional control group consisting of *pair fed* animals with daily energy intake identical to alcohol consuming animals on previous day.

Serum alcohol concentration (202 ± 33 mg/100 ml) proved the intake of a medium dose of alcohol (Yamakami et al. 1995; Miki et al. 2000). It is important to point out that since 6 % (v/v) alcohol intake significantly attenuated solid food intake, the reduction of energy intake (~ 10 % vs. C) does not represent any serious starvation (Arai et al. 2004). It seems unambiguous that the reduced energy intake in A and PF rats could rationalize diminished body mass gain, lower epididymal adipose tissue mass and smaller fat cells (vs. controls). These characteristics suggest the prevalence of catabolic processes to cover energy requirements. The hypothesis is partially proved by doubled serum NEFA levels in both, A and PF animals (vs. controls) as a sign of intensified lipolysis.

Here we present serum glucose and insulin concentrations to characterize metabolic condition of experimental animals. Significantly elevated glycemia was present in alcohol treated rats only (vs. both control and *pair fed* rats), while identical insulin levels were in all experimental groups. This combination of glucose and insulin values provides evidence of alcohol induced impairment of glucose homeostasis. Leptin is involved in regulation of glucose homeostasis and lipid metabolism indirectly, via central nervous system (Shen et al. 2007) and directly, through acting on insulin sensitive tissues (Reidy and Weber 2000). Moreover, leptin in adipose tissue by autocrine mechanism stimulates lipolysis and inhibits triacylglycerol synthesis (Wang et

al. 1999; Zhou et al. 1999), while in the liver stimulates gluconeogenesis and glucose output (Cohen et al. 1996). Significantly elevated leptin in alcohol treated rats (50 % increase vs. C; 78 % vs PF) clearly demonstrates that alcohol intake *per se* is the stimulus for increased leptinemia. We suppose that elevated leptin by the central effect contributed to reduced food intake and by the peripheral effect contributed to elevated glycemia by: 1. stimulating gluconeogenesis in liver, 2. inhibiting insulin stimulated glucose transport in adipocytes (Pravdova et al. 2007). The tight relation between leptin and glycemia in our alcohol treated animals is proved by the positive correlation between serum leptin and glucose.

However, in the literature, there is an inconsistency how alcohol intake modifies leptin levels, since either elevated (Nicolas et al. 2001; Obradovic and Meadows 2002; Szkudelski et al. 2004), reduced (Calissendorff et al. 2004) and even unchanged leptin levels (Strbak et al. 1998; Wannamethee et al. 2007; Beulens et al. 2008) were observed. Almost all studies are failing the precise measurement of dietary/energy intake of control/experimental group. This drawback is also distinctive for the recent human study (Buelens et al. 2008) describing that moderate alcohol consumption during three weeks did not modify leptin and resistin concentrations in lean and overweight men. The use of *pair fed* group in our experiment allowed to distinguish the effects of alcohol consumption and reduced energy intake. Thus, alcohol intake in our experimental schedule is characterized by unambiguously elevated leptin levels.

We failed to find any relation between serum leptin and leptin gene expression in epididymal adipose tissue. Thus, elevated serum leptin could result from: 1. increased synthesis and/or secretion from other adipose tissue locations and additional tissues expressing leptin (Morash et al. 1999; Jin et al. 2000) and/or 2. attenuated leptin degradation.

Adiponectin is unique among adipocyte-derived hormones in that its circulating concentrations are inversely proportional to adiposity. While adiponectin concentrations are unchanged after meal ingestion (Swarbrick and Havel, 2008), long-term nutritional status (satiety/malnutrition) and changes in body weight are main determinants of circulating adiponectin levels (Merl et al. 2002; Housova et al. 2005). We suppose that substantial elevation of adiponectin in alcohol treated rats (doubled) and in *pair fed* counterparts (above 50 %) as compared to control ones, is rather specific response to reduced energy intake then the effect of alcohol ingestion. Recently, it was described that moderate alcohol consumption for three weeks (three glasses of beer daily) increased adiponectin in lean and overweight man (Beulens et al. 2008). The authors hypothesize elevated adiponectin as the benefit for the improvement of insulin sensitivity in subjects under observation. Actually, we do not reject the above role of elevated adiponectin, as our experimental animals with local insulin resistance in isolated adipocytes do not demonstrate *in vivo* insulin resistance as expressed by HOMA, Quicki and McAuley indexes (Pravdova et al. 2007). It is rather possible that elevated adiponectin with beneficial effects on insulin sensitivity eliminated opposite effects of leptin and resistin (see further).

In parallel with elevated adiponectin levels (in A and PF), increased expression of adiponectin gene in epididymal adipose tissue was present. As adiponectin is produced exclusively in adipocytes, unambiguous positive correlation between these parameters was confirmed for A and PF animals.

Resistin induces insulin resistance in peripheral tissues thus acting mainly in an opposite way than adiponectin. Moreover the latest observations have shown, that central resistin downregulates mRNA expression of NPY (Vazquez et al. 2008), and inhibits food intake (Cifani et al. 2009). Elevated resistin in our alcohol treated rats (vs. both, control and pair-fed rats) clearly demonstrated the effect of alcohol intake *per se* and not the effect of caloric intake restriction. In addition to affecting food intake, elevated resistin could participate in mechanism(s) for impaired glucose homeostasis in these rats (Pravdova et al. 2007), the association is proved by positive correlation between serum resistin and serum glucose. Since the main sources of resistin are monocytes and macrophages (Bokarewa et al. 2005), it appears most likely why we failed to detect any changes in resistin gene expression in epididymal adipose tissue.

Our model of chronic alcohol intake was not accompanied by any serious liver injury (for details see Pravdova et al. 2007).

The finding of the present study give the evidence of altered endocrine activity of adipose tissue due to moderate alcohol intake. Of particular note are the elevated serum levels of leptin and resistin involved in reduced food intake and participating in impaired glucose homeostasis. Increased serum adiponectin most likely reflects only extended deficiency of energy intake and by insulin sensitizing effect neutralizes opposite effects of leptin and resistin.

Gene expression for leptin and resistin is not altered and did not correspond to respective serum levels. Only serum adiponectin was simultaneously elevated with increased gene expression. The addition of second control group, e.g. *pair fed* animals with identical energy intake as alcohol treated rats makes the present study exceptional for defining specific effects of moderate alcohol intake on adipokines production and secretion.

## Acknowledgements

This study was supported by the grant VEGA 2/7044/27.

## References

Arai K, Soga T, Ohata H, Otagiri A, Shibasaki T: Effects of food restriction on peroxisome proliferator-activated receptor-γ and glucocorticoid receptor signaling in adipose tissues of normal rats. Metabolism 53, 28-36, 2004. doi:10.1016/j.metabol.2003.08.007

Avogaro A, Sambataro M, Marangoni A, Pianta A, Vettor R, Pagano C, Marescotti MC: Moderate alcohol consumption, glucose metabolism and lipolysis: the effect on adiponectin and tumor necrosis factor alpha. J Endocrinol Invest 26, 1213-1218, 2003

Beulens JW, Beers RM, Stolk RP, Schaafsma G, Hendriks HF: The effect of moderate alcohol consumption on fat distribution and adipocytokines. Obes Res 14, 60-66, 2006. doi:10.1038/oby.2006.8

Beulens JW, de Zoete EC, Kok FJ, Schaafsma G, Hendriks HF: Effect of moderate alcohol consumption on adipokines sensitivity in lean and overweight men: a diet interventional study. Eur J Clin Nutr 62, 1098-1105, 2008. doi:10.1038/sj.ejcn.1602821

Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A: Resistin, an adipokine with potent proinflammatory properties. J Immunol 174, 5789-5795, 2005

Calissendorff J, Brismar K, Rojdmark S: Is decreased leptin secretion after alcohol ingestion catecholamine-mediated? Alcohol 39, 281-286, 2004

Campfield LA, Smith FJ, Burn P: The OB protein (leptin) pathway - a link between adipose tissue mass and central neural networks. Horm Metab Res 28, 619-632, 1996. doi:10.1055/s-2007-979867

Ceddia RB, William WN, Lima FB, Curi R: Leptin inhibits insulin-stimulated incorporation of glucose into lipids and stimulates glucose decarboxylation in isolated rat adipocytes. J Endocrinol 158, R7-R9, 1998. doi:10.1677/joe.0.158R007

Chandran M, Phillips SA, Ciaraldi T, Henry RR: Adiponectin: more than just another fat cell hormone? Diabetes Care 26, 2442–2450, 2003. doi:10.2337/diacare.26.8.2442

Cifani C, Durocher Y, Pathak A, Penicaud L, Smih F, Massi M, Rouet P, Polidori C: Possible common central pathway for resistin and insulin in regulating food intake. Acta Physiol (Oxf). 2009 Jan 24. (Epub ahead of print). doi:10.1111/j.1748-1716.2008.01949.x

Cnop M, Havel PJ, Utyschneider KM, Carr DB, Sihna MK, Boyko EJ: Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 46, 459-469, 2003

Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. Science 274, 1185-1188, 1996. doi:10.1126/science.274.5290.1185

Corella D: Gene-alcohol interactions in the metabolic syndrome. Nutr Metab Cardiovasc Dis 17, 140-147, 2007. doi:10.1016/j.numecd.2006.02.007

Davies MJ, Baer DJ, Judd JT et al.: Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 287, 2559–2262, 2002. doi:10.1001/jama.287.19.2559

Djousse L, Arnett DK, Eckfeldt JH et al.: Alcohol consumption and metabolic syndrome: does the type of beverage matter? Obes Res 12, 1375–1385, 2004. doi:10.1038/oby.2004.174

Friedman JM, Halaas JL: Leptin and the regulation of body weight in mammals. Nature 395, 763-770, 1998. doi:10.1038/27376

Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543-546, 1995. doi:10.1126/science.7624777

Hillemacher T, Weinland C, Heberlein A, Gröschl M, Schanze A, Frieling H, Wilhelm J, Kornhuber J, Bleich S: Increased levels of adiponectin and resistin in alcohol dependence--possible link to craving. Drug Alcohol Depend 99, 333-337, 2009. doi:10.1016/j.drugalcdep.2008.07.019

Housova J, Anderlova K, Krizova J, Haluzikova D, Kremen J, Kumstyrova T, Papezova H, Haluzik M:. Serum adiponectin and resistin concentrations in patients with restrictive and binge/purge form of anorexia nervosa and bulimia nervosa. J Clin Endocrinol Metab 90, 1366-1370, 2005. doi:10.1210/jc.2004-1364

Jin L, Zhang S, Burguera BG, Couce ME, Osamura RY, Kulig E, Lloyd RV: Leptin and leptin receptor expression in rat and mouse pituitary cells. Endocrinology 141, 333-339, 2000. doi:10.1210/en.141.1.333

Joosten MM, Beulens JW, Kersten S, Hendriks HF: Moderate alcohol consumption increases insulin sensitivity and ADIPOQ expression in postmenopausal women: a randomised, crossover trial. Diabetologia 51, 1375-1381, 2008. doi:10.1007/s00125-008-1031-y

Kern PA, Gregorio GB, Lu T, Rassouli N, Ranganathan G: Adiponectin expression from human adipose tissue. Diabetes 52, 1779-1785, 2003. doi:10.2337/diabetes.52.7.1779

Kim KH, Lee K, Moon YS, Sul HS: A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J Biol Chem 276, 11252-11256, 2001. doi:10.1074/jbc.C100028200

Li J, Yu X, Pan W, Unger RH: Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. Am J Physiol Endocrinol Metab 282, E1334-E1341, 2002

Merl V, Peters A, Oltmanns KM, Kern W, Born J, Fehm HL, Schultes B: Serum adiponectin concentrations during a 72-hour fast in over- and normal-weight humans. Int J Obes 29: 998-1001, 2002. doi:10.1038/sj.ijo.0802971

Miki T, Harris SJ, Wilce P, Takeuchi Y, Bedi KS: Neurons in the hilus region of the rat hippocampus are depleted in number by exposure to alcohol during early postnatal life. Hippocampus 10, 284-295, 2000. doi:10.1002/1098-1063(2000)10:3<284::AID-HIPO9>3.0.CO;2-K

Moon B, Kwan JJ, Duddy N, Sweeney G, Begum N: Resistin inhibits glucose uptake in L6 skeletal muscle cells independent of changes in insulin signaling components and Glut-4 translocation. Am J Physiol Endocrinol Metab 258, E106-E115, 2003

Morash B, Li A, Murphy PR, Wilkinson M, Ur E: Leptin gene expression in the brain and pituitary gland. Endocrinology 140, 5995-5998, 1999. doi:10.1210/en.140.12.5995

Muller G, Ertl J, Gerl M, Preibish G: Leptin impairs metabolic actions of insulin in isolated rat adipocytes. J Biol Chem 272, 10585-10593, 1997. doi:10.1074/jbc.272.16.10585

Nicolas JM, Fernandez-Sola J, Fatjo F, Casamitjana R, Bataller R: Increased circulating leptin levels in chronic alcoholism. Alcohol Clin Exp Res 25, 83-88, 2001. doi:10.1111/j.1530-0277.2001.tb02130.x

Obradovic T, Meadows GG: Chronic ethanol consumption increases plasma leptin levels and alters leptin receptors in the hypothalamus and the perigonadal fat of C57BL/6 mice. Alcohol Clin Exp Res 24, 255-262, 2002

Onishi Y, Honda M, Ogihara T, Sakoda H, Anai M, Fujishiro M, Ono H, Shojima N, Fukushima Y, Inukai K, Katagiri H, Kikuchi M, Oka Y, Asano T: Ethanol feeding induces insulin resistance with enhanced PI 3-kinase activation. Biochem Biophys Res Commun. 303, 788-794, 2003. doi:10.1016/S0006-291X(03)00407-8

Pravdova E, Macho L, Hlavacova N, Fickova M: Long-time alcohol intake modifies resistin secretion and expression of resistin gene in adipose tissue. Gen Physiol Biophys 26, 221-229. 2007

Rajala MW, Obici S, Scherer PE, Rossetti L: Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. J Clin Invest 111, 225-230, 2003

Reidy SP, Weber J: Leptin: an essential regulator of lipid metabolism. Comp Biochem Physiol A Mol Integr Physiol 125, 285-298, 2000. doi:10.1016/S1095-6433(00)00159-8

Rodbell M: Metabolism of isolated fat cells. I. Effect of hormones on glucose metabolism and lipolysis. J Biol Chem 239, 375-380, 1964

Schweinsburg BC, Taylor MJ, Videen JS, Alhassoon OM, Patterson TL, Grant I: Elevated myo-inositol in gray matter of recently detoxified but not long-term abstinent alcoholics: A preliminary MR spectroscopy study. Alcohol Clin Exp Res 24, 699-705, 2000. doi:10.1111/j.1530-0277.2000.tb02042.x

Shen J, Tanida M, Niijima A, Nagai K: In vivo effects of leptin on autonomic nerve activity and lipolysis in rats. Neurosci Lett 416, 193-197, 2007. doi:10.1016/j.neulet.2007.02.003

Sierksma A, Patel H, Ouchi N, Kihara S, Funahashi T, Heine RJ, Grobbee DE, Kluft C, Hendriks HFJ: Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-α, and insulin sensitivity. Diab Care 27, 184-189, 2004. doi:10.2337/diacare.27.1.184

Singhal NS, Lazar MA, Ahima RS: Central resistin induces hepatic insulin resistance via neuropeptide. NY J Neurosci 27, 12924-12932, 2007

Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS: The hormone resistin links obesity to diabetes. Nature 409, 307-312, 2001. doi:10.1038/35053000

Strbak V, Benicky J, Macho L, Jezova D, Nikodemova M: Four-week ethanol intake decreases food intake and body weight but does not affect plasma leptin, corticosterone, and insulin levels in pubertal rats. Metabolism 47, 1269-1273, 1998. doi:10.1016/S0026-0495(98)90335-3

Swarbrick MM, Havel PJ: Physiological, pharmacological, and nutritional regulation of circulating adiponectin concentrations in humans. Metab Syndr Relat Disord 6, 87-102, 2008. doi:10.1089/met.2007.0029

Szkudelski T, Bialik I, Szkudelska K: Adipocyte lipolysis, hormonal and metabolic changes in ethanol-drinking rats. J Anim Physiol Anim Nutr 88, 251-258, 2004. doi:10.1111/j.1439-0396.2004.00478.x

Wang MY, Lee Y, Unger RH: Novel form of lipolysis induced by leptin. J Biol Chem 25, 17541-17544, 1999. doi:10.1074/jbc.274.25.17541

Wannamethee SG, Tchernova J, Whincup P, Lowe GD, Kelly A, Rumley A, Wallace AM, Sattar N: Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. Atherosclerosis 191, 418-426, 2007. doi:10.1016/j.atherosclerosis.2006.04.012

Vazquez MJ, Gonzalez CR, Varela L, Lage R, Tovar S, Sangiao-Alvarellos S, Williams LM, Vidal-Puig A, Nogueiras R, Lopez M& Dieguez C: Central resistin regulates hypothalamic and peripheral lipid metabolism in a nutritional-dependent fashion. Endocrinology 149, 4534-4543, 2008. doi:10.1210/en.2007-1708

Weiss R, Dufour S, Groszmann A, Petersen K, Dziura J, Taksali SE, Shulman G, Caprio S: Low adiponectin levels in adolescent obesity: a marker of increased intramyocellular lipid accumulation. J Clin Endocrinol Metab 88, 2014-2018, 2003. doi:10.1210/jc.2002-021711

Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA: Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86, 1930-1935, 2001. doi:10.1210/jc.86.5.1930

Xu A, Wang Y, Keshaw H, Xu LY, Lam KSL, Cooper GJS: The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 112, 91-100, 2003

Yamakami I, Vink R, Faden AI, Gennarelli TA, Lenkinski R, McIntosh TK: Effects of acute ethanol intoxication on experimental brain injury in the rat: neurobehavioral and phosphorus-31 nuclear magnetic resonance spectroscopy studies. J Neurosurg 82, 813-821, 1995. doi:10.3171/jns.1995.82.5.0813

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7, 941-946, 2001. doi:10.1038/90984

Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL: Plasma adiponectin levels in overweight and obese Asians. Obes Res 10, 1104-1110, 2002. doi:10.1038/oby.2002.150

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425-432, 1994. doi:10.1038/372425a0

Zhou YT, Wang ZW, Higa M, Newgard CB, Unger RH: Reversing adipocyte differentiation: implications for treatment of obesity. Proc Natl Acad Sci USA 96, 2391-2395, 1999. doi:10.1073/pnas.96.5.2391