Physiol. Res. 54: 73-78, 2005

Adipocyte-Derived Hormones in Heroin Addicts: the Influence of Methadone Maintenance Treatment

J. HOUSOVÁ[1], H. WILCZEK[1], M. M. HALUZÍK[1, 2], J. KŘEMEN[1], J. KŘÍŽOVÁ[1], M. HALUZÍK[1]

[1]*Third Department of Medicine, First Faculty of Medicine and University Hospital, Prague and* [2]*Department of Chemistry, Faculty of Sciences, University of Ostrava, Ostrava, Czech Republic*

Received December 16, 2003
Accepted March 5, 2004

Summary

Heroin addiction markedly affects the nutritional and metabolic status and frequently leads to malnutrition. The aim of our study was to compare circulating concentration of adipose tissue-derived hormones leptin, adiponectin and resistin in 12 patients with heroin addiction before and after one-year methadone maintenance treatment with the group of 20 age- and body mass index-matched healthy subjects. Basal serum leptin and adiponectin levels in heroin addicts were significantly decreased (3.4±0.4 vs. 4.5±0.6 ng/ml and 18.9±3.3 vs. 33.9±3.1 ng/μl, respectively; $p<0.05$) while serum resistin concentrations were increased compared to healthy subjects (10.1±1.2 vs. 4.6±0.3 ng/ml; $p<0.05$). Moreover, positive correlation of serum leptin levels with body mass index was lost in the addicts in contrast to control group. One year of methadone maintenance treatment normalized serum leptin, but not serum adiponectin and resistin concentrations. In conclusion, circulating concentrations of leptin, adiponectin and resistin are markedly altered in patients with chronic heroin addiction. These alterations appear to be relatively independent of nutritional status and insulin sensitivity.

Key words

Heroin • Methadone • Addiction • Resistin • Adiponectin • Leptin

Introduction

Drug addiction is at present one of the most severe social-economical problems (Cartwright and Solano 2003). This problem is, among others, accompanied by many pathophysiological consequences influencing health and nutritional status. Drug addicts commonly suffer from malnutrition owing primarily to the limited food availability (Mohs *et al.* 1990). They also frequently develop infectious complications e.g. (most frequently) infections of urinary tract and subcutaneous tissue as a result of secondary immune dysfunction. Transmission of infectious diseases (such as HIV or hepatitis) following intravenous drug administration is another cause of excessive infection dissemination in this group of patients (Robles *et al.* 1998).

Methadone maintenance treatment (pioneered in the early 1960s) represents one of the possible ways to prevent and control heroin addiction. Methadone is a long-acting opioid mu receptor agonist acting on the surfaces of opioid-sensitive neurons (Vetulani 2001).

Opioid-sensitive neurons located in the ventral tegmental area (VTA) produce dopamine and release it into the nucleus accumbens, giving rise to pleasurable feeling. Conditioned associations of the feeling of pleasure with the circumstances and environment of addiction create a long-lasting memory record. Another opioid brain circuit includes locus ceruleus secreting noradrenaline and distributing it to other parts of the brain, which participate in opioid dependence. Methadone, though acting similarly to other opioids as dependence-producing drug, has minimal tolerance and alleviates craving and compulsive drug use (Nestler 2001). Moreover, it tends to normalize many aspects of the hormonal disruptions found in addicted individuals, reduces relapse rates and enables patients to concentrate on everyday tasks.

The discovery of leptin and other hormones produced by the adipose tissue revealed new interconnections of nutritional status with immune system and metabolic regulations (Havel 2002). Leptin is a protein hormone produced almost exclusively by adipocytes (Zhang *et al.* 1994), although there are some reports suggesting that other tissues, in particular the brain, may contribute significantly to the overall circulating leptin pool (Eikelis *et al.* 2004). Serum leptin concentrations positively correlate with body fat content (Maffei *et al.* 1995, Haluzík *et al.* 1998). Leptin´s major role is to signal the status of peripheral energy stores to the hypothalamic satiety center. Malnutrition with decreased adiposity is accompanied by hypoleptinemia that has important pathophysiological consequences (Haluzík *et al.* 1999). Low leptin levels act as a trigger of the complex neuroendocrine adaptive response to fasting. This response includes the drop of energy expenditure, amenorrhea in females, decreased sexual function in males, delayed onset of puberty in prepubertal children and immune dysfunction with decreased CD4+/CD8+ ratio (Sanchez-Margalet *et al.* 2003).

Recently, two more adipocyte-derived hormones that affect insulin action and immune function were discovered. The first, resistin, was proposed to link obesity to insulin resistance (Steppan *et al.* 2001). In the original report, serum resistin levels were increased in animal models of obesity and insulin resistance and anti-resistin antibody partially blunted insulin resistance. The original hypothesis was not fully supported by later studies and the role of resistin in the regulation of insulin sensitivity remains controversial (Savage *et al.* 2001). Another adipocyte-derived hormone – adiponectin – is in contrast to leptin and resistin inversely related to body fat content, i.e. its serum concentrations are increased in lean relative to obese subjects (Arita *et al.* 1999). Experimental data suggest that adiponectin has insulin-sensitizing and anti-inflammatory properties (Berg *et al.* 2002, Haluzík *et al.* 2004).

The adipose tissue thus produces a number of substances that can affect metabolic regulations and immune function. We hypothesized that drug addiction could be accompanied by alterations in circulating adipocytokines primarily related to nutritional status of drug addicts. We show here that circulating levels of adipose tissue-derived hormones in patients with drug addiction significantly differ from those of healthy subjects and that methadone maintenance treatment partially normalizes the differences.

Methods

Twelve patients (8 males and 3 females) with chronic heroin addiction (mean addiction duration 9±0.5 years, mean age 26.2±2.1 years) and 20 healthy age-matched control subjects (13 males and 7 females, mean age 25.1±1.1 years) were included into the study. While in healthy subjects no signs of acute infectious disease were present, 60 % of heroin addicts were positive for hepatitis B and/or C infection. None of the heroin addicts was HIV positive.

Control subjects underwent a single physical examination and blood withdrawal only, while addicts were examined before the start and after one year of methadone maintenance treatment.

The study protocol was approved by the local ethical committee. The subjects were informed about the purpose of the study and provided informed consent.

Subjects were measured and weighed. The blood samples for all measurements were withdrawn after an overnight fast between 8:00 and 9:00 h. Serum leptin, soluble leptin receptor (SLR), resistin and TNF-α levels were measured by commercial ELISA kits (BioVendor, CR and R&DS, USA, respectively), insulin, cortisol and adiponectin levels by RIA kits (Immunotech, CR and Linco Research, USA, respectively). Serum total and C-reactive protein were measured at the Department of Clinical Biochemistry of the University Hospital, Prague, by standard laboratory methods.

The statistical analysis was performed with SigmaStat software (Jandel Scientific, USA). The results are expressed as means ± S.E.M. Analysis of variance followed by Student-Neuman-Keuls test and paired t-test

were used for comparison of the groups as appropriate. The correlations between the variables were calculated by Pearson correlation test.

Results

The body mass index of the addicts did not significantly differ from the control group of healthy subjects (Table 1). The same was found for serum total protein, insulin and TNF-α. In contrast, serum cortisol, leptin, soluble leptin receptor and adiponectin levels were significantly lower in the addict group relative to healthy subjects. Serum C-reactive protein (CRP) and resistin levels were markedly higher in the addicts relative to the control group (Table 1).

Table 1. Anthropometric, hormonal and biochemical parameters of control and heroin addicts group (HA) before and after one year of methadone maintenance treatment.

	Control group	HA - basal	HA - after methadone
BMI (kg/m^2)	22.9 ± 0.5	22.1 ± 1.3	22.4 ± 1.1
TP (g/l)	79.4 ± 0.9	76.0 ± 1.3	79.0 ± 1.0$^+$
CRP (mg/l)	3.2 ± 2.2	13.6 ± 2.6*	11.14 ± 2.1*
Insulin ($mU.l^{-1}$)	15.7 ± 0.7	18.8 ± 3.1	16.6 ± 1.7
Cortisol (ng/ml)	507 ± 47	332 ± 36*	441 ± 53
TNF-α (ng/ml)	3.55 ± 0.57	3.50 ± 0.63	3.07 ± 0.80
Leptin (ng/ml)	4.5 ± 0.6	3.4 ± 0.4*	6.5 ± 0.6$^+$
SLR ($U.ml^{-1}$)	27.9 ± 2.3	12.6 ± 1.0*	13.5 ± 1.0*
Adiponectin (ng/μl)	33.9 ± 3.1	18.9 ± 3.3*	19.6 ± 2.5*
Resistin (ng/ml)	4.6 ± 0.3	10.1 ± 1.2*	10.2 ± 1.3*

BMI – body mass index, TP – total protein, CRP – C-reactive protein, SLR – soluble leptin receptor. Data are means ± S.E.M., * $p<0.05$ vs. Control group, $^+$ $p<0.05$ vs. HA before methadone maintenance treatment

Table 2. Correlation of serum leptin levels with anthropometric, biochemical and hormonal parameters in control and heroin addicts group (HA) before and after one year of methadone maintenance treatment.

	Control group	HA - basal	HA - after methadone
BMI	r=0.69, p<0.05	NS	r=0.78, p<0.05
TP	NS	NS	r=0.80, p<0.05
CRP	NS	NS	NS
Insulin	NS	NS	NS
Cortisol	NS	NS	NS
TNF-α	NS	NS	NS
SLR	NS	NS	NS
Adiponectin	NS	NS	NS
Resistin	NS	NS	NS

BMI – body mass index, TP – total protein, CRP – C-reactive protein, SLR – soluble leptin receptor. r: correlation coefficient, p: significance level ($p<0.05$), NS: non-significant (Pearson test)

One year of methadone maintenance treatment did not significantly affect the body mass index of addicts group (Table 1). No change after methadone maintenance treatment was found in serum insulin, TNF-α and soluble leptin receptor concentrations. Serum CRP levels tended to decrease after methadone maintenance treatment but the difference did not reach statistical significance and CRP levels in addicts group after one of methadone

maintenance treatment still remained significantly increased relative to control group (Table 1). One year of methadone maintenance treatment increased serum total protein and leptin levels and tended to increase serum cortisol levels (p=0.063 for addicts before vs. after methadone maintenance treatment). On the contrary, methadone maintenance treatment did not significantly affect serum soluble leptin receptor and adiponectin levels which remained lower relative to the control group. Serum resistin levels in addicts group after one year of methadone maintenance treatment still remained markedly increased being two-fold higher than in healthy subjects (Table 1).

Serum leptin levels positively correlated with body mass index in controls and addicts group after but not before methadone maintenance treatment (Table 2). Serum leptin levels correlated positively with total protein concentrations in addicts after one year of methadone maintenance treatment only (Table 2). No other significant relationship of serum leptin levels with other biochemical and hormonal parameters were found.

Discussion

The most important finding of our study is that circulating levels of adipose tissue-derived hormones in heroin addicts are markedly different from those of healthy subjects with comparable body weight. Serum leptin levels were significantly decreased in patients with heroin addiction and this decrease was normalized after one year of methadone maintenance treatment. Moreover, basal serum leptin concentrations in heroin addicts did not correlate with body mass index and methadone maintenance treatment restored this correlation. We suggest that the regulation of leptin production in heroin addicts appears to be relatively independent of body fat content. One possible factor that may lead to hypoleptinemia in the addicts is the decrease of cortisol levels detected in our group of drug users. Glucocorticoids are strong stimulators of leptin production and their deficiency can eventually result in decreased leptin levels (Dagogo-Jack *et al.* 2003, Wabitsch *et al.* 1996). Alternatively, leptin production could be influenced directly by heroin or its metabolites. However, to our best knowledge, no experimental or clinical data directly addressing this question are available. The experimental data rather suggest an inverse relationship showing that leptin administration attenuates acute food deprivation-induced relapse to heroin seeking (Shalev *et al.* 2001). Another possible factor in the regulation of serum leptin levels is the concentration of its major plasma binding protein – soluble leptin receptor (Křížová *et al.* 2002, 2003). Here we found significantly decreased soluble leptin receptor concentrations in the addict group. It remains to be elucidated whether lower soluble leptin receptor can lead to a decrease of serum leptin levels and which mechanism is possibly involved.

Adiponectin concentrations, which are normally increased in lean and decreased in obese subjects, positively correlate with insulin sensitivity (Weyer *et al.* 2001). Here we found significantly lower adiponectin concentrations in the addicts. In contrast to leptin, adiponectin concentration was not affected by methadone maintenance treatment. Interestingly, serum resistin levels were twofold higher in the addicts relative to healthy subjects. Both decreased adiponectin and increased resistin levels are commonly found in patients with insulin resistance (Havel 2002, Ukkola 2002). Insulin sensitivity was not directly assessed in this study, however, insulin concentrations were not different from the control group, suggesting no major perturbations in insulin sensitivity in the addicts. The changes of both adiponectin and resistin in the addicts thus appear to be unrelated to insulin sensitivity and could be directly caused by other factors including the activation of chronic inflammatory response.

In contrast to the normalization of leptin and cortisol levels after one year of methadone maintenance treatment neither resistin nor adiponectin concentrations were affected by methadone maintenance treatment. These results argue against direct and specific effect of methadone and/or heroin on resistin and adiponectin levels.

Since serum concentrations of adipocyte-derived hormones were markedly altered in patients with heroin addiction, it is important to consider possible patho-physiological consequences of such changes. Lower serum leptin levels could contribute both to disturbances of menstrual cycle in women with drug addiction and to immune dysfunction commonly found in these patients (Sanchez-Margalet *et al.* 2003). Recently, brain was identified as a significant source of circulating leptin (Eikelis *et al.* 2004). It is tempting to speculate that decreased leptin production in the brain rather than in the periphery could be responsible for lower serum leptin levels in drug addicts before methadone maintenance treatment.

Decreased adiponectin and increased resistin

levels could in concert lead to a decreased overall insulin sensitivity and increased tendency towards atherosclerosis. Further studies are necessary to assess whether and to what extent the changes of insulin sensitivity present in patients with drug addiction are induced by the changes in circulating adiponectin and resistin levels.

In conclusion, our study shows that circulating levels of adipose tissue-derived hormones in patients with heroin addiction significantly differ from those of healthy control subjects. One-year methadone maintenance treatment normalizes leptin, but not adiponectin or resistin concentrations. The clarification of pathophysiological consequences of altered circulating adipocyte-derived hormones in drug addicts requires further investigation.

Acknowledgements

Supported by grants of IGA MH CR No. 6663-3 and 6632-3 and 7429-3.

References

ARITA Y, KIHARA S, OUCHI N, TAKAHASHI M, MAEDA K, MIYAGAWA J, HOTTA K, SHIMOMURA I, NAKAMURA T, MIYAOKA K, KURIYAMA H, NISHIDA M, YAMASHITA S, OKUBO K, MATSUBARA K, MURAGUCHI M, OHMOTO Y, FUNAHASHI T, MATSUZAWA Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* **257**: 79-83, 1999.

BERG AH, COMBS TP, SCHERER PE: ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends Endocrinol Metab* **13**: 84-89, 2002.

CARTWRIGHT WS, SOLANO PL: The economics of public health: financing drug abuse treatment services. *Health Policy* **66**: 247-260, 2003.

DAGOGO-JACK S, UMAMAHESWARAN I, ASKARI H, TYKODI G: Leptin response to glucocorticoid occurs at physiological doses and is abolished by fasting. *Obes Res* **11**: 232-237, 2003.

EIKELIS N, LAMBERT G, WIESNER G, KAYE D, SCHLAICH M, MORRIS M, HASTINGS J, SOCRATOUS F, ESLER M: Extra-adipocyte leptin release in human obesity and its relation to sympathoadrenal function. *Am J Physiol* **286**: E744-E752, 2004.

HALUZÍK M, BOUDOVÁ L, NEDVÍDKOVÁ J, HALUZÍKOVÁ D, BARÁČKOVÁ M, BRANDEJSKÝ P, VILIKUS Z: Lower serum leptin concentrations in rugby players in comparison with healthy non-sporting subjects--relationships to anthropometric and biochemical parameters. *Eur J Appl Physiol Occup Physiol* **79**: 58-61, 1998.

HALUZÍK M, PAPEŽOVÁ M, NEDVÍDKOVÁ J, KÁBRT J: Serum leptin levels in patients with anorexia nervosa before and after partial refeeding, relationships to serum lipids and biochemical nutritional parameters. *Physiol Res* **48**: 197-202, 1999.

HALUZÍK M, PAŘÍZKOVÁ J, HALUZÍK MM: Adiponectin and its role in the obesity-induced insulin resistance and related complications. *Physiol Res* **53**: 123-129, 2004.

HAVEL PJ: Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol* **13**: 51-59, 2002.

KŘÍZOVÁ J, PAPEŽOVÁ H, HALUZÍKOVÁ D, PAŘÍZKOVÁ J, JISKRA J, KOTRLÍKOVÁ E, HAAS T, HALUZÍK M: Soluble leptin receptor levels in patients with anorexia nervosa. *Endocr Res* **28**: 199-205, 2002.

KŘÍZOVÁ J, SULKOVÁ S, BEDNAŘOVÁ V, KOTRLÍKOVÁ E, HALUZÍK M: Soluble leptin receptor levels in patients with chronic renal failure. *Physiol Res* **52**: 347-351, 2003.

MAFFEI M, HALAAS J, RAVUSSIN E, PRATLEY RE, LEE GH, ZHANG Y, FEI H, KIM S, LALLONE R, RANGANATHAN S, KERN PA, FRIEDMAN JM: Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* **1**: 1155-1161, 1995.

MOHS ME, WATSON RR, LEONARD-GREEN T: Nutritional effects of marijuana, heroin, cocaine, and nicotine. *J Am Diet Assoc* **90**: 1261-1267, 1990.

NESTLER EJ: Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* **2**: 119-128, 2001.

ROBLES RR, MARRERO CA, REYES JC, COLON HM, MATOS TD, FINLINSON HA, MUNOZ A, MELENDEZ M, SAHAI H: Risk behaviors, HIV seropositivity, and tuberculosis infection in injecting drug users who operate shooting galleries in Puerto Rico. *J Acquir Immune Defic Syndr Hum Retrovirol* **17**: 477-483, 1998.

SANCHEZ-MARGALET V, MARTIN-ROMERO C, SANTOS-ALVAREZ J, GOBERNA R, NAJIB S, GONZALEZ-YANES C: Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action. *Clin Exp Immunol* **133**: 11-19, 2003.

SAVAGE DB, SEWTER CP, KLENK ES, SEGAL DG, VIDAL-PUIG A, CONSIDINE RV, O'RAHILLY S: Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes* **50**: 2199-2202, 2001.

SHALEV U, YAP J, SHAHAM Y: Leptin attenuates acute food deprivation-induced relapse to heroin seeking. *J Neurosci* **21** (RC129): 1-5, 2001.

STEPPAN CM, BAILEY ST, BHAT S, BROWN EJ, BANERJEE RR, WRIGHT CM, PATEL HR, AHIMA RS, LAZAR MA: The hormone resistin links obesity to diabetes. *Nature* **409**: 307-312, 2001.

UKKOLA O: Resistin - a mediator of obesity-associated insulin resistance or an innocent bystander? *Eur J Endocrinol* **147**: 571-574, 2002.

VETULANI J: Drug addiction. Part II. Neurobiology of addiction. *Pol J Pharmacol* **53**: 303-317, 2001.

WABITSCH M, JENSEN PB, BLUM WF, CHRISTOFFERSEN CT, ENGLARO P, HEINZE E, RASCHER W, TELLER W, TORNQVIST H, HAUNER H: Insulin and cortisol promote leptin production in cultured human fat cells. *Diabetes* **45**: 1435-1438, 1996.

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.

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.

Reprint requests

Martin Haluzík, MD, PhD, Third Department of Medicine, First Faculty of Medicine, Charles University, U nemocnice 1, 128 08, Prague 2, Czech Republic, E-mail: mhalu@lf1.cuni.cz