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Increased proinflammatory cytokine production in adipose tissue of obese patients with chronic kidney disease

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Erhöhte Expression von proinflammatorischen Zytokinen im Fettgewebe von adipösen Patienten mit chronischer Niereninsuffizienz

Zusammenfassung. *Hintergrund:* Die Adipositas ist ein Hochrisikofaktor sowohl für die Entwicklung von Gefäßerkrankungen als auch für chronische Niereninsuffizienz (CKD). Ziel dieser Studie ist es, den Einfluss des Fettgewebes auf den Entzündungsstatus bei adipösen Patienten mit CDK zu erfassen.

Patienten und Methoden: In einer prospektiven Querschnitt-Studie analysierten wir 40 CDK (Stadium 3–4) Patienten mit milder Proteinurie (2,3–3,5 g/Tag): 20 Patienten mit Adipositas (Gruppe 1) und 20 normalgewichtigen Patienten (Gruppe 2) wurde während einer elektiven Abdominaloperation (laparoskopische Cholecystektomie) einmalig Blut abgenommen, sowie Proben des subkutanen und des viszeralen Fettgewebes entnommen. Die Serumkonzentrationen folgender Parameter wurden bestimmt: Asymmetrisches Dimethylarginin (ADMA), Adiponektin (ADPN), C-reaktives Protein (CRP), Interleukin-6-(IL-6), Tumor Nekrose Faktor-α (TNF-α), Pentosidin, Monocyte Chemoattractant Protein-1 (MCP-1). Mit Hilfe von Real-Time-PCR wurde die Expression der Messenger RNA (mRNA) von TNF-α, MCP-1 und der Adiponektin Rezeptoren 1 und 2 sowie des immunkompetenten Zellmarkers CD68 im subkutanen sowie im viszeralen Fettgewebe bestimmt. Das Fettgewebe wurde immunhistochemisch auf CD68 positive Zellen geprüft. Außerdem wurden in beiden Gruppen folgende weitere biochemische Parameter bestimmt: Insulin, HbA1c, Cholesterin, LDL-Cholesterin, und Triglyzeride.

Ergebnisse: Die Serumkonzentrationen von ADMA, CRP, Pentosidin, Interleukin-6, TNF-α, and MCP-1 waren bei den adipösen CDK-Patienten signifikant höher. Das Adiponektin war signifikant im Vergleich zur Kontrollgruppe erniedrigt. Die subkutane und viszerale mRNA Expression von TNF-α, CD68, Adiponektin Rezeptor-1 and MCP-1 war bei den adipösen CDK Patienten signifikant erhöht. Die mRNA Expressionen waren im viszeralen Fettgewebe signifikant höher als im subkutanen Fettgewebe ($p < 0{,}01$ *vs.* $p < 0{,}05$). Die Expressionen der mRNA von Adiponektin, Interleukin-6, und des Adiponektin Rezeptors-2 beider Fettdepots waren nicht unterschiedlich in den beiden Gruppen. Bei den adipösen CDK-Patienten wurde im subkutanen und im viszeralen Fettgewebe eine erhöhte Infiltration mit CD68 positiven immunkompetenten Zellen gefunden. Die Fettstoffwechsel-Parameter waren in der Gruppe 1 gering, aber signifikant ($p < 0{,}02$) erhöht. Ausgeprägter waren die Veränderungen in den Triglyzeriden ($p < 0{,}01$). Ein ähnlicher Anstieg wurde bei den Insulin und HbA1c Werten der Gruppe 1 beobachtet ($p < 0{,}02$).

Schlussfolgerungen: Im Fettgewebe adipöser Patienten mit CKD im Stadium 3–4 wurde eine erhöhte Expression von proinflammatorischen Zytokinen und eine gesteigerte Infiltration mit immunkompetenten Zellen gefunden. Diese hinauf-regulierte Entzündung könnte zur Auslösung eines systemischen proinflammatorischen Zustands bei Patienten mit CDK beitragen und das Fortschreiten der Störung der Nierenfunktion beschleunigen.

Summary. *Background:* Obesity is a known high-risk factor for the development of vascular diseases and chronic kidney disease (CKD). In this study we aimed to elucidate the impact of adipose tissue on the inflammatory state in CDK patients with obesity.

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Patients and methods: A cohort of 40 patients with CKD (stages 3–4) with mild proteinuria (2.3–3.5 g/day) were analyzed in a prospective cross-sectional study: single blood samples and visceral and subcutaneous samples of adipose tissue were taken from 20 patients with obesity and 20 without obesity (control group) during elective abdominal surgery (laparoscopic cholecystectomy). Serum concentrations of asymmetric dimethylarginine (ADMA), adiponectin, C-reactive protein, interleukin-6, tumor necrosis factor-α, pentosidine and monocyte chemoattractant protein-1 were measured. Messenger RNA expression of tumor necrosis factor-α, monocyte chemoattractant protein-1, adiponectin receptors 1 and 2, and immunocompetent cell marker CD68 was measured in subcutaneous and visceral fat samples using real-time PCR. Adipose tissue was examined immunohistochemically for CD68-positive cells. Other biochemical parameters (insulin, glycated hemoglobin, cholesterol, LDL cholesterol, and triglycerides) were assessed in the two groups of patients at the same time.

Results: Serum concentrations of ADMA, C-reactive protein, pentosidine, interleukin-6, tumor necrosis factor-α and monocyte chemoattractant protein-1 were significantly higher in obese CKD patients than in the control group; adiponectin was lower in the obese group. Subcutaneous and visceral mRNA expressions of tumor necrosis factor-α, CD68, adiponectin receptor-1, and monocyte chemoattractant protein-1 were significantly increased in the obese patients, whereas expression of adiponectin, interleukin-6, and adiponectin receptor-2 did not significantly differ between the patient groups. In general, mRNA expressions were higher in visceral than in subcutaneous samples ($P<0.01$ *vs.* $P<0.05$). Increased infiltration of subcutaneous and visceral adipose tissue by CD68-positive immunocompetent cells was found in the obese CKD group. With respect to lipid metabolism parameters, a small but significant increase in levels was found in the obese patients ($P<0.02$). Changes in triglycerides were more marked in this group ($P<0.01$) and a similar increase was noted in insulin and HbA1c levels ($P<0.02$).

Conclusion: Increased expression of proinflammatory cytokines and increased infiltration by immunocompetent cells were found in adipose tissue of obese patients with CKD stages 3–4. This upregulated inflammation may contribute to the induction of a systemic proinflammatory state in patients with CKD and could accelerate the progression of renal dysfunction.

Key words: Chronic kidney disease, adipose tissue, inflammation, ADMA, adiponectin.

Introduction

During the past decade much knowledge has been gained on the metabolic and hormonal functions of adipose tissue. Such tissue is no longer viewed as a passive reservoir of energy but has been shown to be a very active metabolic organ secreting numerous hormones and cytokines related to inflammatory processes, insulin resistance, and endothelial dysfunction. Adipocytes and other cell types residing in adipose tissue, such as immunocompetent cells, contribute to systemic endocrine functions [1].

Obesity is accompanied by an increased local inflammatory response in adipose tissue, as measured by immunocompetent cell infiltration, that in turn leads to increased production of proinflammatory factors such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and others [2–4]. Increased release of proinflammatory factors from adipose tissue may contribute to a systemic subclinical inflammatory response in patients with obesity and thus promote the development of insulin resistance and atherosclerosis in these patients [5]. Clinical and experimental studies have demonstrated that administration of several adipose tissue-derived hormones such as leptin or adiponectin may exert significant insulin-sensitizing, anti-inflammatory, and antiatherogenic effects [6–11].

Patients with chronic kidney disease (CKD) and end-stage renal disease represent a group with massively increased mortality from cardiovascular diseases [12–15]. Adipose tissue has been shown to serve as a source of proinflammatory factors in patients with obesity, atherosclerosis and/or type 2 diabetes mellitus, and also in critically ill patients [16–17]. It has been suggested that a high mass of metabolically active visceral adipose tissue could also be the primary source of the subclinical inflammatory response in patients with CKD.

Asymmetric dimethylarginine (ADMA) has been described as an autocrine regulator of endothelial activity of nitric oxide (NO) synthase, and elevated ADMA levels in CKD may be responsible for the endothelial dysfunction observed in these patients [18]. Increased plasma levels of ADMA in CKD depend on the degree of impairment of renal function but might also be influenced by counter-regulatory responses to other metabolic disorders of CKD and obesity. Among non-CKD patients, plasma concentrations of ADMA were higher in obese insulin-resistant individuals and weight loss was associated with decreased ADMA levels [19]. Massively elevated ADMA concentrations have been found in morbidly obese patients with body mass index (BMI) >35 and a significant decrease was seen after weight loss following gastroplasty [20].

In the present study, by comparing findings in obese and non-obese patients with CKD stages 3 and 4, we tested the hypothesis that adipose tissue might contribute to the systemic inflammatory state through increased production of proinflammatory cytokines. We also evaluated the relationship between serum concentrations and mRNA expression of selected adipokines in subcutaneous and visceral adipose tissue sampled during elective abdominal surgery in obese and non-obese CKD patients.

Patients and methods

The study was approved by the Human Ethical Review Committee, Institute for Clinical and Experimental Medicine, Prague and complied with the Declaration of Helsinki, including the current revision and good Clinical Practice Guidelines. The procedures followed were in accordance with institutional guidelines. All patients gave written informed consent before being enrolled in the study.

A cohort of 40 CKD patients undergoing elective laparoscopic cholecystectomy were analyzed in this prospective cross-sectional metabolic study: 20 patients with CKD stages 3–4 and obesity (mean age 54.5 years, mean BMI 32.2) formed Group I; 20 non-obese CKD patients (mean age 58.1 years, BMI 25.2) formed Group II, the control group. In Group I the causes of CKD were diabetic nephropathy ($n=8$), polycystic kidney disease ($n=2$), chronic glomerulonephritis ($n=3$), tubulointerstitial nephritis ($n=3$), vascular nephrosclerosis ($n=3$), and Alport's syndrome ($n=1$). Among this group, 15 patients had arterial hypertension and 12 had hyperlipidemia; 10 were treated with statins (atorvastatin 10 mg/day).

In group II, nine patients had diabetic nephropathy, five had chronic glomerulonephritis, three had vascular nephrosclerosis and three tubulointerstitial nephritis; 14 suffered from arterial hypertension and nine were treated with statins in doses similar to those used in Group I.

All patients with hypertension were receiving adequate antihypertensive therapy (ACEI and ARBs). There were no significant differences in age, sex, or race between the groups.

The patients were evaluated anthropometrically at basal state one day before surgery. All were weighed and measured and BMI calculated. Blood samples for hormonal measurement were taken at basal state (before the beginning of anesthesia). Serum was obtained by centrifugation and the samples stored at –70°C until further analysis.

Samples of subcutaneous and visceral adipose tissues for analysis of mRNA expression and histopathologic examination were taken from the abdominal region at the beginning of surgery. To avoid the influence of local damage on tissue parameters, all samples were taken from approximately the same location in all patients from tissue that had not been previously traumatized mechanically or by cauterization. Tissue samples were collected into RNA later reagent (Qiagen, Hilden, Germany) and stored at –70°C until further analysis.

Serum levels of adiponectin were measured using a commercial ELISA kit (BioVendor, Brno, Czech Republic; Linco Research, St. Charles, MI, USA). Serum concentrations of IL-6, TNF-α and MCP-1 were determined using a human serum adipokine LINCOplex kit on a Luminex 200 instrument (Linco Research). Serum concentrations of C-reactive protein (CRP) were measured using an ultrasensitive CRP ELISA kit (DSL, Oxon, UK).

Total RNA was extracted from 60 to 100 mg of subcutaneous and visceral adipose tissue samples by homogenization using an MagNA Lyser instrument (Roche Diagnostics, GmbH, Mannheim, Germany), followed by isolation of RNA using an MagNA Pure Compact RNA isolation kit (Roche Diagnostics, GmbH) on an automatic MagNA Pure Compact isolator (Roche Diagnostics, GmbH). Concentration and purity of RNA samples were determined using spectrophotometry (BioPhotometer Eppendorf AG, Hamburg, Germany).

The average RNA concentration was 70.2 mg/ml, and the R260/280 nm ratio was 1.79. The integrity of the RNA was checked using ethidium-bromide visualization of 18S and 28S ribosomal bands on 1% agarose gels. Total RNA (0.1–1 mg) was used for reverse transcription to synthesize first-strand cDNA using the oligo(dT) primers of the RevertAid First-Strand cDNA Synthesis Kit (Fermentas Life Science, Vilnius, Lithuania). Complementary DNA was used for determination of gene expression of leptin, adiponectin, resistin, adiponectin receptor-1 (AdipoR1), adiponectin receptor-2 (AdipoR2), IL-6, TNF-α, MCP-1 and the immunocompetent cell marker CD68 using real-time PCR on an ABI PRISM 7500 instrument (Applied Biosystems, Foster City, CA, USA); TaqMan Universal PCR Master Mix, NO AmpErase UNG and specific TaqMan gene expression assays (Applied Biosystems) were used. PCRs for each gene were amplified separately. Controls lacking template cDNA were included in each assay and all samples were run at least in duplicate. The increase in fluorescence was measured in real time and data were obtained as threshold cycle (Ct) values. To compensate for variation in amounts of input RNA and the efficiency of reverse transcription, β2-microglobulin was used as an endogenous reference and results were normalized to these values. The relative expression of genes was calculated using the formula $2^{-\Delta\Delta(\text{CT cytokine}-\text{CT B2M})}$.

Sections (5 μ thick) cut from formalin-fixed, paraffin-embedded tissue samples were embedded in paraffin and xylene and rehydrated. Endogenous peroxidase activity was inhibited with 3% H_2O_2 in methanol for 30 min followed by 15 min of rinsing in tap water. Non-specific reactivity was avoided by pretreatment of sections for 2 h with 1% normal goat serum (Dako Cytomation, Glostrup, Denmark) containing 1% bovine fetal albumin diluted in ChemMate antibody diluent (Dako Cytomation). Slides were incubated with primary mouse monoclonal antibody CD68 clone KP1 (Dako Cytomation) and diluted with ChemMate antibody diluent for 1 h at room temperature. The HistofineR kit (Nichirei, Tokyo, Japan) was used to visualize sections incubated with the primary antibody. The chromogen 3,3-diaminobenzidine (Liquid DAB Substrate, Dako Cytomation) was added to all sections and Mayer's hematoxylin was used as a counterstain. Tissue sections incubated without primary antibody were used as negative controls. Sections were analyzed in random order by a pathologist who was unaware of clinical data, and the number of CD68-positive cells per high-power field was counted.

All patients were in CKD stages 3–4 with glomerular filtration rates 28–48 ml/min per 1.73 m^2 estimated by inulin clearance. Metabolic acidosis was corrected before surgery in all patients. In Group I all patients had a BMI ≥ 30 and signs of central obesity (waist/hip ratio >0.85). The antihypertensive therapy was not changed (ACEI + ARB), but its dose was modified when monitoring blood pressure with goal levels of 125/80 mmHg. Proteinuria was in the range 2.3–3.5 g per 24 h. Inulin clearance and corrected creatinine clearance were determined for the estimation of renal function in the clearance laboratory of the Department of Nephrology [21].

For estimation of ADMA concentration patients fasted for at least 10 h before sampling to avoid the influence of methionine from food. An ADMA ELISA kit (DLD Diagnostika GmbH, Hamburg, Germany) and an AUTO-EIA II microplate reader (Labsystems Oy, Espoo, Finland) were used for ADMA quantification; this competitive method uses the microtiter plate format. Plasma pentosidine concentrations were measured using a commercially available competitive FSk pentodine ELISA kit (Fushimi Pharmaceutical, Kagawa, Japan) [20]. Serum albumin was determined using bromocresol purple in a routine procedure in the Department of Clinical Biochemistry. Total cholesterol, HDL-cholesterol and triglycerides were determined using an enzymatic colorimetric method with an Olympus AU 600 analyzer and reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). LDL cholesterol was calculated using Friedewald's formula. Inulin (polyfructosane S) was analyzed using anthrone on a spectrophotometer at wavelength 580 nm (Antelie Light Secoman, France).

Serum insulin concentrations were measured using a commercial RIA kit (CisBio International, Lyon, France); glycated hemoglobin (HbA1c) was analyzed using liquid chromatography on a Tosoh HLC-723 G7 (Shiba, Minato-Ku, Tokio, Japan) and proteinuria per 24 h by photometry with pyrogallol red using an Olympus 800 system (Hamburg, Germany).

Statistical analysis

SigmaStat software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. A *t*-test or Mann–Whitney rank-sum test was used to compare data from the two groups of CKD patients. The relations between respective variables were assessed using Pearson's or Spearman's correlation coefficient as appropriate. Results were considered statistically significant at $P<0.05$.

Results

Basic clinical characteristics of the two groups of CKD patients are listed in Table 1: the groups did not significantly differ with respect to age, sex, or renal function measured by inulin clearance. Group I had significantly higher serum levels of ADMA, pentosidine, HbA1c, proteinuria, LDL cholesterol, triglycerides, blood pressure and insulin and significantly lower adiponectin levels than Group II, the control group. Group I also showed a significant increase in hsCRP levels compared with Group II.

With respect to lipid metabolism parameters, there were small but significant increases in the total serum cholesterol and LDL cholesterol levels in Group I (6.1±1.2 *vs.* 5.8±2.2 and 3.9±1.2 *vs.* 3.6±1.0 mmol/l, $P<0.02$). More marked were changes in triglycerides in Group I (3.9±1.6 *vs.* 2.8±1.0 mmol/l, $P<0.01$). There was no significant difference between statin-treated and untreated patients with respect to proinflammatory cytokines or CRP.

Similarly, Group I showed an increase in HbA1c (5.3±1.4 *vs.* 4.2±0.9%, $P<0.02$) and plasma insulin concentration (365.3±40.1 *vs.* 292.4±49.1 mU/l, $P<0.02$).

Moreover, there were also differences between the two groups in proteinuria, which was significantly higher in Group I (3.5±2.2 *vs.* 2.8±1.1 g/l, $P<0.02$). A slight but significant difference was also found in systolic and diastolic blood pressure ($P<0.02$ for systolic and $P<0.05$ for diastolic blood pressure).

As shown in Fig. 1, levels of ADMA, IL-6, and TNF-α were significantly higher in Group I than Group II. Significant increases in serum MCP-1 and pentosidine were confirmed in Group I (Fig. 2).

The mRNA expression of the proinflammatory factor TNF-α and the immunocompetent cell marker CD68 were significantly increased in subcutaneous and visceral adi-

Table 1. Clinical and biochemical characteristics Group I (CKD obese subjects) and Group II (control CKD non–obese subjects

Parameter	Group I (*N*=20)	Group II (*N*=20)	Statistical significance Gr I *vs.* Gr II
C_{in} (mL/min/1.73 m²)	29.7±9.9	31.2±9.7	NS
BMI	32.2±3.3	25.1±4.0	$P<0.01$
WHR	0.88±0.05	0.81±0.04	$P<0.02$
ADMA (μmoL/L)	2.4±0.5	1.3±0.4	$P<0.01$
ADPN (μg/mL)	14.4±6.6	21.4±8.2	$P<0.01$
TNF-α (pg/mL)	8.6±2.3	5.3±1.7	$P<0.05$
MCP-1 (pg/mL)	195±53	112±40	$P<0.05$
Il-6 (pg/mL)	14.8±4.2	9.0±3.1	$P<0.05$
Pentosidine (μg/L)	485±170	395±80	$P<0.02$
HbA1c (%)	5.3±1.4	4.2±0.9	$P<0.02$
Insulin (pg/mL)	365.3±40.1	292.4±49.1	$P<0.02$
CRP (mg/L)	14.9±4.5	7.4±2.5	$P<0.02$
Albumin (g/L)	32.1±1.5	31.8±2.3	NS
Proteinuria (g/24 h)	3.5±2.2	2.3±2.1	$P<0.02$
Cholesterol (mmol/L)	6.1±1.2	5.8±2.2	$P<0.02$
LDL-cholesterol (mmol/L)	3.9±1.2	3.6±1.0	$P<0.05$
Triglycerides (mmol/L)	3.9±1.6	2.8±1.0	$P<0.01$
Syst BP (mm Hg)	135±10	125±7	$P<0.02$
Diast BP (mm Hg)	90±9	82±7	$P<0.05$

C_{in} inulin clearance; *BMI* body mass index; *WHR* waist-hip ratio; *ADMA* asymmetric dimethylarginine; *ADPN* adiponectin; *TNF-α* tumor necrosis factor α; *IL-6* interleukin 6; *MCP-1* monocyte chemoatracttant protein; *HbA1c* glycated Hb; *CRP* C-reactive protein.
* Values are means±SEMs. Statistical significance is from unpaired *t*-test or Mann–Whitney rank-sum test. *NS* non significant.

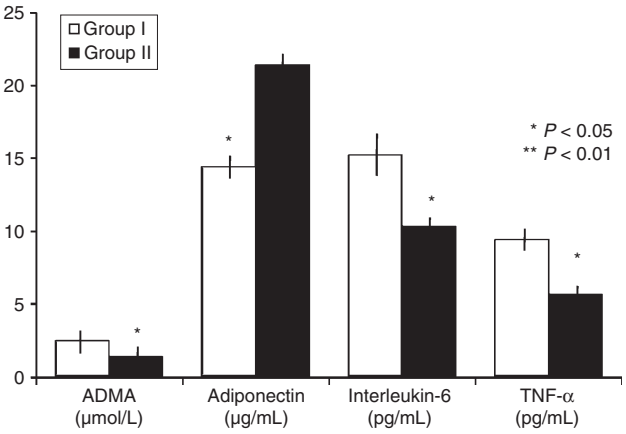

Fig. 1. Serum concentrations of asymmetric dimethylarginine (ADMA), adiponectin, interleukin-6, and TNF-α in obese chronic kidney disease patients (Group I – open bars) and in non-obese chronic kidney disease patients (Group II – filled bars)

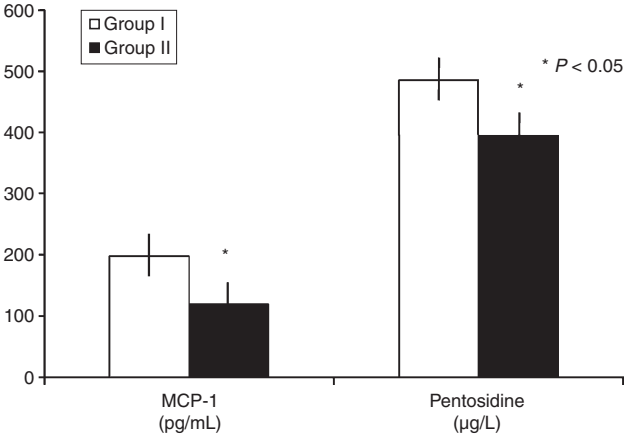

Fig. 2. Serum concentrations of MCP-1 and pentosidine in obese chronic kidney disease patients (Group I – open bars) and in non-obese chronic kidney disease patients (Group II – filled bars)

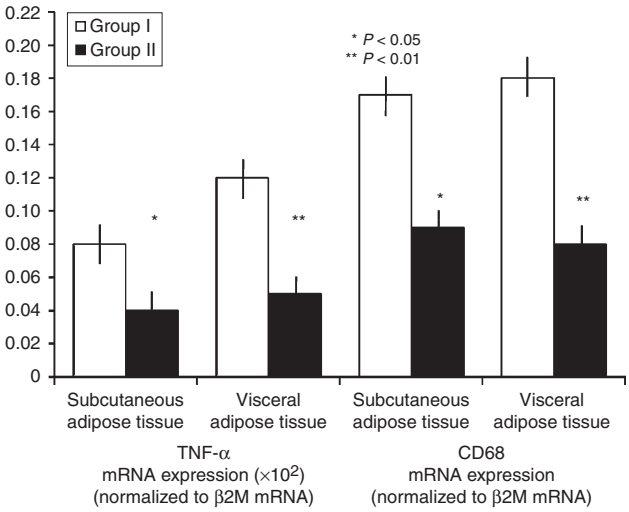

Fig. 3. Messenger RNA expression of TNF-α and CD 68 in subcutaneous and visceral adipose tissue samples from obese chronic kidney disease patients (Group I – open bars) and in non-obese chronic kidney disease patients (Group II – filled bars)

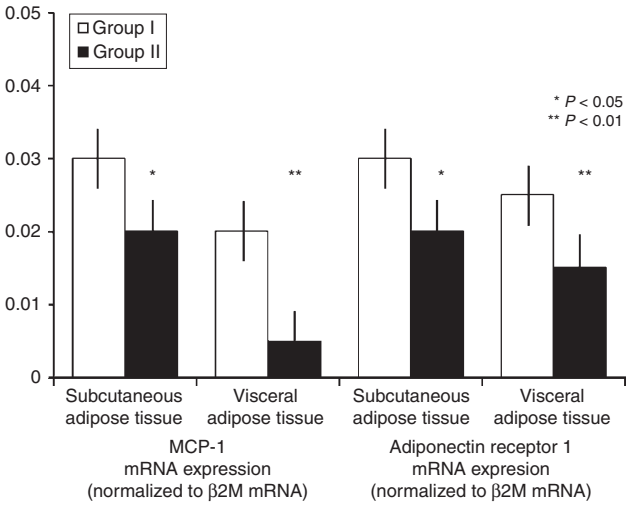

Fig. 4. Messenger RNA expression of MCP-1 and adiponectin receptor in subcutaneous and visceral adipose tissue samples from obese chronic kidney disease patients (Group I – open bars) and in non-obese chronic kidney disease patients (Group II – filled bars)

pose tissues sampled from patients in Group I (Fig. 3). Expressions of AdipoR1 and MCP-1 were significantly higher in Group I than Group II (Fig. 4), mainly in visceral adipose tissue.

No other significant relations were found between mRNA expression and circulating concentrations of adipokines, and no significant differences in expression of the adipokines were found when comparing subcutaneous with visceral adipose tissue depots in the two groups of patients.

Group I showed increased infiltration of visceral and subcutaneous adipose tissue by CD68-positive immunocompetent cells (Group I *vs.* Group II: subcutaneous fat 0.162 *vs.* 0.083, $P<0.05$; visceral fat 0.172 *vs.* 0.071, $P<0.01$).

Discussion

Adipose tissue has been recognized as an important endocrine organ producing numerous hormones and cytokines, including proinflammatory factors such as TNF-α, IL-6, and many others [22, 23]. Furthermore, production of proinflammatory cytokines is increased in obese patients, suggesting the pathogenetic contribution of these factors in development of insulin resistance, vascular disease, and possibly other related pathologies [24, 25]. Experimental and clinical studies have shown that adipose tissue of obese individuals is characterized by increased infiltration by immunocompetent cells that become the most important producers of proinflammatory cytokines [3, 4]. The hypothesis of a causal contribution of 'inflamed' fat tissue to the development of atherosclerosis has been further supported by studies demonstrating that partial inhibition of inflammation in fat by transgenic [26] or pharmacologic manipulations improved insulin sensitivity and delayed the progression of atherosclerosis [27, 28].

Similar to, and in extension of, previous studies we found that patients with CKD and obesity have markedly increased circulating levels of proinflammatory cytokines when compared with a non-obese CKD group [13]. The increase has been explained as the combination of increased systemic production of these factors because of subclinical inflammation and decreased degradation in the kidney because of renal insufficiency. Our present study confirms that adipose tissue of patients with advanced CKD is an important producer of proinflammatory cytokines.

Similar to our study, it has been demonstrated that systemic activation of the immune response by cardiac surgery markedly upregulates the production of proinflammatory cytokines in subcutaneous and epicardial adipose tissues [16, 17, 29, 30]. We suggest that the stimulation of immunocompetent cells in adipose tissue may be an important and universal part of the overall systemic inflammatory reaction, which can be activated by various stimuli. Nevertheless, in our study we measured mRNA expression of proinflammatory factors, which may not always reflect their protein levels.

In both groups of CKD patients we found characteristic dyslipidemia with elevated triglycerides, similar to previous studies [31, 32]. Hypertriglyceridemia in patients in renal failure can be explained by the accumulation of triacylglycerol-rich very-low-density lipoproteins resulting from inhibition of lipolysis rather than from overproduction [32]. Patients with hyperlipidemia in both groups were treated with statins.

Although statins could exert some anti-inflammatory effects, this was not confirmed in patients with advanced CKD [33], and in our study we did not observe any significant differences between statin-treated and untreated patients with respect to proinflammatory cytokines or CRP (data not shown).

Among our 40 CKD patients, 17 had diabetes mellitus (Group I: 8 patients; Group II: 9 patients, no significant difference). Both HbA1c and plasma insulin levels were significantly increased in group I. Elevated insulin levels in this group could represent increased insulin resistance,

potentially caused by proinflammatory cytokines and/or other factors [34, 35]. Whether elevated insulin or triglyceride levels themselves had an impact on the proinflammatory profile of adipose tissue of patients with CKD requires further investigation.

Visceral and to some extent subcutaneous adipose tissues of patients with CKD were characterized by significantly increased expression of CD68 and increased infiltration by CD68-positive immunocompetent cells, as previously described in obese non-renal patients [8]. This finding confirms increased presence of immunocompetent cells in the fat tissue of patients with advanced CKD. These cells, together with adipocytes, may markedly contribute to increased production of proinflammatory factors by adipose tissue.

Although some of the characteristics of endocrine function of adipose tissue in patients with CKD were similar to those of non-renal patients with obesity, there were also several important differences. An increase in circulating levels of proinflammatory cytokines in CKD is accompanied by increased levels of adiponectin, an adipose tissue-derived hormone with anti-inflammatory and antiatherogenic properties. In contrast, decreased adiponectin levels are normally found in non-renal patients with obesity, atherosclerosis, insulin resistance, and type 2 diabetes mellitus [36, 37], whereas leanness and increased levels of physical activity are accompanied by hyperadiponectinemia [38, 39]. Adiponectin circulates in multiple isoforms and its high-molecular-weight isoform is thought to be the one most closely related to overall insulin sensitivity [40]. The distribution of adiponectin isoforms has been recently studied in patients with CKD and found to be similar to the distribution in healthy control persons [41].

In the present study we found that patients with CKD and obesity had slightly decreased circulating adiponectin levels but significantly increased mRNA expression of AdipoR1 in visceral adipose tissue. Our results support findings by Shen et al., who observed increased mRNA expression of AdipoR1 on peripheral blood mononuclear cells from patients with CKD in comparison with cells from healthy controls [42]. We suggest that an increase in AdipoR1 in visceral adipose tissue could represent a compensatory anti-inflammatory response to the uremic milieu and that AdipoR1 (and AdipoR2) may respond to a stimulus specific to CKD, as proposed by Shen et al. from their observations in peripheral blood mononuclear cells [42].

The increased levels of ADMA in our patient groups could be attributed to renal impairment. Humans generate approximately 300 μmol/day (60 mg) of ADMA, of which approximately 50 μmol/day is excreted in the urine, thus ADMA accumulates in patients with renal failure. Kidney transplantation normalizes symmetric DMA, whereas ADMA levels remain elevated. This may be due to persistent decrease in ADMA degradation, and impaired activity of the enzyme dimethylarginine dimethylaminohydrolaze (DDAH) in the kidney has been proposed.

There is sparse evidence that plasma ADMA levels can be reduced by pharmacotherapy. But, in contrast to the conflicting data on the effect of ACEIs and ARBs, oral antidiabetic drugs (such as metformin and glitazone) improve insulin resistance and can also reduce ADMA levels; this effect probably results from the upregulation of DDAH [43].

In CKD patients, plasma ADMA concentrations have been found higher in obese insulin-resistant individuals and weight loss was associated with decrease in plasma ADMA concentrations [2]. Highly elevated ADMA levels have also been found in morbidly obese patients (BMI > 35), together with significant decrease in ADMA after weight loss post-gastroplasty [20]. Miazaki et al. observed significant correlation between plasma ADMA and age, arterial blood pressure and blood glucose [44]; in healthy volunteers, plasma ADMA was not correlated with cholesterol or triglycerides but was inversely correlated with creatinine clearance ($P < 0.001$) and BMI.

In humans, ADMA may be excreted by the kidney or metabolized by DDAH, both entities being widely distributed in tissues. Ito et al. suggested that lipoproteins or cytokines may increase endothelial secretion of ADMA by reducing DDAH activity [45], which in turn may lead to local accumulation or release of intracellular ADMA and inhibition of NO synthase in states of hypercholesterolemia. In addition, a plausible explanation is the effect of increased mass of visceral fat tissue on increasing production of inflammatory factors and on development of insulin resistance, which may impair DDAH activity and subsequently lead to elevated ADMA levels [46].

Obesity and weight reduction have been shown to be associated with changes in endothelial function [47]. In fact, this clinical study demonstrated reduced NO-dependent vasodilatation in obese compared with lean persons, and it is possible that increased concentrations of ADMA could contribute to the development of this endothelial dysfunction in obesity. Recently, Spoto et al. measured ADMA concentrations in cultured adipocytes, together with mRNA expression of enzymes involved in ADMA metabolism in the cultured cells and in adipose tissue harvested from healthy persons. It was found that human adipocytes express the whole gene set that encodes for the enzymatic system responsible for biosynthesis and degradation of ADMA; these findings may be of relevance in clarifying the role of fat mass expansion in human disease [48].

Dietary changes before surgery cannot be excluded as the reason for changes in ADMA levels, because the obese patients under study slightly reduced their calorie intake and composition of their diet. Although increased carbohydrate intake is associated with reduced ADMA plasma concentrations, protein and fat intake appears to have no influence [49].

The limitations of our study are the lack of a healthy control group of obese and non-obese persons without CKD and the small number of patients studied. In addition, the patients' heterogenous underlying diagnoses (diabetes mellitus in 17 cases) might have had an impact on the findings.

Conclusion

Our data show that expression of proinflammatory cytokines and infiltration of adipose tissue by immunocompetent cells are increased in obese persons with CKD stages 3–4. Moreover, serum concentrations of several proinflammatory cytokines are higher and insulin resistance is more pronounced in these patients. It remains to be determined how important this adipose tissue-mediated inflammation is in contributing to the systemic proinflammatory state in uremia, to accelerated vascular disease and to progression of renal failure in these patients and whether interventions suppressing such inflammation may have beneficial effects.

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Conflict of interest
The authors declare no conflict of interest.

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