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# Selected Adipokines - Plasma Concentrations and Adipose Tissue Expressions during 24-Hour Lipid Infusion in Healthy Men

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**Summary**

Our aim was to assess the reaction of TNFα, resistin, leptin and adiponectin to lipid infusion. Eight healthy subjects underwent a 24-hour lasting infusion of lipid emulsion. Plasma concentrations and expressions of selected cytokines in subcutaneous fat were measured. TNFα plasma concentration did not change during the first 4 hours of hypertriglyceridemia, but a significant increase after 24 hours was detected ($p<0.001$ for 0; 30; 240 min vs. 24 h). Plasma concentration of resistin significantly increased at 30 min of infusion and remained elevated ($p<0.01$ for 0 min vs. 30; 240 min; $p<0.001$ for 0 min vs. 24 h). Plasma concentrations of leptin and adiponectin did not show any significant changes. Although the expression of resistin in the subcutaneous adipose tissue tended to increase, the change was not significant. Expressions of TNFα, leptin and adiponectin were unaffected. In conclusions, our results indicate that acutely induced hyperlipidemia could influence the secretion of TNFα and resistin.

**Key words**

Resistin • TNFα • Leptin • Adiponectin • Insulin resistance • NEFA • Triglycerides • Lipotoxicity

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## Introduction

Hyperlipidemia of either dietary origin or caused by lipid infusion promotes insulin resistance (IR) in rodents and humans (Boden and Chen 1995, Griffin *et al.* 1999, Leung *et al.* 2004). Pathophysiological mechanisms underlying the association between the elevation of plasma non-esterified fatty acids (NEFA) and IR have not been fully determined – for the evidence of interplay between glucose and NEFA see review by Cahová *et al.* (2007). Some of the pathways that lead to IR might include following cytokines produced by adipose tissue: TNFα, resistin, leptin and adiponectin.

TNFα is a promising candidate for mediating IR, although most of it comes from other sources than adipose tissue (Fain *et al.* 2003, Gabriely *et al.* 2002). Plasma concentrations of TNFα are related to obesity and IR (Hotamisligil *et al.* 1993). TNFα knock-out mice are more insulin sensitive than controls and there is the evidence that TNFα may directly interfere with insulin signaling by inhibiting phosphorylation of the insulin receptor (Hotamisligil *et al.* 1994). It impairs human preadipocyte differentiation, in mature adipocytes it decreases the activity of markers of differentiation, (Petruschke and Hauner 1993) and increases their apoptosis (Prins *et al.* 1997). TNFα has been shown to mediate NEFA-induced IR in 3T3-L1 adipocytes *in vitro* (Nguyen *et al.* 2005)

Resistin was named for its putative role in mediating IR in obesity. In mice, it is a product of an adipocyte-specific gene whose expression is down-regulated by rosiglitazone (Arner 2005). Serum levels of resistin were found to be elevated in rodent models of obesity and diabetes implicating a dysregulation of resistin in these disease states (Arner 2005). In humans,

however, the role of resistin is unclear. The human homologue of resistin is only expressed at very low levels in adipose tissue and in circulating monocytes, and the reports on its function are controversial (Janke *et al.* 2002, Savage *et al.* 2001).

Leptin is a cytokine secreted mainly by adipose tissue, its circulating levels are in direct proportion to adipose tissue mass and nutritional status (Maffei *et al.* 1995). Therefore, it is a likely candidate for mediating insulin sensitivity. It influences the human energy balance by altering energy intake and expenditure (Hukshorn and Saris 2004). It was shown in experimental animals that leptin infusion could prevent IR induced by acute lipid infusion (Dube *et al.* 2007).

Consistent inverse association between adiponectin and IR as well as the pro-inflammatory state has been established (Nedvídková *et al.* 2005). Several mechanisms of its metabolic effects have been described (Kadowaki *et al.* 2006, Whitehead *et al.* 2006): induction of glucose uptake and NEFA oxidation in muscle, increased insulin sensitivity and NEFA oxidation and reduced glucose output and NEFA influx in liver.

Our aim was to assess the reaction of TNFα, resistin, leptin and adiponectin to lipid infusion. We have evaulated both the plasma concentrations and expression of their genes in the abdominal subcutaneous adipose tissue in healthy humans.

## Subjects and methods

*Subjects*

We examined eight young healthy males. All of them were euthyroid and none had a concomitant disease. They were not taking any drugs, and none had a family history of diabetes. Clinical characteristics are in Table 1. The study was conducted after approval by local ethics committee. Informed consent was obtained from all the individuals after the purpose, nature, and potential risks of the study had been explained.

*Experimental protocol*

The subjects were instructed to adhere to their ordinary lifestyle, to avoid changes in food intake, alcohol consumption, and vigorous exercise on the day before examination. They were examined after overnight fasting with only tap water allowed *ad libitum*. The study always started between 7:45-8:00 AM. Subjects were excluded from the study if the weight change was more than 3 kg in 6 months.

**Table 1.** Characteristics of the study group.

| | |
|---|---|
| *n* | 8 |
| *Age (years)* | 25.8±3.7 |
| *Weight (kg)* | 83.4±4.7 |
| *Body mass index (kg.m$^{-2}$)* | 25±1.7 |
| *Waist circumference (cm)* | 88.5±5.0 |
| *Fasting blood glucose (mmol.l$^{-1}$)* | 4.35±0.7 |
| *Blood glucose at 120 min of OGTT (mmol.l$^{-1}$)* | 4.55±0.7 |
| *Fasting IRI (mIU.l$^{-1}$)* | 4.50±2.85 |
| *HbA1c (%) - according to IFCC* | 2.58±0.61 |
| *Albuminuria (μg.min$^{-1}$)* | 2.52±2.6 |
| *Serum creatinine (μmol.l$^{-1}$)* | 87±10.5 |
| *Total cholesterol (mmol.l$^{-1}$)* | 4.24±0.73 |
| *Triglycerides (mmol.l$^{-1}$)* | 0.87±0.30 |
| *HDL-cholesterol (mmol.l$^{-1}$)* | 1.45±0.34 |
| *LDL-cholesterol (mmol.l$^{-1}$)* | 2.48±0.55 |

Data are expressed as mean ± SD (IFCC, International Federation of Clinical Chemistry and Laboratory Medicine).

A 24-h infusion of lipid emulsion (Intralipid 20 %; Fresenius Kabi AB, Uppsala, Sweden) was administered through teflon cannula in the antecubital vein. Intralipid 20 % contained soya oil 200 g, egg lecithin 12 g and glycerol 22 g per 1 liter. The speed was set to 125 ml.h$^{-1}$ for the first 4 h, and then until the 24$^{th}$ hour to a constant rate that was calculated to achieve a total dose of 3 g of fat.kg body weight$^{-1}$.d$^{-1}$ This two-step setting of infusion was used to achieve both maximum effect and to stay within the clinically allowed boundaries. Thirty minutes before blood sampling a second cannula was inserted retrogradely into a wrist vein for blood collection. To assess the plasma levels of selected adipokines, blood samples were taken at 0 min, 30 min, 240 min and 24 h of the infusion. Thirty minutes before taking each sample the hand was placed in a heated (65 °C) box in order to achieve venous blood arterialization. Needle biopsy of abdominal subcutaneous adipose tissue was performed before (0 min), and at the 4$^{th}$ and 24$^{th}$ hour of the lipid infusion. Under local anesthesia (1 % trimecain in a field block pattern) an incision (3-4 mm) was made through the skin at the lower abdomen and a subcutaneous fat specimen (200 mg) was obtained by needle (Braunüle MT, no.4; B. Braun, Melsungen, Germany) aspiration. Different place and incision was used each time, but the same depth in the adipose tissue mass when performing the needle aspiration was attempted. The samples were washed in

NaCl 0.9 % solution, immediately frozen by liquid nitrogen and stored at –80 ºC until used for RNA extraction.

*Analytical procedures*

*Infusion monitoring*

Plasma glucose concentrations were measured on a Beckman analyzer (Beckman Instruments Inc, Fullerton, CA, USA) using the glucose oxidase method. Hemoglobin A1c was measured by fully automated analyzer Tosoh HLC-723 G7 (Tosoh Corporation, Tokyo, Japan). Method was calibrated to IFCC reference procedure (Mosca *et al.* 2007). Immunoreactive insulin (IRI) was determined by radioimmunoassay (Insulin IRMA kit, IMMUNOTECH as, Prague, Czech Republic). Plasma concentrations of NEFA were estimated by Free fatty acids, Half-micro test (Roche Diagnostics GmbH, Penzberg, Germany), whereas plasma concentrations of triglycerides (TG) by enzymatic assay (BIO-LA-TEST, PLIVA-Lachema, Brno, Czech Republic).

*Adipokine assessment in plasma*

Plasma concentrations of TNFα were measured by immunoassay (Human TNF-α UltraSensitive; BioSource Int., Camarillo, CA, USA) and the detection limit was <0.09 pg/ml, intra-assay and inter-assay coefficients of variation (CV) of 5.3 to 6.7 % and 8.2 to 9.7 %, respectively). Plasma concentrations of resistin were determined using a Human Resistin ELISA kit (BioVendor Lab. Med. Inc., Brno, Czech Republic) the detection limit was 0.033 ng/ml, intra-assay and inter-assay CV of 2.8 to 3.4 % and 5.1 to 6.9 %, respectively. Pplasma concentrations of leptin were measured by a Human Leptin ELISA kit (BioVendor Lab. Med. Inc., Brno, Czech Republic), the detection limit was 0.5 ng/ml, intra-assay and inter-assay CV of 3 to 7.5 % and 3.2 to 9.2 %, respectively. Plasma concentrations of adiponectin were determined using a Human Adiponectin ELISA kit (BioVendor Lab. Med. Inc., Brno, Czech Republic) the detection limit was 210 ng/ml, intra-assay and inter-assay CV of 6.4 to 7 % and 7.3 to 8.2 %, respectively (all according to manufacturers instructions).

*Assessment of expressions in adipose tissue*

Expression of each adipokine was analyzed by the real-time PCR method. Subcutaneous adipose tissue (100 mg) was excised from the biopsy, and homogenized in 1 ml of a QIAzol Lysis Reagent (Guanidin Thiocyanate – Phenol solution) for 2 min. The RNA was isolated from the liquid nitrogen frozen biopsy using the RNeasy Lipid Tissue Mini Kit (QIAGEN, Valencia, CA, USA) and QIAzol Lysis Reagent (QIAGEN, Valencia, CA, USA). Possible contamination of RNA with genomic DNA remains was taken off by DNase digestion (RNase-free DNase Set; QIAGEN, Valencia, CA, USA). The cDNA was synthesized using a recombinant Omniscript Reverse Transcriptase (QIAGEN, Valencia, CA, USA), Ribonuclease Inhibitor from human placenta (SIGMA, St. Louis, MO, USA), and $(dT)_{16}$ oligonucleotides.

The real-time PCR procedure itself has been carried out on the DNA Engine Opticon 2 System (MJ Research, Waltham, MA, USA). HotStar Taq DNA polymerase and SYBR Green fluorescent dye (QuantiTec SYBR Green PCR Kit, QIAGEN, Valencia, CA, USA) were used for the RT-PCR reaction. To eliminate the influence of primer dimmers, negative controls were used. The human gene cyclophilin was used as a reference. Primers used are shown in Table 2. The data were processed by Q-gene 96 software.

*Statistical analysis*

The time profile was evaluated using a repeated measures ANOVA model consisting of the time and subject factors. To evaluate the differences between basal values and individual stages of the time profile, the ANOVA testing was followed by least significant difference multiple comparisons. A probability level of $p<0.05$ was considered as statistically significant in all statistical tests. Due to non-Gaussian data distribution in most of the dependent variables, these data underwent a power transformation to attain distributional symmetry and constant variance. Non-homogeneities were detected using residual diagnostics. The experimental points showing absolute values of studentized residuals greater than 3 were excluded from the analysis. With the exception of serum leptin levels (6.3 %), the proportions of such data never exceed 5 % of the total number. Statistical software Statgraphics Plus v. 5.1 from Manugistics (Rockville, MD, USA) was used for calculations.

## Results

Our primary goal was to assess the reaction of selected adipokines to acute lipid infusion and the following results were obtained. TNFα plasma concentration did not change during the first 4 h of hypertriglyceridaemia, but a significant increase after 24 h

**Table 2.** Primers used for RT-PCR of the subcutaneous adipose tissue samples taken during the lipid infusion.

| Gene | accession number | forward primer | reverse primer |
|---|---|---|---|
| *Adiponectin* | XM_290602 | HACRP30-F: 5′-GGT TCA ATG GCT TGT TTG C -3′ | HACRP30-R: 5′-TCA TCC CAA GCT GAT TCT G-3′ |
| *Leptin* | NM_000230 | Hleptin-F: 5′-CCC TAA GCC TCC TTT TGC T-3′ | Hleptin-R: 5′-GCT AAG AGG GGA CAA GAC A-3′ |
| *TNFα* | X02910 X02159 | HTNFa-F: 5′-CTA TCT GGG AGG GGT CTT C-3′ | HTNFa-R: 5′-TTG GGA AGG TTG GAT GTT C -3′ |
| *Resistin* | AY207314 | HRETN-F: 5′-ATA AGC AGC ATT GGC CTG G-3′ | HRETN-R: 5′-TGG CAG TGA CAT GTG GTC T-3′ |
| *Cyclophilin* | XM_090070 | HCLPNa-F: 5′-CAA ATG CTG GAC CCA ACA CA -3′ | HCLPNa-R: 5′-TGC CAT CCA ACC ACT CAG TC-3′ |

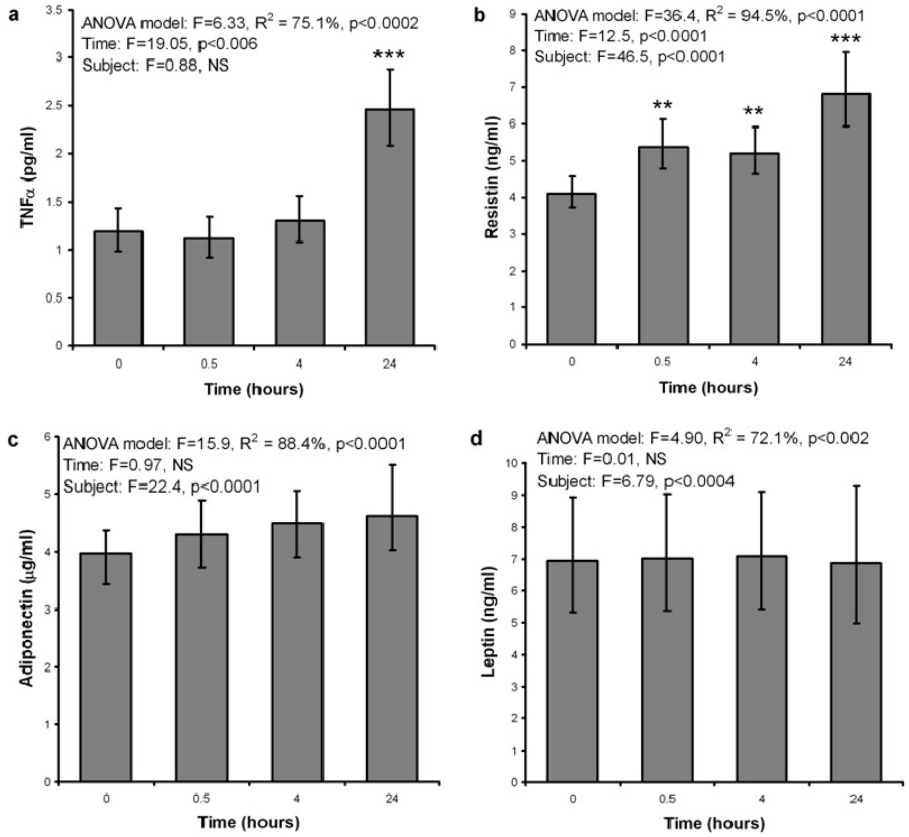


**Fig. 1a-d.** Plasma levels of selected adipokines during 24-hour lipid infusion. The bars with error bars represent re-transformed means with their 95 % confidence intervals. F in the embedded tables represents Fisher's statistics for individual factors. $R^2$ (squared correlation coefficient of the linear model) symbolize the proportion of the total variability in the dependent variable, which is explained by the ANOVA model. Asterisks symbolize significant differences between individual stages of the time profiles and basal values (** for $p<0.01$, *** for $p<0.001$) as detected by least significant difference multiple comparisons.

was detected ($p<0.001$ for 0; 30; 240 min vs. 24 h; Fig. 1a). The expression of TNFα in subcutaneous adipose tissue did not change (Fig. 2a). Plasma concentration of resistin significantly increased at 30 min of infusion and remained elevated throughout the 24 h ($p<0.01$ for 0 min vs. 30; 240 min; $p<0.001$ for 0 min vs. 24 h; Fig. 1b). The expression of resistin in the subcutaneous adipose tissue tended to increase, but the change was not significant (Fig. 2b). Plasma concentrations of leptin and adiponectin (Fig. 1c-d) did not show any significant changes and their expressions were not significantly altered.

To validate the effectiveness of lipid infusion,

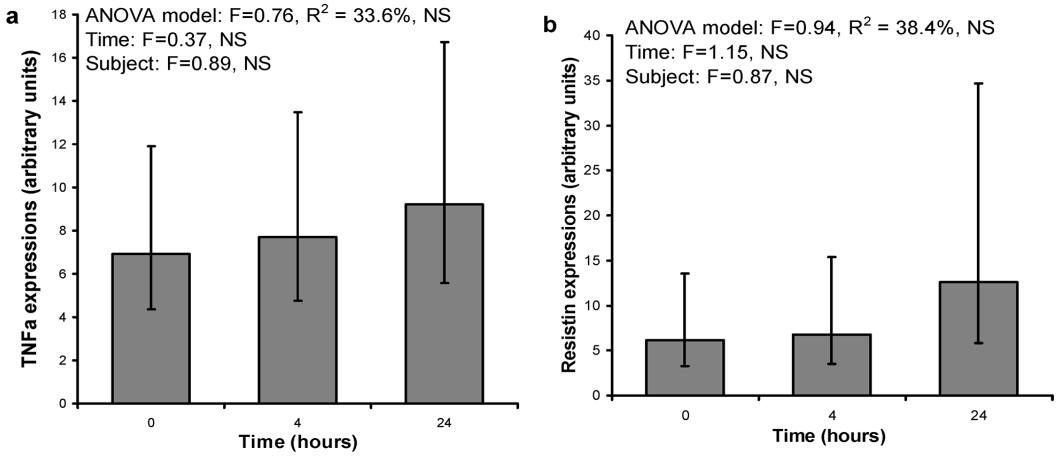


**Fig. 2a,b.** Expressions of selected adipokines in subcutaneous abdominal adipose tissue during 24-hour lipid infusion. The bars with error bars represent re-transformed means with their 95 % confidence intervals (expression of selected cytokine related to expression of cyclophilin). The changes in time were assessed by ANOVA and are not statistically significant.

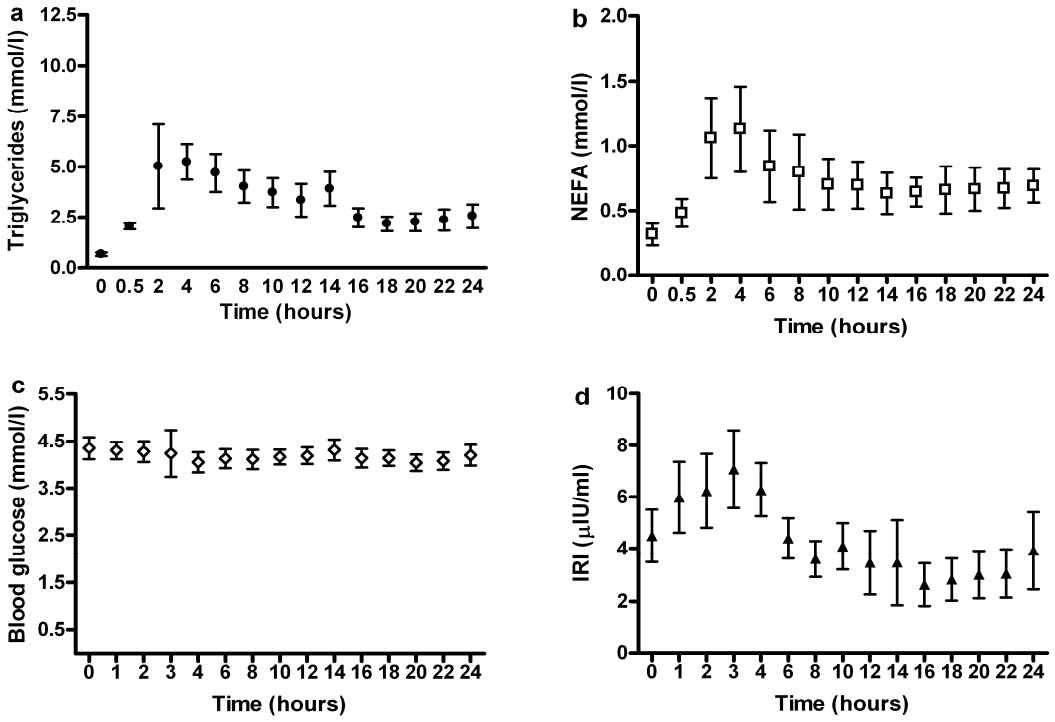


**Fig. 3a-d.** Concentrations of serum triglycerides (●), non-esterified free fatty acids (NEFA, □), blood glucose (◇) and immunoreactive insulin (IRI,▲) during the 24-hour lipid infusion. Data are shown as means with their 95 % confidence intervals.

plasma levels of TG, NEFA, glucose and IRI were monitored. TG increased almost 8-fold and NEFA about 3.5-fold in the 4th hour and they remained elevated until the end of infusion at more than 3-fold and 2-fold, respectively (Figs 3a and 3b). Blood glucose was stable throughout the 24 h (Fig. 3c). After initial modest rise, IRI concentrations were also stable (Fig. 3d). Cytokine plasma concentrations did not correlate with IRI concentrations (data not shown).

## Discussion

We have characterized the plasma levels of adipose tissue cytokines and their expression in adipose tissue during pharmacologically-induced hyperlipidemia. This was achieved by an intravenous lipid infusion, in which the effectiveness in rise of plasma TG and NEFA was verified. We have decided not to use heparin infusion alongside because heparin as a co-factor of a lipoprotein lipase would decrease plasma TG concentrations and

increase plasma NEFA concentrations, which was not our aim. It could also add further bias through possible unknown effects. Since heparin was used in some further cited experiments (Garcia-Lorda *et al.* 2003, Nisoli *et al.* 2000, Yang *et al.* 2005), this sole fact could be the reason for differences in results obtained.

Our finding of an increase in TNFα plasma concentration during lipid infusion is novel. It is partly in accordance with the report of Nisoli *et al.* (2000) who found the increase in TNFα gene expression in subcutaneous gluteal fat tissue after a 5-h intralipid/ heparin infusion. In our results, the expressions in adipose tissue showed only a tendency to increase but not a significant rise. This can be explained by the differences in expressions in different fat depots. Both in human (Fain *et al.* 2003) and rodent (Gabriely *et al.* 2002) studies, it has been reported that production of both of resistin and TNFα is much higher in visceral than in subcutaneous fat. Moreover, most of the free circulating TNFα comes from circulating monocytes and significant amounts of resistin, although originally reported as adipocyte-specific (Kim *et al.* 2001), might be of the same source (Savage *et al.* 2001).

During lipid infusion, plasma concentrations of resistin have increased significantly. This is in accordance with the reports from animal models (Yang *et al.* 2005) where intralipid/heparin infusion induced hepatic and peripheral IR and was associated with elevated plasma resistin levels. In human, it is a novel observation. Since the increase in expression of resistin mRNA in subcutaneous adipose tissue was not significant, we can conclude that this adipocyte depot is not responsible for its increased plasma concentration.

Plasma concentration of leptin did not show any significant changes and its expression was not significantly altered. Although most studies have not shown direct effect of lipid infusion on leptin, as when consistent release from adipose tissue during lipid without heparin infusion was reported (Samra *et al.* 1998), some other results were also obtained: no change in plasma levels together with increase in leptin RNA expression in adipocytes in gluteal region during lipid and heparin infusion (Nisoli *et al.* 2000) and modest decline of its plasma levels during lipid and heparin infusion (Garcia-Lorda *et al.* 2003).

Response of adiponectin to acute lipid load was also not statistically significant. The same result of unchanged plasma concentrations was observed during intralipid/heparin infusion (Krzyzanowska *et al.* 2007).

Lipid infusion resulting in a subsequent increase in NEFA plasma concentrations is a well-known method used for inducing IR (Boden and Chen 1995, Griffin *et al.* 1999, Leung *et al.* 2004). Therefore, although we have not measured insulin sensitivity, we suggest that the changes we have observed might be implicated in the pathogenesis of lipid-induced IR. Our results are in agreement with a theory that TNFα is implicated in NEFA-induced IR as shown *in vitro* (Nguyen *et al.* 2005). For resistin it is an unclear result because it is often found not to be associated with IR (Utzschneider *et al.* 2005), although the studies are usually limited to correlations between resistin and various measures of metabolic syndrome. Rather exception is a report of Sheng *et al.* (2008) where overexpression of resistin in human hepatocytes induced IR most likely by blocking insulin signal transduction pathways of PI-3K/Akt and of CAP/c-cbl.

We are aware that there are limits to our study. Increase in plasma lipid concentrations by intravenous infusion certainly differs from dietary and lifestyle induced hyperlipidemia observed in real life. Small number of subjects examined might have prevented us from seeing some other possibly significant results and therefore the value of "negative" results is limited. In addition, the lack of placebo control, which was not done due to the potential troublesome interpretation of a 24-h long fasting, to some extent limits the reliability of "positive" results. Circadian variations were partly assessed by 24-h duration. Glycerol (a component of intralipid emulsion) fortunately does not affect insulin secretion (Boden and Chen 1999, Pelkonen *et al.* 1968), glucose effectiveness (Hawkins *et al.* 2003) or insulin sensitivity (Ferrannini *et al.* 1983). However, the effect of glycerol on resistin and TNFα can not be excluded. Resistin could also be affected by hyperinsulinemia, although this observation was made at several-fold higher insulin concentrations (Krušinová *et al.* 2007) than observed during our lipid infusion. We have also found significant inter-individual and intra-individual differences of the mRNA content in adipose tissue samples for each adipokine. We must, however, note that this could partly be due to different adipocytes/stroma-vascular cells ratio in each sample. In future studies, this can be assessed by separation of these fractions by collagenase tissue digestion (Rodbell 1963).

In conclusion, our results indicate that acutely induced hyperlipidemia could increase the secretion of TNFα and resistin. This finding supports the hypothesis

that these adipokines could be involved in the pathogenesis of lipid-induced IR.

## Conflict of Interest

There is no conflict of interest.

## Acknowledgements

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## References

ARNER P: Resistin: yet another adipokine tells us that men are not mice. *Diabetologia* **48**: 2203-2205, 2005.

BODEN G, CHEN X: Effects of fat on glucose uptake and utilization in patients with non-insulin-dependent diabetes. *J Clin Invest* **96**: 1261-1268, 1995.

BODEN G, CHEN X: Effects of fatty acids and ketone bodies on basal insulin secretion in type 2 diabetes. *Diabetes* **48**: 577-583, 1999.

CAHOVÁ M, VAVŘÍNKOVÁ H, KAZDOVÁ L: Glucose-fatty acid interaction in skeletal muscle and adipose tissue in insulin resistance. *Physiol Res* **56**: 1-15, 2007.

DUBE JJ, BHATT BA, DEDOUSIS N, BONEN A, O'DOHERTY RM: Leptin, skeletal muscle lipids, and lipid-induced insulin resistance. *Am J Physiol* **293**: R642-R650, 2007.

FAIN JN, CHEEMA PS, BAHOUTH SW, LLOYD HILER M: Resistin release by human adipose tissue explants in primary culture. *Biochem Biophys Res Commun* **300**: 674-678, 2003.

FERRANNINI E, BARRETT EJ, BEVILACQUA S, DEFRONZO RA: Effect of fatty acids on glucose production and utilization in man. *J Clin Invest* **72**: 1737-1747, 1983.

GABRIELY I, MA XH, YANG XM, ATZMON G, RAJALA MW, BERG AH, SCHERER P, ROSSETTI L, BARZILAI N: Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? *Diabetes* **51**: 2951-2958, 2002.

GARCIA-LORDA P, NASH W, ROCHE A, PI-SUNYER FX, LAFERRERE B: Intralipid/heparin infusion suppresses serum leptin in humans. *Eur J Endocrinol* **148**: 669-676, 2003.

GRIFFIN ME, MARCUCCI MJ, CLINE GW, BELL K, BARUCCI N, LEE D, GOODYEAR LJ, KRAEGEN EW, WHITE MF, SHULMAN GI: Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. *Diabetes* **48**: 1270-1274, 1999.

HAWKINS M, TONELLI J, KISHORE P, STEIN D, RAGUCCI E, GITIG A, REDDY K: Contribution of elevated free fatty acid levels to the lack of glucose effectiveness in type 2 diabetes. *Diabetes* **52**: 2748-2758, 2003.

HOTAMISLIGIL GS, SHARGILL NS, SPIEGELMAN BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* **259**: 87-91, 1993.

HOTAMISLIGIL GS, BUDAVARI A, MURRAY D, SPIEGELMAN BM: Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-alpha. *J Clin Invest* **94**: 1543-1549, 1994.

HUKSHORN CJ, SARIS WH: Leptin and energy expenditure. *Curr Opin Clin Nutr Metab Care* **7**: 629-633, 2004.

JANKE J, ENGELI S, GORZELNIAK K, LUFT FC, SHARMA AM: Resistin gene expression in human adipocytes is not related to insulin resistance. *Obes Res* **10**: 1-5, 2002.

KADOWAKI T, YAMAUCHI T, KUBOTA N, HARA K, UEKI K, TOBE K: Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* **116**: 1784-1792, 2006.

KERN PA, SAGHIZADEH M, ONG JM, BOSCH RJ, DEEM R, SIMSOLO RB: The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* **95**: 2111-2119, 1995.

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.

KRUŠINOVÁ E, KLEMENTOVÁ M, KOPECKÝ J, WOHL P, KAZDOVÁ L, MLEJNEK P, PRAVENEC M, HILL M, PELIKÁNOVÁ T: Effect of acute hyperinsulinaemia with and without angiotensin II type 1 receptor blockade on resistin and adiponectin concentrations and expressions in healthy subjects. *Eur J Endocrinol* **157**: 443-449, 2007.

KRZYZANOWSKA K, MITTERMAYER F, KRUGLUGER W, RODEN M, SCHERNTHANER G, WOLZT M: Adiponectin concentrations increase during acute FFA elevation in humans treated with rosiglitazone. *Horm Metab Res* **39**: 769-772, 2007.

LEUNG N, SAKAUE T, CARPENTIER A, UFFELMAN K, GIACCA A, LEWIS GF: Prolonged increase of plasma non-esterified fatty acids fully abolishes the stimulatory effect of 24 hours of moderate hyperglycaemia on insulin sensitivity and pancreatic beta-cell function in obese men. *Diabetologia* **47**: 204-213, 2004.

MAFFEI M, HALAAS J, RAVUSSIN E, PRATLEY RE, LEE GH, ZHANG Y, FEI H, KIM S, LALLONE R, RANGANATHAN S, et al.: 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.

MOSCA A, GOODALL I, HOSHINO T, JEPPSSON JO, JOHN WG, LITTLE RR, MIEDEMA K, MYERS GL, REINAUER H, SACKS DB, WEYKAMP CW: Global standardization of glycated hemoglobin measurement: the position of the IFCC Working Group. *Clin Chem Lab Med* **45**: 1077-1080, 2007.

NEDVÍDKOVÁ J, SMITKA K, KOPSKÝ V, HAINER V: Adiponectin, an adipocyte-derived protein. *Physiol Res* **54**: 133-140, 2005.

NGUYEN MT, SATOH H, FAVELYUKIS S, BABENDURE JL, IMAMURA T, SBODIO JI, ZALEVSKY J, DAHIYAT BI, CHI NW, OLEFSKY JM: JNK and tumor necrosis factor-alpha mediate free fatty acid-induced insulin resistance in 3T3-L1 adipocytes. *J Biol Chem* **280**: 35361-35371, 2005.

NISOLI E, CARRUBA MO, TONELLO C, MACOR C, FEDERSPIL G, VETTOR R: Induction of fatty acid translocase/CD36, peroxisome proliferator-activated receptor-gamma2, leptin, uncoupling proteins 2 and 3, and tumor necrosis factor-alpha gene expression in human subcutaneous fat by lipid infusion. *Diabetes* **49**: 319-324, 2000.

PELKONEN R, MIETTINEN TA, TASKINEN MR, NIKKILA EA: Effect of acute elevation of plasma glycerol, triglyceride and FFA levels on glucose utilization and plasma insulin. *Diabetes* **17**: 76-82, 1968.

PETRUSCHKE T, HAUNER H: Tumor necrosis factor-alpha prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. *J Clin Endocrinol Metab* **76**: 742-747, 1993.

PRINS JB, NIESLER CU, WINTERFORD CM, BRIGHT NA, SIDDLE K, O'RAHILLY S, WALKER NI, CAMERON DP: Tumor necrosis factor-alpha induces apoptosis of human adipose cells. *Diabetes* **46**: 1939-1944, 1997.

RODBELL M: Metabolism of isolated fat cells. *J Biol Chem* **239**: 375-380, 1963.

SAMRA JS, GILES SL, SUMMERS LK, EVANS RD, ARNER P, HUMPHREYS SM, CLARK ML, FRAYN KN: Peripheral fat metabolism during infusion of an exogenous triacylglycerol emulsion. *Int J Obes Relat Metab Disord* **22**: 806-812, 1998.

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.

SHENG CH, DI J, JIN Y, ZHANG YC, WU M, SUN Y, ZHANG GZ: Resistin is expressed in human hepatocytes and induces insulin resistance. *Endocrine* **33**: 135-143, 2008.

UTZSCHNEIDER KM, CARR DB, TONG J, WALLACE TM, HULL RL, ZRAIKA S, XIAO Q, MISTRY JS, RETZLAFF BM, KNOPP RH, KAHN SE: Resistin is not associated with insulin sensitivity or the metabolic syndrome in humans. *Diabetologia* **48**: 2330-2333, 2005.

WHITEHEAD JP, RICHARDS AA, HICKMAN IJ, MACDONALD GA, PRINS JB: Adiponectin – a key adipokine in the metabolic syndrome. *Diabetes Obes Metab* **8**: 264-280, 2006.

YANG G, LI L, FANG C, ZHANG L, LI Q, TANG Y, BODEN G: Effects of free fatty acids on plasma resistin and insulin resistance in awake rats. *Metabolism* **54**: 1142-1146, 2005.