Insulin Up-Regulates Natriuretic Peptide Clearance Receptor Expression in the Subcutaneous Fat Depot in Obese Subjects: A Missing Link between CVD Risk and Obesity?

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Context: Natriuretic peptides (NP) regulate cardiovascular homeostasis and have multiple metabolic properties. Decreased levels of NP or “natriuretic handicap” are signs of insulin resistance such as central obesity. Increased expression of NP clearance receptor (NPRC) in sc adipose tissue (SAT) was observed in insulin-resistant subjects.

Objective: We hypothesized that insulin acutely regulates NP receptor expression in adipose tissue.

Design and Participants: NPRA, NPRB, and NPRC mRNA expression was measured in paired samples of visceral adipose tissue (VAT) and SAT from 157 subjects (108 with type 2 diabetes). The effect of insulin on NPR gene expression in SAT was studied in euglycemic-hyperinsulinemic and hyperglycemic-hyperinsulinemic clamp experiments. Additionally, the effect of insulin and glucose on NPR expression in the culture of primary human monocytes and macrophages was tested.

Results: NPRA and NPRC gene expression was higher in VAT compared with SAT (P < 0.01), but only NPRC gene expression strongly correlated with fasting insulin levels (r = 0.65, P = 0.04 × 10⁻³; and r = 0.54, P = 0.002, for VAT and SAT, respectively). NPRB expression was lower in VAT than in SAT in subjects with type 2 diabetes and was lower compared with nondiabetic subjects. NPRC gene expression was up-regulated in SAT during both euglycemic- and hyperglycemic-hyperinsulinemic clamp experiments. Additionally, the effect of insulin and glucose on NPR expression in the culture of primary human monocytes and macrophages was tested.

Conclusion: Insulin increased expression of NPRC in SAT independently of circulating glucose concentrations. Thus, insulin might suppress circulating NP via up-regulation of NPRC expression in obesity, providing a novel link between hyperinsulinemia and obesity. (J Clin Endocrinol Metab 97: E731–E739, 2012)
Central obesity and metabolic syndrome are independent risk factors for cardiovascular disease (1) and heart failure (2). Despite the strong epidemiological evidence, the underlying pathophysiological mechanism remains unclear.

Natriuretic peptides (NP) play important roles in the regulation of cardiovascular homeostasis and have potent growth-regulating properties (3). The NP family includes atrial NP (ANP), B-type NP (BNP), and C-type NP (CNP). ANP and BNP are secreted from the cardiomyocytes of cardiac atria and ventricles and directly influence both blood pressure (BP) and body fluid homeostasis (3, 4). CNP is predominantly expressed in the brain, but it is also produced in chondrocytes, vascular endothelial cells, and other tissues (4). NP activate two membrane-bound guanylyl cyclase receptors [NP receptor A (NPRA) and NP receptor B (NPOR)], leading to an increase in intracellular cGMP and thus affecting the activity of cGMP-regulated enzymes and ion channels (3, 4). NPRA responds to ANP and to a lesser degree to BNP; NPRB responds primarily to CNP. NP receptor C (NPRC) lacks intrinsic enzymatic activity and controls the local concentrations of NP through constitutive receptor-mediated internalization and degradation (4, 5). NPRC or NP clearance receptor interacts with all three NP in the order ANP>BNP>CNP (4, 5). NPRC is the most widely expressed NPR in both animals (5) and humans (4, 5). Moreover, ANP and BNP have been shown to exert potent lipolytic action in adipocytes (6) and to have antiinflammatory properties (7).

ANP and BNP increase dramatically in relation to hypertension and particularly heart failure or myocardial ischemia in an attempt of the organism to correct the dysregulation of cardiovascular risk markers (8). In contrast, recent large-cohort epidemiological studies showed that NP levels are suppressed in obesity, and the phrase “natriuretic handicap” has been used to describe this phenomenon (9–11). However, in a population without manifest cardiovascular disease, a decrease of cardiovascular protective hormones may well increase long-term risk of cardiovascular disease such as occurs for ANP in the metabolic syndrome. Because obesity is associated with salt retention and increased cardiac output, it would be expected to produce elevated NP levels. The opposite effect of obesity appeared counterintuitive and has been attributed to nonhemodynamic factors (10, 11). Another mechanism may be due to an increased expression of the NPRC by adipose tissue, resulting in increased clearance of NP in obesity (11). However, in rodents, insulin has been suggested as the major regulator of the NPR expression in the body (12).

The “insulin-resistance syndrome,” as suggested by Reaven (13), proposed that elevated insulin levels resulting from insulin resistance and typically accompanying central obesity induce the multiple metabolic disturbances. We therefore hypothesized that insulin may regulate NPR expression in adipose tissue. First, we studied NPR gene expression in different adipose tissue depots. Furthermore, we investigated the acute effect of insulin infusion on NPR expression in sc adipose tissue (SAT) in nondiabetic subjects with moderate obesity. Based on interactions between adipocytes and macrophages in adipose tissue, we examined the influence of insulin and glucose on NPR expression in cultures of primary human macrophages and in monocytes as macrophage precursor cells.

Subjects and Methods

**Cohort I**

Paired samples of visceral adipose tissue (VAT) and SAT were obtained from 157 Caucasian men (n = 62) and women (n = 95) who underwent abdominal surgery, as described (12). Percentage body fat was measured by dual-energy x-ray absorptiometry. Fasting plasma insulin was measured using an enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products Corporation, Los Angeles, CA). The study was approved by the ethics committee of the University of Leipzig, and all subjects gave written informed consent before taking part in the study.

**Cohort II**

Fourteen healthy, moderately obese male subjects had normal glucose tolerance in the 75-g oral glucose loading (13), and all participants reported a stable body weight for at least 12 months. Subjects with elevations in liver enzymes or with elevated serum creatinine concentrations (>1.3 mg/dl) were excluded. Subjects were instructed to maintain their normal physical activity and to consume a diet containing at least 200 g of carbohydrate for 3 d before and 14 d during the study. Percentage body fat was measured by air displacement plethysmography (BOD POD; Life Measurement Inc., Concord, CA). Subjects meeting the inclusion criteria were recruited for participation in one or both of the following procedures in the randomized design: 1) a hyperinsulinemic-euglycemic clamp of 4-h duration with continuous infusion of 40 mU/m² of body surface/min of human insulin at a steady-state capillary plasma glucose concentration of 4.4 mmol/liter (80 mg/dl) (n = 10); and 2) a hyperinsulinemic-hyperglycemic clamp of 4-h duration with continuous infusion of 40 mU/m² of body surface/min human insulin at a steady-state capillary plasma glucose concentration of 7.8 mmol/liter (140 mg/dl) (n = 7). SAT needle biopsy samples were taken at 40 min before and during the last 30 min of clamp tests.

**Cohort III**

Twenty-five overweight volunteers underwent hyperinsulinemic-euglycemic clamp experiments, which were performed as described in the methods of clamp experiments. Hypertension (16), use of antihypertensive therapy, type 2 diabetes (T2DM), and unstable body weight for at least 2 months before the study
were exclusion criteria. N-terminal fragment of pro-brain (B-type) natriuretic peptide (NT-proBNP) levels were measured additionally to midregional-proANP (MR-proANP) in 10 subjects [age, 59.0 ± 8.8 yr; 40% female; body mass index (BMI), 30.6 ± 4.0 kg/m²; body fat, 34.6 ± 7.9%; waist circumference, 105.7 ± 9.4 cm; systolic BP, 127 ± 7 mm Hg; and diastolic BP, 78 ± 3 mm Hg]. For detailed subjects characteristics of all three cohorts see Supplemental Table 1 (published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org).

Both studies were approved by the Ethics Commission of Brandenburg, Germany (registration no., AS 2(a)/2005), and registered at www.clinicaltrials.gov (NCT00774488).

Glucose clamp experiments

All glucose clamp tests (17) were performed in the morning after a 12-h overnight fast. In cohort II, insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) at an infusion rate of 40 mU/m² of body surface/min and glucose infusions (glucose 10%; Serag Wiessner, Naila, Germany) were started at \( t = 0 \) min, following the first sc periumbilical adipose tissue biopsy. In the hyperinsulinemic-euglycemic clamp, capillary blood glucose was measured every 5 min to adjust the glucose infusion rate to the required glucose level of 4.4 mmol/liter. A deviation of a single capillary glucose concentration of more than 10% during assumed steady-state conditions was defined as non-steady state. Hyperinsulinemic-hyperglycemic clamp tests aiming at a steady-state condition was defined as non-steady state. Venous blood samples for the analysis of hormones were taken at -20, -10, 100, 110, and 120 min, timed from the start of the insulin infusion. SAT biopsies were taken before and after infusions \( (t = -40 \) min and 240 min) from contralateral sites at the level of the umbilicus, stored in an RNA protecting buffer (RNAlater; Qiagen, Hilden, Germany) at 4 C until the procedure was finished, and shock-frozen in liquid nitrogen.

In cohort III, hyperinsulinemic-euglycemic clamps (5.5 mmol/liter of capillary blood glucose) were performed for at least 120 min using 100 mU/m² of body surface/min human insulin and a variable infusion of 20% glucose. In the steady-state condition of the clamp, the glucose infusion rate was held constant for at least 60 min. Venous blood samples for the analysis of hormones were taken at -20, -10, 100, 110, and 120 min, timed from the start of the insulin infusion.

Biochemical measurements

Capillary blood glucose concentrations were measured using a glucose-oxidase method on a Dr. Müller Super GL (Dr. Müller Glucose analyzer, Freital, Germany), and glycosylated hemoglobin was measured using a Hi-Auto A1C HA-8140 system (Menarini Diagnostics, Neuss, Germany). Serum triglycerides, total cholesterol, and high-density lipoprotein-cholesterol were measured by standard enzymatic assays, and low-density lipoprotein-cholesterol was calculated from these data. Serum insulin was measured using the commercial ELISA (Insulin ELISA; Merckodia AB, Uppsala, Sweden). Human plasma MR-proANP was measured using a chemiluminescence immunoassay (BRAHMS AG, Hennigsdorf, Germany) as described elsewhere (18). NT-proBNP was measured by an electrochemiluminescence immunoassay (ELICIA, Roche Diagnostics, Basel, Switzerland).

Monocyte and macrophage cell culture

Monocytes were isolated from whole blood of healthy donors \( (n = 3) \); one male and two females) by magnetic cell sorting using CD14 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany). For the treatment, monocytes were suspended in DMEM (Biocrom AG, Berlin, Germany) supplemented with 1% HyClone FCS (Thermo Scientific, Hamburg, Germany) and 10% HyClone FCS as described elsewhere (19). The regulation of NPR expression in monocytes and mature macrophages was studied under three different conditions: normal glucose (5.5 mmol/liter), high glucose (30 mmol/liter) and high glucose (30 mmol/liter) with insulin (10 nM) (Sigma, Taufkirchen, Germany) for 4 h.

Quantitative real-time PCR (qRT-PCR)

In cohort I, qRT-PCR was carried out as described previously (14). In cohort II, total RNA from sc adipose tissue biopsy samples was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany).
and cDNA were synthesized with TaqMan Reverse Transcription Reagents (Applied Biosystems, Darmstadt, Germany). qRT-PCR was performed in ABI Prism 7900 sequence detection system (Applied Biosystems) using Power SYBR Green PCR Master Mix (Applied Biosystems). Quantification of mRNA levels was performed by the standard curve method. RPLP0 and HPRT1 genes were used as internal control. Primer sequences are shown in Supplemental Table 2.

**Microarray analysis and gene network reconstruction**

Total RNA was extracted from SAT biopsy samples (RNeasy Lipid Tissue Mini Kit; QIAGEN), the grade of quality was measured by ND-1000 spectrophotometer (Nanodrop; PeqLab, Erlangen, Germany), and was further hybridized to the Agilent 60-mer Whole Human Genome (44K) single-color DNA microarrays (Agilent Single Color 12391; Agilent Technologies, Böblingen, Germany) according to the manufacturer’s protocol (for details, see Supplemental Data). The reconstruction of NPRC and NPRB gene correlations network in the hyperinsulinemic-euglycemic clamp was performed as described in detail previously (20).

**Calculations and statistical analyses**

Data are shown as means ± sd unless stated otherwise. Insulin sensitivity was measured as glucose infusion rate per kilogram of body weight in the steady-state period of the clamp or calculated by homeostasis model assessment (21). Correlation analysis was performed using Spearman correlation. Differences between two groups were analyzed by the Mann-Whitney test. The nonparametric Wilcoxon signed-rank test for paired samples was used to compare data from the baseline with end of clamp data. Statistical significance was defined as \( P < 0.05 \). Statistical analyses were performed using SPSS for Windows 16 (SPSS Inc., Chicago, IL).

**Results**

**NPR gene expression in SAT and VAT**

The main baseline characteristics of the cohorts I–III are summarized in Supplemental Table 1. As expected, subjects with T2DM were more insulin resistant and had higher fasting plasma glucose (FPG) and fasting insulin concentrations. The VAT NPRA- and NPRC-mRNA expression was higher than the expression in the SAT in both groups of subjects in cohort I (Fig. 1, A and C). In contrast, NPRB-mRNA expression was lower in VAT compared with SAT in T2DM subjects and lower compared with the nondiabetic subjects (Fig. 1B). The SAT expression of

![FIG. 2. Correlation of NPRB- and NPRC-mRNA expression with fasting insulin concentrations in nondiabetic subjects (n = 85). Data for VAT (A) and SAT (B) are shown. vis, Visceral fat.](https://academic.oup.com/jcem/article-abstract/97/5/E731/2536565)
NPR correlated with VAT NPR expression in the general cohort I (NPRA, r = 0.274, P = 0.001; NPRB, r = 0.746, P < 0.001; NPRC, r = 0.659; P < 0.001).

Fasting insulin correlated with NPRB- and NPRC-mRNA, but not with NPRA-mRNA expression in both VAT and SAT in nondiabetic subjects (n = 108) (Fig. 2), also after adjustment for age, gender, and BMI (for NPRB, r = 0.87, P = 0.02 x 10^{-5}, and r = 0.63, P = 0.02 x 10^{-2}; for NPRC, r = 0.65, P = 0.04 x 10^{-3}, and r = 0.54, P = 0.002, respectively). NPBR- and NPRC-mRNA expression in VAT correlated inversely with FPG and age (NPBR, r = -0.28, P = 0.009 for FPG, and r = -0.25, P = 0.024 for age; NPRC, r = -0.28, P = 0.008 for FPG, and r = -0.39, P = 0.0001 for age). In the entire cohort (n = 157), fasting insulin correlated with NPBR- and NPRC-mRNA expression in both SAT and VAT (for NPBR, r = 0.22, P = 0.14, and r = 0.32, P = 0.0001; for NPRC, r = 0.27, P = 0.002, and r = 0.30, P = 0.001, respectively). Additionally, NPBR- and NPRC-mRNA expression in VAT correlated inversely with age (r = -0.27, P = 0.002; and r = -0.36, P = 0.0001, respectively). No correlation with BMI, body fat, glycosylated hemoglobin, and fasting glucose concentrations were observed (data not shown).

**Regulation of NPR-mRNA expression and circulating levels of NP by insulin and simultaneous effects of high insulin and high glucose in the clamp experiments**

In the steady state of the hyperinsulinemic-hyperglycemic clamp experiments, circulating insulin levels increased to 754.4 ± 321.7 pmol/liter and were higher as in the hyperinsulinemic-euglycemic clamp (392.7 ± 77.3 pmol/liter; P < 0.001). We observed a significant up-regulation of NPRC-mRNA in both euglycemic- and hyperglycemic-hyperinsulinemic clamps (74.7%, P = 0.038; and 26.2%, P = 0.048, respectively) (Fig. 3, A–C).

In addition, we measured circulating levels of MR-proANP, a newly described stable fragment of N-terminal proatrial NP (18). In both clamp experiments, a significant reduction of MR-proANP at about 20% of basal values was observed (Fig. 3D). Furthermore, we evaluated the effect of insulin on circulating NP levels in the normotensive subjects with normal glucose tolerance of cohort III (Supplemental Table 1). During the hyperinsulinemic-euglycemic clamp experiments, circulating insulin levels increased to 1275.1 ± 285.6 pmol/liter at 120 min. MR-proANP levels were 57.6 ± 27.7 pmol/liter at baseline and decreased during insulin infusions to 43.9 ± 17.2 pmol/liter (P < 0.0001; 23.6% from baseline MR-proANP) (Fig. 4A). In insulin-sensitive subjects (determined as glucose infusion rate above the 75th percentile), the suppression of MR-proANP was higher as in the insulin-resistant subjects (determined as glucose infusion rate below the 25th percentile), but this difference was not statistically significant (Fig. 4C). In contrast to MR-proANP, levels of NT-proBNP (n = 10) did not decline during the insulin infusion: 50.7 (13.8–123.3) pg/ml at −20 min of the clamp test vs. 55.4 (15.6–112.1) pg/ml at 120 min of the test (P = 0.8; Fig. 4B). For this subgroup, we observed a decrease of MR-proANP levels from 50.3 (range, 44.4–99.2) pmol/liter at −20 min to 47.7 (range, 32.1–74.1) pmol/liter at the end of the test (P < 0.001).
Fasting MR-proANP levels correlated with age, diastolic BP, and insulin sensitivity index (calculated as ratio of glucose infusion rate to mean of insulin concentration during the steady state of the clamp) \((r = 0.539, P = 0.005; r = -0.396, P = 0.049; r = 0.410, P = 0.042)\).

**Regulation of NPR expression in human primary monocytes and macrophages**

Macrophages expressed all three types of NPR, whereas the monocytes showed only expression of NPRB and NPRC, which was higher than in macrophages. NPRC expression was increased after the simultaneous insulin and glucose treatment for 4 h in monocytes (70.2%; \(P = 0.01\)), but not in mature macrophages (Fig. 5C). The trend to increase NPRA expression in macrophages and NPRB expression in monocytes was also detected under high glucose and insulin concentrations, but these alterations were not significant (Fig. 5, A and B).

**Reconstruction of the NPRC and NPRB gene correlations network of hyperinsulinemic-euglycemic clamp**

We attempted to understand the complexity of insulin-dependent NPR gene regulation by network-based analysis of the SAT transcriptome (Fig. 6 and Supplemental Table 3). In this network, NPRC and NPRB genes were directly opposed to each other in their actions regarding other network partners. About 30% of network genes have known functions in cell proliferation, cell differentiation, and apoptosis. NPRA had no direct connections to the NPRB and NPRC and was not included in the reconstructed network.

**Discussion**

We conducted a systematic investigation of NPR gene expression in human paired sc and visceral fat tissue samples in a cohort of subjects with different degrees of glucose tolerance. Visceral NPRA-mRNA and NPRC-mRNA were higher than sc mRNA expressions in both groups. In contrast, NPRB expression showed an inverse relationship and was down-regulated in subjects with T2DM. NPRB and NPRC expression in both fat depots positively correlated with fasting insulin levels. Furthermore, 4-h infusion of insulin in conditions of both clamped euglycemia and hyperglycemia up-regulated NPRC expression in SAT in subjects with moderate obesity and normal glu-
cose tolerance. This supported our hypothesis that insulin may regulate NPR expression in adipose tissue in humans. Moreover, insulin infusion during the euglycemic or hyperglycemic clamps led to a significant decrease of circulating MR-proANP levels. Additionally, we studied the expression of all three NPR on macrophages and monocytes in vitro, and we observed up-regulation of NPRC expression only after simultaneous high glucose and insulin treatment in monocytes but no effect of insulin or glucose in mature macrophages.

In contrast to the existing animal data (12), fat depot-specific localization of NPR has not been investigated previously in humans. In animals, the relation of NPRA to NPRC expression in adipose tissue influences ANP-stimulated lipolysis, which was observed in primates but not in rodents (4). Otherwise, an up-regulation of all three types of NPR expression in the white and brown adipose tissue under high-fat diet conditions associated with hyperinsulinemia was found in rodents (22, 23). Moreover, the NPRC expression was dramatically down-regulated in an insulin-deficient mice model (12). In the first observation in humans, up-regulation of NPRC was observed in obese subjects with hypertension (11). In contrast, moderate weight loss in humans leads to a decrease in the NPRA gene expression in the sc fat depot (24). High expression levels of NPRA and NPRC in the VAT compared with the SAT suggested that the visceral fat depot is the target organ for NP effects, which needs further investigation. Interestingly, ANP inhibits the proliferation of visceral adipocytes in vitro (25); furthermore, conditions associated with increased half-life of ANP in the circulation (5) or increased levels of BNP, the other ligand of NPRA (22), have been shown in a lean phenotype in rodents. Moreover, ANP-stimulated lipolysis in the sc fat tissue is independent of coapplication of insulin (26). Thus, ANP appears to be a natural physiological antagonist of insulin action in the adipose tissue.

Based on the inverse relationship between circulating ANP levels and up-regulated NPRC expression in hypertensive obese patients, Dessì-Fulgheri et al. (11) hypothesized the existence of the phenomenon “natriuretic peptide handicap” in obesity. In a population without manifest cardiovascular disease, a decrease of ANP and BNP, both hormones with assumed cardioprotective properties, may well increase long-term risk of cardiovascular disease such as occurs in central obesity and the metabolic syndrome (1–3). In our study, NPRC mRNA in VAT and SAT correlated strongly with fasting insulin levels, independently of other anthropometric or glycemic traits, suggesting that insulin was a major regulator of NPRC expression in adipose tissue. This hypothesis was supported by data from our clamp experiments: acute insulin infusion increased expression of NPRC in sc fat tissue independent of glucose concentration in obese subjects. Furthermore, recently published data of in vitro experiments suggested that insulin elevates NPRC expression through the phosphatidylinositol phosphatase-3 kinase pathway (12), the same pathway that leads to inhibition of lipolysis in adipocytes. The insulin-induced up-regulation of NPRC, which could increase the elimination of ANP from circulation, therefore coincides with the opposed metabolic functions of ANP and insulin in lipolysis (27). Similarly, the antinatriuretic action of insulin can be counteracted by ANP infusion (26). Otherwise, it should be noted that the potential relevance of the insulin-induced NPRC up-regulation on circulating NP levels in humans needs to be investigated in further long-term observations. The ablation of NPRC in mice leads to a remarkable increase of half-life of ANP, but had no effect on circulating levels of both hormones, ANP and

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**FIG. 5.** A–C, Regulation of NPR expression in human primary monocytes and macrophages with respect to different concentrations of insulin and glucose. Data are expressed as mean ± SD. Expression levels of NPRA (A), NPRB (B), and NPRC (C) were studied in the three different conditions: normal glucose (5.5 mM) (white columns), high glucose (30 mM) (gray columns), and high glucose (30 mM) with insulin (10 nm) (dark columns). P < 0.05 in the paired t test. HPRT, Hypoxanthine phosphoribosyltransferase.
BNP (5), suggesting the existence of a feedback mechanism for ANP and BNP synthesis in vivo (4). Moreover, NPRC has been shown to stimulate phospholipase C in a G protein-dependent manner (4). Thus, it is possible that NPRC mediates similar yet to be discovered NP functions in adipose tissue.

In this study, we observed an insulin-mediated reduction of MR-proANP, but not of NT-proBNP, in the hyperinsulinemic-euglycemic clamp. In healthy subjects, levels of BNP are significantly lower than those of ANP (4). ANP might be physiologically more relevant because genetic deletion of ANP in mice resulted in hypertension, atherosclerosis, and cardiac hypertrophy, whereas deletion of BNP caused cardiac fibrosis, only without the more generalized vascular phenotype (28). Some previous studies investigated the role of ANP or BNP in euglycemic clamps or in iv glucose tolerance tests (26, 29–32). These studies yielded variable results that may be related to differences in glucose infusion rates, hyperglycemia, and the high variability of assays determining ANP (33) rather than the more stable N-terminal fragments. The assays of MR-proANP and of NT-proBNP used in our study were shown to correlate closely with clinical end points of cardiovascular diseases (34) as well as with markers of the metabolic syndrome (35).

Based on the possible contribution of macrophages to effects described in adipose tissue, we also examined the influence of acute insulin and glucose treatment on NPR expression in cultures of primary human monocytes and macrophages. In accordance with known links between NP and the immune system (7), we detected all three types of NPR on macrophages but only NPRB and NPRC at higher expression levels on monocytes. NPRC expression was increased after simultaneous insulin and glucose treatment in monocytes, but not in mature macrophages. Thus, the NPRC up-regulation in the SAT in our experiments in vivo originated most likely from adipocytes or adipocyte-macrophage interactions but not from adipose tissue macrophages alone.

In conclusion, we conducted the first systematic investigation of NPR gene expression in human paired sc and visceral fat tissue samples in a cohort of subjects with varying degrees of glucose tolerance. Acute insulin treatment increased expression of NPRC in the sc fat depots and decreased circulating levels of MR-proANP, independent of glucose concentrations. Thus, in the obese state insulin might suppress circulating levels of NP via up-regulation of NPRC expression. Our observations provide a novel and direct link from hyperinsulinemia to cardiovascular components of the metabolic syndrome.

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