Towards an integrated approach on RAAS-blockade

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LOW DIETARY SODIUM AND EXOGENOUS ANGIOTENSIN II INFUSION DECREASE PLASMA ADIPONECTIN CONCENTRATIONS IN HEALTHY MEN

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Stephan Bakker
Frans Boomsma
Robin Dullaart
Bruce Wolffentbuttel
Gerjan Navis
Abstract

Adiponectin has anti-inflammatory and vascular protective effects, and may improve insulin sensitivity. Animal data suggest a role of the renin-angiotensin aldosterone system (RAAS) in the regulation of adiponectin.

Investigate the role of the RAAS in regulation of adiponectin in humans in vivo. To this purpose we studied the effects of physiological (change in sodium status) and pharmacological modulation of RAAS activity (angiotensin II infusion and enalapril treatment) on plasma adiponectin.

Design, setting and patients 35 healthy male volunteers (aged 26 ± 9 years) were studied after two 7 day periods; one on low sodium (LS: 50 mmol Na+/day) and one on high sodium (HS: 200 mmol Na+/day). At the end of each period, adiponectin was measured and its response to angiotensin II infusion (0.3, 1 and 3 ng/kg/hr all during 1 hour) was determined. Additionally, all subjects received administrated to one week treatment of enalapril 20 mg once daily (ACEI) during HS.

Plasma adiponectin concentrations during LS and HS and in response to angiotensin II infusion. The suppression of the RAAS by HS elicited a significant rise in adiponectin (LS baseline 11.9 (8.3-16.2) ug/L; HS baseline 14.4 (11.2-20.4) ug/L, p<0.05). All dosages angiotensin II elicited a profound decrease in adiponectin during both conditions (LS 3 ng/kg/hr 7.4 (6.3-8.9) ug/L, HS 3 ng/kg/hr 8.4 (7.3-9.9) ug/L both p<0.001 vs. baseline). ACEI induced a significant rise in adiponectin (16.6 (10.6-20.9) ug/L, p<0.05 vs HS).

Physiological and pharmacological modulation of RAAS affects plasma adiponectin with lower concentrations during the high angiotensin II conditions. The therapeutic potential of RAAS blockade as a tool to correct hypoadiponectinemia should be further explored.
**Introduction**

Adiponectin is specifically and abundantly produced in adipose tissue, and has direct effects on glucose and lipid metabolism [1]. The anti-inflammatory and cardioprotective properties of this adipokine are increasingly recognized. Adiponectin circulates in plasma at high concentrations [2]. Plasma adiponectin concentrations are inversely associated with insulin resistance and obesity. Furthermore lower concentrations of adiponectin have been described in patients type 2 diabetes mellitus. Low adiponectin concentrations have also been documented in subjects with essential hypertension and may predict incident cardiovascular disease.

Furthermore, lower concentrations of adiponectin are associated with an increased risk for the development of diabetes [3;4] essential hypertension [5] and myocardial infarction [6]. These associations are attributed to anti-atherogenic actions of adiponectin, as well as its favourable effects on insulin sensitivity [7].

The mechanisms involved in the regulation of plasma adiponectin are of considerable interest, but are largely unknown. Several factors have been proposed to determine plasma adiponectin, such as its renal clearance [8] and activation of the peroxisome proliferator-activated receptor gamma (PPAR-γ) [9]. Plasma adiponectin concentrations have been shown to be upregulated by thiazolidinediones [10]. They have been proposed to ameliorate insulin resistance by binding and activating PPAR-γ in adipose tissue, thereby promoting adipocyte differentiation and increasing the number of small adipocytes that are more sensitive to insulin [11].

Interestingly, in diabetic [12] and hypertensive patients [13], inhibition of the renin-angiotensin aldosterone system (RAAS) by angiotensin receptor type 1 blockade (ARB) or angiotensin converting enzyme inhibition (ACEi) increases plasma adiponectin. Moreover, very recently infusion of angiotensin II (ang II) in rats was shown to decrease adiponectin concentrations, and this response was prevented by ang II receptor blockade [14]. These observations elicit the hypothesis that the RAAS is involved in regulation of adiponectin in humans.

To test this hypothesis we studied the effects of physiological and pharmacological modulation of the RAAS on plasma adiponectin in healthy subjects. Physiological modulation of RAAS-activity was induced by a shift in dietary sodium intake. Pharmacological modulation was achieved by infusion of exogenous ang II (stimulation) on both sodium intakes, and ACEi during high sodium intake.

**Methods**

*Subjects*

Thirty-five healthy men (age 26 ± 9 years (mean ± SD)) were recruited for the study. They were normotensive, defined as sitting systolic blood pressure <140 mmHg and diastolic blood pressure <80 mmHg. All subjects underwent normal routine physical examination. Exclusion criteria were any
abnormalities during routine physical examination, a body mass index > 30 kg/m², diabetes mellitus and drug use. Written informed consent was obtained from each subject after a full explanation of the study. The study protocol was approved by the Ethics Committee of the University Medical Centre Groningen.

**Study Protocol**

The protocol consisted of two periods, in which all the subjects were first studied after a 7-day period on a low sodium diet (LS; aim: 50 mmol/d) and afterwards a 7-day period on a high sodium diet (HS; aim: 200 mmol/d). The diets were based on personal food habits. We achieved differences in sodium intake by replacing sodium-rich products with a low-sodium product of the product group in order to remain isocaloric with a similar balance between protein, carbohydrate and fat. On day 4 and day 6 of each dietary period, subjects collected 24-hour urine to assess dietary compliance and achievement of a stable sodium balance.

On day 7 the subjects reported to the research unit at 8:00 a.m. after an overnight fast. Body weight, length, waist and hip values were measured. An intravenous canula was inserted into each forearm, one for drawing blood samples, the other for infusion of ang II and renal function tracers. Subjects remained in semi-supine position after a light standardized breakfast in a quiet room for 3 hours to standardize their activities and posture before the ang II infusion and blood sampling. We measured blood pressure at 15-minute intervals using a non-invasive device (Dinamap®; GE Medical systems, Milwaukee, WI, USA).

**Glomerular filtration rate**

Glomerular filtration rate (GFR) was measured by constant infusion of radioactive-labelled tracers, $^{125}$I-iothalamate (Tyco Health Care, Petten, The Netherlands) and $^{131}$I-hippurate (QOL Medical, Woodinville, WA, USA), respectively, as previously described [15]. After drawing a blank blood sample, a priming solution containing 0.4 ml/kg body weight of the infusion solution (0.04 MBq of $^{125}$I-iothalamate and 0.03 MBq of $^{131}$I-hippurate) plus an extra of 0.6 MBq of 125I-iothalamate was given at 8 am, followed by infusion at 12 ml/h. In order to attain stable plasma concentration of both tracers, a

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Sodium</td>
</tr>
<tr>
<td>24h urinary sodium excretion (mmol/24h)</td>
<td>40 ± 25</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>79.1 ± 9.4</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>85 ± 7</td>
</tr>
<tr>
<td>Glomerular filtration rate measured (ml/min/1.73m²)</td>
<td>103 (99-113)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD or median (25% and 75% percentile), * p<0.05 for low vs. high sodium (Student paired t-test), # p<0.05 for low vs. high sodium (Wilcoxon Signed Rank test)
two hour stabilization period followed, after which baseline measurements started at 10:00 am. The clearances were calculated as \((U \times V)/P\) and \((I \times V)/P\), respectively. \(U \times V\) represents the urinary excretion of the tracer, \(I \times V\) represents the infusion rate of the tracer; \(P\) represents the tracer value in plasma at the end of each clearance period.

This method corrects for incomplete bladder emptying and dead space, by multiplying the urinary clearance of \(^{125}\)I-iothalamate with the ratio of the plasma and urinary clearance of \(^{131}\)I-hippuran [16].

**Angiotensin II infusion**

Baseline values for blood pressure were obtained from 10 to 12 am. Between 12 a.m. and 3 p.m. ang II (Clinalfa, Merck Biosciences AG, Läufelfingen, Switzerland) was administered in the left antecubital vein. Between 12 a.m. and 1 p.m. ang II was infused at a constant rate in a dose of 0.3 ng/kg/h. Thereafter, ang II was infused at a constant rate of 1 ng/kg/h and 3 ng/kg/h each during one hour. At the end of each infusion step (at 1, 2 and 3 pm) blood samples were drawn. During the ang II infusions blood pressure was measured at 5-minute intervals.

**ACE-inhibition**

Additionally all subjects received one week of enalapril 20 mg once daily (ACEi) while being on high sodium intake (200 mmol/d). At the end of the week blood samples were drawn for adiponectin measurement and blood pressure was measured by Dinamap.

**Blood sampling and analysis**

Blood samples for baseline assessment of adiponectin were drawn at 11 am. Samples were drawn in semi-supine position, in pre-chilled tubes and immediately centrifuged at 4°C. Plasma and serum for measurement of adiponectin, aldosterone, renin activity and insulin was stored at -20°C until analysis. Aldosterone was measured with a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma renin activity (PRA) was measured as described previously with a radioimmunoassay that detects the amount of angiotensin I produced per hour in the presence of excess angiotensinogen (nanograms of angiotensin I produced per milliliter of plasma per

### Table 2 | Circulating values of RAAS components, glucose, insulin and insulin sensitivity

<table>
<thead>
<tr>
<th></th>
<th>Low Sodium</th>
<th>High Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity (ng angI/ml/h)</td>
<td>5.9(4.4-8.1)</td>
<td>2.5(1.6-3.5)*</td>
</tr>
<tr>
<td>Aldosterone (ng/L)</td>
<td>130(81-174)</td>
<td>43(24-57)*</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>4.5 (4.1-4.9)</td>
<td>4.5 (4.2-4.8)</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>8.3 (6.1-13.5)</td>
<td>8.0 (6.3-11.6)</td>
</tr>
<tr>
<td>HOMA: homeostasis model assessment ([\text{glucose} \times \text{insulin} / 22.5])</td>
<td>1.7 (1.3-2.5)</td>
<td>1.6 (1.2-2.4)</td>
</tr>
</tbody>
</table>

Data are expressed as median (25% and 75% percentile), *p < 0.05 for low vs. high sodium (Wilcoxon Signed Rank test)
hour). This assay measures the enzymatic activity of active plasma renin in the presence of an exogenous excess of its substrate [17]. Fasting serum insulin was determined using a radioactive immunoassay (DSL-1600; Diagnostic Systems Laboratories, Webster, TX). Plasma glucose was determined by the glucose-oxidase method (YSI 2300 Stat plus; Yellow Springs, OH, USA). Homeostasis model assessment (HOMA) was calculated by \( \text{glucose (in millimoles per liter) \times insulin (in microunits per milliliter)}/22.5 \).

Blood samples for determination of ang II was drawn in cold, standard 3ml vacuum tubes containing 5.4 ug K3EDTA and an additional 0.2ml ACE inhibitor cocktail containing 1.704 ug phenantroline, 0.16mg enalaprilat, 1ml ethanol and 4mg neomycin. After centrifugation at 4°C, the plasma for determination of ang II was snap-frozen and stored at -80°C until analysis. Plasma concentration of ang II was measured by a specific radioimmunoassay after SepPak extraction of plasma samples and HPLC separation [18]. Plasma adiponectin and leptin were measured by enzyme-linked immunosorbent assays using a kit from Linco Research Inc. (St. Charles, MO, USA, cat no EZHADP-61K). Within assay coefficient of variation for adiponectin and leptin was 3.4% and 3.7% respectively.

**Data analysis**

Mean values and standard deviations were calculated for normally distributed variables after checking for normality. Medians and quartiles were computed for variables with non-normal distribution. After testing for normality, we used Student paired t-test or Wilcoxon Signed Rank test to compare values between the different periods, each subject being its own control. A value of \( p<0.05 \) was considered to be statistically significant.

![Figure 1 | Plasma angiotensin II and adiponectin](image)

Ang II: plasma angiotensin II, data are expressed as median (25% and 75% percentile), * \( p<0.05 \) for low vs. high sodium. (Wilcoxon Signed Rank test)
Results

Effects of altered sodium status

The 35 healthy male subjects had a BMI of 22.5 (21.2-24.4) kg/m2 and waist circumference was 81 (76-85) cm. Urinary sodium excretion was consistent with good dietary compliance at both sodium intakes, as shown in table 1. HS induced a small but significant rise in weight and blood pressure. HS also elicited an increase in GFR.

The effects on PRA and aldosterone are given in table 2. The shift from LS to HS intake suppressed PRA and aldosterone concentration significantly to approximately one-third of the values on LS. The shift in sodium intake did not affect glucose or insulin concentration nor the HOMA index.

The shift from LS to HS suppressed ang II concentration in proportion to the decreases in PRA and aldosterone (figure 1, left y-axis). HS elicited a significant rise in adiponectin by 21 % from 11.9 (8.3-16.2) to 14.4 (11.2-20.4) (figure 1, right y-axis). During baseline, ang II levels did not correlate
Angiotensin II infusion

Ang II infusion induced a dose-dependent significant increase in MAP (figure 2, left panel) during both low and high sodium. During low sodium the lowest dose of ang II did not induce a significant rise in MAP (LS baseline vs. 0.3 ng ang II: p=0.45). During both low and high sodium, all other dosages of ang II induced a significant rise in MAP compared to the MAP at the preceding lower rate of infusion (p<0.001). Plasma adiponectin concentrations (figure 2, right panel) decreased significantly (LS/HS baseline vs. 0.3, 1 and 3 ng, p<0.001) during all steps of ang II infusion compared to baseline, both during LS and HS, with a prominent decrease during the lowest dose of ang II already. Figure 3 shows the changes from baseline in blood pressure and adiponectin during ang II infusion (3 ng/kg) on the two different sodium intakes, allowing a comparison of the ang II responses on LS and HS. As anticipated, the overall blood pressure response to ang II was higher during HS (Δ MAP: LS vs. HS, p<0.05). Likewise, the overall decrease in adiponectin was larger during HS as well (Δ adiponectin: LS vs HS, p<0.05).

ACE-inhibition

MAP decreased significantly during ACEi (88±8 vs 83±8 mmHg, p<0.05). ACEi induced a significant rise in adiponectin (14.4 (11.2-20.4) vs 16.6 (10.6-20.9) ug/L, p<0.05).
Leptin

Sodium intake did not significantly affect leptin concentrations (figure 4). During low sodium intake ang II infusion did not influence leptin concentrations. However, during high sodium there was a small but significant decrease in leptin concentrations during ang II infusion.

Discussion

This study is the first study that shows that physiological suppression of ang II by high sodium intake and ACEi are associated with an increase in plasma adiponectin concentrations. Moreover, plasma adiponectin is profoundly decreased by infusion of exogenous ang II. Thus, physiological and pharmacological modulation of ang II is associated with reciprocal changes in adiponectin concentrations. Together, these findings support the hypothesis that the RAAS contributes to a relevant extent in plasma adiponectin regulation in humans in vivo, most likely via ang II.

The shift in sodium intake, a physiological modifier of the endogenous RAAS-activity, affected adiponectin. The changes in circulating RAAS parameters, renin, aldosteron and ang II, mirrored the changes in plasma adiponectin. This suggests that the influence of the RAAS on adiponectin is likely to be physiologically relevant, and is not limited to pharmacological interventions. This holds all the more true because the range of sodium intake that we studied was not excessive, and is well within the range encountered in the normal population [19]. Our observations are in line with primarily data, published in abstract form showing a decrease in adiponectin concentration after three days of sodium restriction [20]. It seems unlikely that an effect on renal elimination contributed to changes in circulating adiponectin in response to modification of sodium intake, because a high sodium diet was accompanied by an increase rather than by a decrease in GFR. To assess the possible clinical
We found that exogenous ang II suppressed adiponectin, supporting a role for the RAAS in regulation of adiponectin in human. These data extend recent data in rats [23;24]. We found that the suppressor effect on adiponectin was present both during low and high dietary sodium, and was already present at non-pressor doses of ang II. As ang II elicited a pronounced drop in adiponectin even at the lowest dose used here, we were not able to identify a threshold dose. During both conditions, after three hours of ang II infusion, the plasma adiponectin concentrations were approximately half of the baseline concentrations. The estimated half life of adiponectin is 2.5 hours [25]. Taken together, these findings thus suggest that the release of adiponectin from adipocytes may to a considerable extent be blocked by ang II.

Which mechanisms could be involved in the effects of ang II on adiponectin? Clasen et al. showed that blockade of the ang II type I receptor can induce stimulation of adiponectin mRNA expression in adipocytes [26], suggesting that the ang II type I receptor is involved. Recent data from Kurata et al. show that olmesartan, an ang II type 1 receptor blocker, attenuates hypoadiponectinemia consequent to obesity and aging in mice [27]. This is in line with observations by Ran et al. in rats [28] who found decreased adiponectin concentrations during ang II infusion which were restored by the olmesartan. They observed changes in adiponectin concentrations after chronic infusion of ang II. However, we observed already changes in adiponectin concentrations after 1 hour of infusion of ang II. The ang II type I receptor is expressed in adipocytes [29]. Therefore, a possible mechanism could be that stimulation of the ang II type I receptor blocks the release of adiponectin from adipose tissue. However, we don’t have direct proof of a role of the ang II type 1 receptor, as we did not study the effects of ang II infusion on adiponectin in combination with an ARB.

Analysis of the changes in adiponectin during ang II infusion in relation to sodium status reveals a parallel between the responses of blood pressure and adiponectin. The blood pressure response to ang II was increased during high sodium, which is in line with prior observations that high sodium potentiates the pressor response to ang II [30]. The mechanism of the potentiation of the pressor response to ang II by high sodium is assumed to be both lower receptor occupancy as well as up regulation of the ang II type I receptor [31]. Interestingly in our study, the response of adiponectin to exogenous ang II was enhanced by high sodium, which parallels the potentiation of the pressor response. Further studies into the role of the ang II type I receptor in the regulation of adiponectin on local adipose tissue level [32] and the impact on plasma concentration are obviously warranted.
Another possible mechanism by which our results could be explained is the sympathetic nervous system. It is known that both dietary sodium restriction and ang II infusion lead to an increased activity of the sympathetic nervous system activity [33] [34]. Other studies have shown that β-adrenergic stimulation inhibits adiponectin gene expression [35]. Moreover, Nowak et al. show that central sympathetic blockade with rilmenidine increases adiponectin concentration [36]. Further elucidation of the interrelationships between ang II, the sympathetic nervous system and adiponectin are necessary.

What could be the implications of an effect of angiotensin II on circulating adiponectin? Several studies reported increased insulin sensitivity on a high sodium intake [37;38], and our findings raise the possibility that effects on adiponectin may be involved. However, we did not find any changes in insulin and glucose concentrations or in the HOMA index, as a measure of insulin sensitivity, in response to a high sodium diet. This lack of effect on parameters of glucose homeostasis could be attributable to the time span of high sodium intake as well as to the magnitude of changes in sodium balance elicited by our diet intervention. Therefore, these assumptions need further substantiation. Moreover, the anti-inflammatory and vascular protective effects of adiponectin would provide an attractive additional mechanism to explain why RAAS-blockade may exert therapeutic effects in conditions where the prior activity of the endogenous RAAS is low – i.e. during high sodium intake and volume excess.

To investigate whether plasma concentration of other adipokines were also affected by sodium intake and ang II infusion, we determined plasma leptin concentration at baseline and during the highest dose of ang II infusion. We observed a small increase of leptin during high sodium. However, this was not significant, albeit in the same direction as adiponectin. Previously, a moderate decrease of plasma leptin concentration by dietary sodium restriction has been described [39]. During high sodium intake ang II infusion induced a small but significant decrease in leptin. Compared to the changes of adiponectin during ang II infusion, the leptin changes were less pronounced (ang II 3 ng/kg/h: adiponectin 35% decrease and leptin 15 %). Previous in vitro studies showed that ang II increases leptin secretion [40;41]. However, Cassis et al. showed in rats that locally produced ang II directly increases leptin release from adipocytes but chronic infusion of ang II in rats decrease plasma leptin concentrations [42]. This study shows the complexity of studying adipokines, depending on the physiological and experimental setting used. Of note is that the leptin concentrations are very low, most probably related to our healthy population.

A possible limitation of our study is, that we did not measure high molecular weight (HMW) adiponectin, which is assumed to be the active form of the protein [43]. However, recently Aso et al. showed that there is very strong correlation ($R^2= 0.938$) between total adiponectin and HMW adiponectin concentrations in type 2 diabetic patients [44]. Moreover, both total and HMW adiponectin concentrations are independent risk factors for the development of type 2 diabetes [45].
In summary, plasma adiponectin is inhibited during acute infusion of ang II in healthy men. Moreover, adiponectin increased during physiological suppression of the RAAS by high sodium intake. Thus adiponectin may provide a link between the RAAS and metabolic status. Further elucidation of the interrelationships between ang II and adiponectin might provide a basis for better intervention in the cardiovascular complications of insulin-resistant states.

**Acknowledgements**

We would like to thank Ms. B. Haandrikman for performing adiponectin measurements
References


