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Profiling of adipokine secretion by adipose tissue in transgenic mice with homotopic overexpression of adiponectin

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Background and aims: We have generated a transgenic (Tg) mouse model allowing persistent and moderate overexpression of native adiponectin (ApN) targeted to white adipose tissue [the transgene being placed under control of aP2 (fatty-acid binding protein) promoter]. Adiposity was reduced in aging mice fed a high-sucrose diet and this anti-obesity effect favored enhanced insulin sensitivity and improved lipid profile. APN appears to be a potent regulator of other adipokines. We took advantage of this unique *in vivo* model to study the influence of APN on adipokine secretion profiling. In order to investigate the early and specific effects of APN, mice were studied before any significant decrease of fat mass or glycemia and circulating lipids occurred.
Materials and methods: 10-wk-old Tg mice receiving a high-sucrose-diet were compared to wild-type (WT) littermates. Subcutaneous adipose tissue was fractionated into adipocytes (A) and stromal-vascular (SV) cells (i.e. non-fat cells that contain macrophages), which were then cultured for 8 h. Medium was screened by medium-scale protein arrays allowing the simultaneous detection of 144 cytokines. Gene expression of some adipokines was measured by RTQ-PCR.
Results: As expected, ApN expression was much higher (30-fold) in A than in SV cells of WT mice, while leptin was virtually undetectable in the SV fraction. ApN was overexpressed in both cellular fractions of Tg mice, in line with the fact that aP2 is co-expressed in adipocytes and macrophages (but at a much lower extent in the latter cells). By using cytokine antibody arrays, we detected more than 40 cytokines secreted by each cellular fraction of WT and Tg mice. Profiling of adipocyte secretory products showed that 9 adipokines were less secreted by Tg than WT mice: one chemokine (regulated upon activation normal T-cell expressed and secreted, RANTES), three pro-inflammatory cytokines (IL-6, IL-17B, IL-21), two extracellular matrix remodeling-/growth arrest-factors and three hematopoietic growth factors (one for megakaryocytes, thrombopoietin; the other two for granulocytes and/or macrophages, GM-CSF/ G-CSF). Profiling of SV cell products showed that 13 peptides were differentially secreted between Tg and WT mice, with usually stronger genotype differences than those observed in adipocytes. Thus, secretion of 12 peptides was downregulated, while one was upregulated in Tg mice. Hyposecreted factors include five adipokines that were also reduced in adipocytes (RANTES, IL-6 and the 3 hematopoietic growth factors) together with another chemokine, four other pro-inflammatory cytokines/ factors (comprising two adhesion molecules), one receptor for an angiogenic factor (VEGF R1), and one factor involved in lipid metabolism (fatty acid translocase). We also found that the secretion of one anti-inflammatory factor (IL-R4, which is an IL1-receptor family member) was increased by SV cells of Tg mice.
Conclusion: At the onset of overexpression, ApN reduced the secretion of 9 to 12 inflammatory adipokines in each cellular fraction of adipose tissue in Tg mice, while upregulating the secretion of one anti-inflammatory factor. Thus, ApN induced a shift of the immune balance in both adipocytes and SV cells toward a less inflammatory phenotype, with a greater effect on SV cells. These adipokines, which are newly identified as downstream targets of ApN *ex vivo*, may have therapeutic potential for the management of the metabolic syndrome.

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Regulation of the expression of angiopoietin-like protein 4 mRNA in diabetic mice

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Background and aims: Angiopoietin-like protein 4 (Angptl4) is mainly secreted from the liver and adipose tissue. It inhibits lipoprotein lipase activity, thereby causing an increase in the serum triglyceride level. The expression and plasma levels of Angptl4 are increased by fasting and decreased by feeding. We have recently shown that insulin negatively regulates Angptl4 mRNA expression in 3T3-L1 adipocytes, suggesting that insulin plays an important role in the regulation of Angptl4 expression. In this study, we aim to elucidate the regulation of Angptl4 expression in diabetic states.
Material and methods: Type 1 diabetes was induced in C57BL/6 mice by treating with streptozotocin (STZ). As a model of type 2 diabetes, the mice were fed with a high-fat diet (HFD) for 18 weeks. We determined Angptl4 mRNA expression by using a quantitative real-time PCR method and compared it with 36B4 mRNA expression.
Results: When compared with the normal mice, the STZ diabetic mice showed significantly higher serum glucose and triglyceride levels. The level of Angptl4 mRNA expression in the STZ diabetic mice had increased by 2.23 ± 0.47 fold in the liver ($p < 0.05$), 2.00 ± 0.37 fold in white adipose tissue (WAT) ($p < 0.05$), and 1.79 ± 0.26 fold in brown adipose tissue (BAT) ($p < 0.05$) when compared with the corresponding values in normal mice. Furthermore, this effect was attenuated by the administration of insulin. In the HFD diabetic mice, a significant increase in insulin resistance and triglyceride level was observed at 18 weeks. The level of Angptl4 mRNA expression in the HFD diabetic mice was increased by 2.15 ± 0.30 fold in the liver ($p < 0.005$), 1.68 ± 0.27 fold in WAT ($p < 0.05$), and 1.77 ± 0.31 fold in BAT ($p < 0.05$) when compared with the corresponding values in the normal chow-fed mice.
Conclusion: Thus, the level of Angptl4 mRNA expression was increased in both the insulin-deficient and insulin-resistant diabetic states, thus indicating that insulin negatively regulates Angptl4 mRNA expression in the liver and adipose tissue of diabetic mice.

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N^{ε}-(Carboxymethyl)lysine trapping in adipose tissue in obesity: implications for obesity-associated changes in adipokine expression

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Background and aims: Obesity is linked to a wide variety of pathophysiological conditions. Dysregulated production of adipokines is associated with obesity and provides a link between obesity and obesity-related complications. Factors that lead to a dysregulated production of adipokines remain unknown. We hypothesize that accumulation of the advanced lipoxidation endproduct N^{ε}-(carboxymethyl)lysine (CML) in adipose tissue contributes to the altered expression of adipokines in obesity.
Materials and methods: Accumulation and localization of CML in adipose tissue of obese patients was assessed by immunohistochemistry using a CML specific antibody. Colocalization studies of CML with macrophages (CD68) and endothelial cells (CD31) were performed. Plasma levels of protein-bound CML were measured by UPLC-tandem MS in controls (n=77), familial combined hyperlipidaemia (FCHL, n=42) and obese patients (n=44). Blood clearance of fluorescently labeled CML-albumin was compared with unmodified-albumin in Db/Db mice at different time periods. Uptake of fluorescently labeled CML-albumin and unmodified albumin in adipose tissue was assessed with two-photon microscopy. To examine the biological effects of CML in adipose tissue, preadipocytes were incubated with CML-albumin, and changes in gene expression of plasminogen activator inhibitor-1 (PAI-1), IL-6 and the receptor for AGE (RAGE) were analyzed by Real Time PCR.
Results: Immunohistochemistry showed the presence of CML in adipocytes, macrophages and endothelial cells of adipose tissue of obese patients. CML staining was significantly higher in visceral (VAT) than in subcutaneous adipose tissue (SAT) (IH score 1.50±0.21 and 1.26±0.20, resp, $p<0.001$). Plasma CML levels were lower in obese (1.3±0.2 μM, $p<0.001$) and FCHL patients (1.3±0.3 μM, $p<0.001$) as compared with controls (1.9±0.5 μM). Weight reduction in obese patients led to an increase of plasma CML levels ($p=0.01$). CML plasma levels were negatively correlated with BMI (r=-0.475, $p<0.001$), and VAT (r=-0.548, $p<0.001$), but not with SAT (r=-0.148, $p=0.238$). This suggested an accumulation and trapping of plasma CML in VAT of obese patients. Experiments in Db/Db mice showed higher clearance of CML-albumin than unmodified albumin, and preliminary results of two-photon microscopy showed accumulation of CML-albumin, but not albumin, in VAT of Db/Db mice. Incubation of human preadipocytes with CML-albumin during 24h and 48h had no effect on gene expression of IL-6, PAI-1 and RAGE, while an incubation of 72h increased their expression by 2.4-, 1.8- and 4.9-fold, respectively.