Influence of Salt Intake on Renin–Angiotensin and Natriuretic Peptide System Genes in Human Adipose Tissue

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Abstract—We tested the hypothesis that changes in sodium intake modulate adipose-tissue renin–angiotensin and natriuretic peptide system gene expression in humans. We studied 9 healthy young men in a metabolic ward at constant room temperature, humidity, and water, potassium, and calcium intake. Subjects were submitted to 4 different periods of sodium intake, and blood samples, microdialysis samples (interstitial fluid), and biopsies from subcutaneous abdominal adipose tissue were obtained at the end of the low-sodium period (0.7 mmol Na/kg per day) and at the end of the high-sodium period (7.7 mmol Na/kg per day). Urinary sodium excretion was 64±4 mmol per day with the low-sodium diet and 521±8 mmol per day with the high-sodium diet. Systemic and microdialysate sodium concentrations were similar with both interventions. With high-sodium intake, systemic renin activity and aldosterone levels were suppressed, angiotensin-converting enzyme activity did not change, and systemic levels of the atrial natriuretic peptide increased. High-sodium diet increased angiotensin-converting enzyme and atrial natriuretic peptide gene expression in adipose tissue. None of the other genes tested were influenced by changes in dietary sodium intake. Our findings suggest that the adipose-tissue renin–angiotensin system is not part of a feedback mechanism regulating sodium homeostasis and blood pressure. Systemic and adipose-tissue renin–angiotensin systems are regulated at least in part independently from each other. In contrast, systemic atrial natriuretic peptide and adipose-tissue atrial natriuretic peptide respond similarly to changes in sodium intake. (Hypertension. 2006;48:1103-1108.)

Key Words: adipose tissue ■ gene expression ■ renin ■ angiotensinogen ■ aldosterone ■ atrial natriuretic peptide ■ sodium intake

Recent studies suggest that adipose tissue modulates the balance between the renin–angiotensin–aldosterone system (RAAS) and the natriuretic peptide system. Adipose tissue accumulation raises blood pressure through volume expansion and increased cardiac output. Systemic RAAS activity is increased in obese subjects, particularly in those with arterial hypertension. In contrast, circulating atrial natriuretic peptide concentrations and atrial natriuretic peptide responsiveness are reduced in obesity. Weight loss reverses systemic RAAS activity and restores the responsiveness to natriuretic peptide infusions. Adipose tissue mechanisms are implicated by the observation that RAAS gene expression is increased in hypertensive obese subjects. Secretion of adipose tissue-derived angiotensinogen (AGT) into the systemic circulation has been demonstrated in animals. We observed a strong relationship between adipose tissue AGT expression and circulating AGT levels in humans. Adipose tissue-related mechanisms may also alter the activity of the natriuretic peptide system. Adipose tissue expresses the natriuretic peptide receptors A and C (NPR-A and NPR-C). The NPR-C receptor, which is thought to clear atrial natriuretic peptide (ANP) from the circulation, is upregulated in obesity. In rats, weight loss reduces NPR-C expression in adipocytes. Thus, the adipose-tissue RAAS and natriuretic peptide systems may have an important bearing on systemic sodium homeostasis, and these changes typically originate in gene expression changes in adipocytes. However, the regulation of these systems at the adipose tissue level is not understood. We tested the hypothesis that changes in sodium intake modulate adipose RAAS and natriuretic peptide system gene expression in humans. Furthermore, we determined whether or not changes in interstitial sodium concentration in adipose tissue might be involved.

Methods

Study Design

Nine healthy men were studied (25.7±0.4 years, 71.0±1.2 kg body weight (BW), and 21.9±0.4 kg/m² body mass index). All of the patients gave their written consent before enrollment. The study was approved by the Ethics Committee of the “Aerztekammer Nor-
Fasting blood samples were drawn at 7:00 AM before standing up (BOSO-Medicus, Bosch and Sohn) 3 times with a 2-minute period next morning. Blood pressure was measured by sphygmomanometry. Protein intake was 1.4 g per kilogram of BW per day, fat intake produced by nutrient oxidation. The diet of the test subjects was recommended intake.11 Needle biopsy from the periumbilical region.3,7 Isolation of total Abdominal subcutaneous adipose tissue samples were taken by expression analysis pump (CMA Microdialysis AB) as described.13,14 After probe Subcutaneous Microdialysis Scale, BP2100S; sensitivity and before breakfast with a precision scale (Sartorius, Precision room temperature and humidity were kept constant during the study (temperature: 24°C; relative humidity: 55%). Strenuous physical exercise, methylxanthine ingestion, and alcohol consumption were not permitted throughout the study. The subjects were constantly monitored by a study nurse.

Dietary Intervention
Subjects were submitted to a 28-day study with 4 separate periods. During the first 6-day period, subjects ingested 0.7 mmol of Na per kilogram of body weight (BW) per day (low sodium). During the second 6-day period, they ingested 2.8 mmol of Na per kilogram of BW per day corresponding with the “normal” sodium intake in Germany. Then, sodium intake was increased to 7.7 mmol of Na per kilogram of BW per day for 10 days (high sodium). Finally, subjects ingested 0.7 mmol of Na per kilogram of BW per day for another 6 days. Approximately 95% of the ingested sodium was sodium chloride. All of the subjects had a constant fluid intake of 40 mL per kilogram of BW per day including ~300 mL per day of water produced by nutrient oxidation. The diet of the test subjects was individually tailored according to their BW and resting metabolic rate. Protein intake was 1.4 g per kilogram of BW per day, fat intake was <30% of the caloric intake, and the remaining calories were provided by carbohydrates. Potassium intake was 110 mg per day. Calcium intake was kept constant at 1000 mg per day. The daily intake level of all other nutrients matched the German dietary recommended intake.11

Measurements
All of the reported results refer to day 5 or 6 (blood samples) of the first low-sodium period and to day 9 or 10 (blood samples) of the high-sodium period. BW was measured every morning after voiding and before breakfast with a precision scale (Sartorius, Precision Scale, BP2100S; sensitivity ±5 g). Urine was collected as 24-hour urine from 7:00 AM one morning (emptying bladder) to 7:00 AM the next morning. Blood pressure was measured by sphygmomanometry (BOSO-Medicus, Bosch and Sohn) 3 times with a 2-minute period between measurements every morning before the subjects stand up. Full blood samples were drawn at 7:00 AM before standing up under standardized conditions. Blood was drawn from the subjects in the supine body position without venostasis through an indwelling catheter in an antecubital vein that was inserted in the supine body position without venostasis through an indwelling catheter in an antecubital vein that was inserted ≥30 minutes before blood sampling. Blood for analysis of arginine vasopressin (AVP), ANP, and renin was collected in ice-chilled tubes. To analyze serum electrolytes, angiotensin-converting enzyme (ACE) activity and aldosterone concentrations blood was collected in tubes without any additive. Renin (IRMA, Nichols Institute) and aldosterone concentrations (MAIA, Adaltis) were measured by radioimmunoassay kits. ACE activity in the serum was determined by a colorimetric assay (Sigma Diagnostics), and ANP was determined by radioimmunoassay as described previously.12

Subcutaneous Microdialysis
One CMA/60 microdialysis probe was inserted into abdominal subcutaneous adipose tissue and connected to a CMA/102 microdialysis pump (CMA Microdialysis AB) as described.13,14 After probe insertion, tissue perfusion with a 50 mmol/L glucose infusion solution (Serumwerk Bernburg AG) was started at a flow rate of 2 µL/min. After instrumentation, subjects recovered for ≥60 minutes. Then, perfusion rate was lowered to 0.3 µL/min to achieve almost complete sodium recovery. Dialysates were collected every 15 minutes for 150 minutes.

Adipose Tissue Biopsies and Gene Expression Analysis
Abdominal subcutaneous adipose tissue samples were taken by needle biopsy from the periumbilical region.3,7 Isolation of total RNA and cDNA synthesis for real-time PCR (TaQMan technology by PE Biosystems) were performed as described previously.3,7 The standard curve method was used for the target genes and internal control genes (GAPDH, 18S rRNA). We determined the expression of AGT, renin, ACE, the angiotensin II type 1 (AT1) receptor, ANP, NPR-A, and NPR-C. Human GAPDH and 18S ribosomal RNA genes were both measured to select an appropriate internal control gene. However, both genes are suitable, because they did not change expression levels between the low- and high-salt periods. Thus, expression of all of the target genes was normalized by GAPDH expression in each sample, and the results are given in arbitrary units. Primer sequences are available on request.

Data Analysis
All of the data are given as mean±SEM. Intraindividual differences were compared by paired t test (parametric data) or the Wilcoxon matched-pairs test (nonparametric data). A P value <0.05 indicates statistical significant differences between groups.

Results
Urine, Blood, and Adipose Tissue Sodium
Urinary sodium excretion was 64±4 mmol per day with the low-sodium diet and 52±8 mmol per day with the high-sodium diet (P=0.0002). Serum sodium concentrations were 144±1 mmol/L with the low-sodium and 147±1 mmol/L with the high-sodium diet (P value not significant). Dialysate sodium concentrations in abdominal adipose tissue were 116±8 mmol/L with the low-sodium and 109±10 mmol/L with the high-sodium diet (P value not significant). In both periods, no fluid retention in adipose tissue during microdialysis was observed (total perfusate volume=total dialysate volume).

BW and Blood Pressure
BW increased from 71.0±1.2 kg at the end of the low-salt period to 72.7±1.3 kg at the end of the high-salt period (P<0.001 by paired-sample t test). Weight gain because of excess caloric intake can be excluded, because the energy content of the diet was adjusted to exactly match daily needs. Furthermore, a 1.7-kg weight gain through increased adipose tissue mass would require a positive energy balance of ≥11 900 kcal (1.700 g of adipose tissue×7 kcal/g of adipose tissue). Mean arterial pressure in the morning was 101±2 mm Hg in the low-sodium period versus 99±3 mm Hg in the high-sodium period (P value not significant, data not shown).

Sodium Regulating Hormones
Venous renin and aldosterone concentrations were profoundly suppressed with the high-sodium diet (Figure 1). Venous ANP was slightly but significantly increased with the high-sodium diet. Serum ACE activity was similar with low- and high-sodium diets. Circulating vasopressin concentration was 1.0±0.2 pg/mL with low- and 1.2±0.2 pg/mL with high-sodium intake (P value not significant).

Adipose-Tissue Gene Expression
We obtained good quality adipose tissue biopsies in 8 subjects. One subject experienced a vasovagal syncope and could not complete the procedure. Figure 2 illustrates AGT, renin, ACE, and AT1-receptor mRNA expression with the low-sodium and high-sodium diet. ACE gene expression was slightly increased with the high-sodium diet. Renin, AGT,
and AT₁-receptor gene expressions were similar with both diets. Adipose tissue gene expression of the NPR-A and NPR-C receptors did not change, whereas ANP mRNA expression increased moderately but significantly with the high-sodium diet (Figure 3).

**Discussion**

We tested the hypothesis that dietary salt intake regulates RAAS and the natriuretic peptide system expression in adipose tissue. Except for a previous study in rats,¹⁵ our study is the first to assess adipose-tissue gene regulation with changes in dietary sodium intake. The issue is scientifically and clinically relevant, considering the sodium retention in obesity associated-hypertension,¹⁶ which is associated with altered adipose RAAS and natriuretic peptide system function.¹⁷ We obtained adipose tissue biopsies after periods with low- and high-sodium diets in a metabolic ward. Because the environment was carefully controlled, complete adherence of

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**Figure 1.** Renin, aldosterone, ACE, and ANP concentrations on low-sodium (LS) and high-sodium (HS) diet in 9 healthy young volunteers. ***P<0.0001.

**Figure 2.** Renin (REN), ACE, AGT, and AT₁ receptor (AT₁-R) mRNA expression in subcutaneous adipose tissue biopsies on low-sodium (low) and on high-sodium (high) diet. *P<0.05.
the subjects to the assigned diets was granted, and this was reflected by an ≈10-fold greater urinary sodium excretion with the high- compared with the low-sodium diet. Serum and interstitial sodium concentrations in adipose tissue did not change with high- or low-sodium intake.

Changes in sodium intake were associated with the expected changes in the activities of the systemic RAAS and the natriuretic peptide system. With the high-sodium diet, circulating renin activity and aldosterone concentrations were strongly suppressed as expected, whereas circulating ANP was slightly increased. Thus, we are confident that the physiological stimulus elicited by the change in dietary sodium intake was sufficiently robust. Further, we suggest that our study encompasses the range of sodium intakes that could be reasonably expected in industrialized countries. To our surprise, we did not find a strong response of the adipose-tissue RAAS to the high-salt diet. Instead, only ACE gene expression increased slightly with high-sodium intake. Renin, AGT, and AT₁-receptor mRNA expression did not change. Our data also suggest that changes in systemic RAAS activity have a rather limited effect on local AT₁ receptors in adipose tissue.

Previous studies assessed systemic and tissue RAAS responses to changes in salt intake in various rat models. Our study is the first to address the issue in human subjects. Salt loading in most but not all of the studies decreased renin expression in the kidney. Salt loading activated AGT expression in the kidney, heart, and aorta in Sabra and Dahl rats. In contrast, salt depletion increased AGT expression in the kidney and aortic smooth muscle layer of Sprague-Dawley and Wistar-Kyoto rats. Hepatic AGT expression was not salt responsive. Cardiac ACE activity and gene expression was activated in response to a high-salt diet in Wistar-Kyoto and stroke-prone spontaneously hypertensive rats (SHR). AT₁ receptor gene expression in the kidney was not responsive to increased dietary sodium intake in Sabra rats but was increased in Sprague-Dawley rats. One study in Wistar-Kyoto rats assessed adipose tissue AGT expression in periaortic adipose tissue. In this study, AGT gene expression was not salt responsive. Thus, RAAS gene expression at the tissue level responds in a tissue-specific and strain-specific fashion. Responses in human adipose tissue cannot be extrapolated from animal studies.

Our findings suggest that the human adipose-tissue RAAS is not part of a feedback mechanism regulating sodium homeostasis and blood pressure. Nevertheless, the adipose RAAS may have impact on blood pressure control. The idea is supported by the observations that adipose tissue-derived ANP is secreted into the systemic circulation and elicits an increase in blood pressure. The strong relationship between adipose-tissue AGT expression and circulating AGT levels in humans further supports this idea. Given the potential contribution of adipose-tissue–derived AGT to systemic blood pressure, mechanisms regulating AGT expression deserve attention. Adipose-tissue AGT expression differs between lean and obese subjects. Weight loss alters AGT expression as well. A recent study showed that insulin stimulates adipose-tissue angiotensin II production in vitro. The response seems to be mediated through tumor necrosis factor-α and is reversed with PPARγ stimulation. Tumor necrosis factor α is upregulated in obesity and declines with weight loss. We suggest that adipose-tissue RAAS activity may be regulated through metabolic factors and tissue inflammation rather than sodium intake.

One possible explanation for the disparate regulation of systemic and adipose tissue RAAS activity is that both systems serve different physiological purposes. We recently applied angiotensin II to the interstitial compartment using the microdialysis technique. Interstitial angiotensin II had a limited effect on tissue perfusion. Yet, carbohydrate and lipid metabolism responded to angiotensin II in a tissue-specific fashion. Bradykinin, which is degraded by ACE, may also influence adipose tissue metabolism. We speculate that an increase in adipose ACE expression with high-sodium diet may lead to secondary changes in tissue metabolism. Indeed, reduction in dietary sodium intake decreases whole body insulin sensitivity and influences brown adipose tissue thermogenesis.

Increased sodium intake was associated with augmented ANP mRNA expression in adipose tissue. ANP secretion by adipocytes has not been demonstrated. Other cells may have contributed to the increase in ANP gene expression on high-sodium diet. Whether or not ANP produced by adipose tissue significantly contributes to systemic ANP concentrations and, thus, volume and blood pressure regulation, is unknown. ANP regulates adipose tissue metabolism. In vitro, ANP activates hormone sensitive lipase (HSL) through an increase in cGMP production and HSL phosphorylation in human adipocytes. We showed recently that ANP in physiological concentrations increases adipose tissue lipolysis in human subjects. Increased ANP production in adipose tissue in response to a salt load could conceivably stimulate lipolysis locally in an autocrine or paracrine fashion.

The mechanism mediating the effect of dietary sodium intake on adipose tissue gene expression is unknown. Sodium could have a direct effect on adipose-tissue gene expression. In rice plants, 57 genes are regulated by direct exposure to...
increased sodium concentrations. To address the issue, we assessed interstitial sodium concentrations in adipose tissue using near equilibrium microdialysis. Microdialysate sodium concentrations were similar with low-sodium and high-sodium diets. Thus, changes in adipose tissue gene expression cannot be explained by differences in interstitial sodium concentrations. The notion of nonosmotically active sodium storage has been raised recently that postulates sodium binding to glycosaminoglycans, which are abundant in the adipose tissue pieces that we sampled. We speculate that nonosmotically active sodium may have influenced adipose tissue gene expression. It is also possible that changes of an unknown circulating factor led to secondary changes in adipose tissue gene expression.

The major limitation of our study is that we relied on mRNA expression in adipose tissue. We do not have data on protein expression or local RAAS function. Furthermore, we changed sodium intake over a relatively brief period of time. We cannot exclude the possibility that the adipose-tissue RAAS shows a delayed response to a change in sodium intake.

Perspectives
Previous studies suggested that systemic RAAS activity is increased in obese subjects, particularly in those with arterial hypertension, whereas the ANP peptide system is suppressed. Adipose tissue mechanisms may contribute to the disbalance between both sodium regulating systems. Our study suggests that the systemic and the adipose-tissue RAAS are regulated at least in part independent from each other. The adipose tissue RAAS is not responsive to increased sodium intake and may be regulated by metabolic factors. In contrast, systemic ANP and adipose tissue ANP seem to respond in a similar fashion to changes in sodium intake.

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