Decreased insulin sensitivity during dietary sodium restriction is not mediated by effects of angiotensin II on insulin action

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ABSTRACT

We have previously reported that modest dietary sodium restriction, as advocated in management guidelines for diabetes, may reduce insulin sensitivity. It has since been suggested that this effect may be mediated via cross-talk between insulin and angiotensin II (AII)-stimulated intracellular second messengers. In order to assess the effect of 5 days of modest sodium restriction (to < 80 mmol/day target sodium intake) on insulin sensitivity, 15 healthy males underwent a double-blind, placebo-controlled, randomized, cross-over euglycaemic hyperinsulinaemic clamp study. One phase was supplemented with sodium tablets and the other with matched placebo. Insulin sensitivity (M) was reduced during dietary sodium restriction [median M value, 10.2 mg/kg per min (interquartile range 9.50–13.85) versus 12.8 mg/kg per min (interquartile range 9.60–14.30), P < 0.05]. To elucidate potential mechanisms that may explain this observation, we investigated the effect of AII on insulin action in isolated adipocytes obtained from healthy females. No effect of AII on insulin-mediated glucose transport or suppression of lipolysis was observed. In conclusion, despite the observation that dietary sodium restriction was associated with a median 15% reduction in insulin sensitivity, we found no evidence of a direct effect of AII on insulin action in human adipocytes.

INTRODUCTION

Insulin sensitivity may be influenced by activity of the renin–angiotensin system (RAS), although the nature of this relationship remains unclear. Although some studies that have examined the metabolic effects of severe dietary sodium restriction have shown a reduction in insulin sensitivity [1,2], others have found no effect [3] or even an increase in insulin sensitivity [4]. Furthermore, observational studies suggest that drugs which inhibit the generation of angiotensin II (angiotensin-converting-enzyme inhibitors) may increase hypoglycaemia in diabetes [5], although there is a lack of compelling evidence to support a direct effect on insulin sensitivity [6].

It has been suggested that if sodium restriction does, indeed, reduce insulin sensitivity, this may be mediated by a direct effect of elevated angiotensin II (AII) on insulin-responsive tissue, such as fat and skeletal muscle. Data from rodent cardiac [7] and aortic vascular smooth muscle cells [8] have suggested that AII may have a direct effect on post-receptor insulin-signalling. AII, acting via the AII type 1 (AT1) receptor, recruits intracellular kinases that reduce the activity of phosphatidylinositol 3-kinase (PI 3-kinase). As such, AII may reduce two of insulin’s

Key words: dietary sodium restriction, glucose transport, insulin resistance, intracellular cross-talk, non-esterified fatty acids, renin–angiotensin system.

Abbreviations: AUC, area under the curve; AII, angiotensin II; AT1, angiotensin II type 1 receptor; BMI, body mass index; IQR, interquartile range; M, insulin sensitivity; NEFA, non-esterified fatty acid; PI 3-kinase, phosphatidylinositol 3-kinase; RAS, renin–angiotensin system.

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PI 3-kinase-mediated downstream metabolic actions: glucose transport and, in adipose tissue, suppression of non-esterified fatty acid (NEFA) release.

In order to clarify the effect of RAS activation on metabolic function and investigate the possible role of AII, we have performed two related studies. First, we investigated the effect of modest dietary sodium restriction on insulin sensitivity in healthy subjects in a double-blind placebo-controlled study. Secondly, we assessed the influence of AII on insulin-mediated glucose uptake and inhibition of NEFA release from human adipocytes ex vivo. Previous studies of intracellular crosstalk between AII and insulin action have been performed in muscle (cardiac muscle or vascular smooth muscle), but effects in adipose tissue are also relevant in view of the presence of a local RAS [9,10]. While the main site of insulin-stimulated glucose disposal is skeletal muscle, it is likely that an effect of AII on insulin action in adipocytes would also have consequences for whole-body insulin sensitivity, as mice which have a specific deficiency of GLUT4 in adipose tissue have a marked reduction in this parameter [11], possibly mediated via adipocyte-derived circulating factors [12,13].

METHODS

Subjects

The Ethics Committee of the West Glasgow Hospitals University NHS Trust approved both the clinical and laboratory study protocols. Informed consent was obtained from every subject. Fifteen healthy males [mean age 26 ± 3.7 years, mean body mass index (BMI) 23 ± 2.4 kg/m²] were recruited to the study by advertisement. Subjects with any intercurrent medical condition, including hypertension and diabetes mellitus, were excluded. All underwent a standard physical examination prior to enrolment.

For the laboratory studies, all subjects were approached on the day prior to elective gynaecological surgery for non-malignant disease. Selection criteria included hypertension, diabetes, age over 60 years, BMI > 30 kg/m² and intercurrent drug therapy that could interfere with insulin, AII or isoproterenol action. Peri-umbilical subcutaneous adipose tissue (1–5 g) was dissected from each subject in theatre. None of the subjects encountered any complications.

Clinical study protocol

Volunteers were studied at the end of two dietary phases, at least 1 week apart. In each phase subjects were instructed to adhere to a low sodium diet for 5 days. Supplemental placebo (10 tablets/day) or slow sodium tablets (Ciba-Geigy) (10 tablets/day; 10 mmol sodium/tablet) were given in random order so that half of the group were given the high sodium intake first. The aim of sodium restriction was to achieve a modest reduction in dietary intake, consistent with that advocated for patients with Type II diabetes and essential hypertension [14]. Urine for measurement of sodium excretion was collected for 24 h from day 4 to 5. On the morning of day 5, volunteers fasted for a euglycaemic hyperinsulinaemic clamp and metabolic profile. A diet sheet was given to each volunteer at screening, detailing low sodium foodstuffs and sample daily diets. The study investigator (C.P.) reinforced dietary advice. Volunteers were asked not to smoke or to drink alcohol during the study.

Biochemical assays

Serum electrolytes, growth hormone and cortisol were analysed by routine auto-analyser in the Department of Biochemistry, Gartnavel General Hospital, Glasgow, U.K. To determine whether modest sodium restriction was associated with activation of the circulating RAS, plasma renin, AII and aldosterone were assayed prior to the commencement of each euglycaemic hyperinsulinaemic clamp. Total plasma renin concentration was measured using a micro-assay based on antibody trapping [15]. Plasma AII was measured using a kit from Diagnostic Products Ltd (Glyn Rhonwy, Gwynedd, Wales, U.K.). Plasma aldosterone was measured using a commercially available RIA kit (Biodata, Milan, Italy), using the method of McKenzie and Clements [16]. Catecholamines were measured by HPLC with electrochemical detection [17]. Samples for insulin and C-peptide were analysed by commercially available RIA (DiaSorin, Wokingham, Berks., U.K.). Glucose was measured using a Beckman II glucose analyser at the bedside during the euglycaemic hyperinsulinaemic clamps.

Haemodynamic salt sensitivity

Mean arterial blood pressure (Dinamap, Critikon) was recorded supine every 2 min for 1 h in a quiet room on the final day of dietary sodium restriction, following an overnight fast. Salt sensitivity, defined as the difference in mean arterial pressure between the sodium deplete and replete phases, was considered as a continuous variable and determined for each volunteer.

Euglycaemic hyperinsulinaemic clamp

The euglycaemic hyperinsulinaemic clamp protocol was carried out as described previously [6,18]. Venous cannulae were inserted into the left antecubital fossa for administration of insulin (Actrapid, Novo-Nordisk) and 20 % glucose (Gemini Volumetric Pump, IMED, Abingdon, Oxon, U.K.), and the right wrist, with the hand heated to 55 °C, to obtain arterialized venous blood. A constant rate of insulin (1.5 m-units·kg of body mass⁻¹·min⁻¹) was infused in a 10 % volume/volume solution of the patients’ own blood in saline (0.9 % NaCl) after an initial priming dose of 4.5 m-units·kg
of body mass^{-1} \cdot \text{min}^{-1}. The 20 % glucose infusion was commenced at 2 min and adjusted to achieve steady-state serum glucose of 5.2 mmol/l. Baseline blood was taken for insulin, C-peptide, electrolytes, plasma renin activity, aldosterone, AII, cortisol, catecholamines, growth hormone, total cholesterol and triacylglycerols. Insulin and electrolytes were also measured throughout the clamp. Blood pressure and heart rate were recorded every 15 min.

**Laboratory protocol**

Adipocyte preparation was by a modification of the method described by Rodbell [19], with the temperature maintained at 37 °C throughout. Adipose tissue was placed immediately in collection buffer (118 mM NaCl, 5 mM NaHCO₃, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM Hepes, 100 nM adenosine, 2.5 mM CaCl₂, pH 7.4, with 1 % BSA and 3 mM glucose). Any attached connective or vascular tissue was dissected from the sample prior to digestion in collagenase (Worthington Type 1; Lorne Laboratories, Reading, Berks., U.K.) for 30 min (4 ml of a 2 mg/ml solution per g of tissue). At the end of this period, the adipocytes were passed through a filter, leaving a layer of cells suspended in buffer.

**Preparation of adipocyte membranes**

Adipocytes were digested as above, and re-suspended at 35 % cytocrit. After two washes in ice-cold PBS, cells were resuspended at 66 % cytocrit in Hes buffer (20 mM Hepes, 1 mM EDTA and 255 mM sucrose), pH 7.4, containing protease inhibitors (Boehringer Mannheim). The remaining steps were carried out at 4 °C. Cells were homogenized by 20 passes in a Dounce homogenizer. They were then centrifuged at 3000 g at 4 °C for 5 min. The resulting fat cake and pellet were discarded, while the supernatant was centrifuged at 100 000 g at 4 °C for 60 min. The membrane pellet was washed then resuspended in binding buffer (10 mM MgCl₂, 1 mM EDTA and 50 mM Tris, pH 7.4) with protease inhibitor cocktail tablets; protein concentration was measured using a commercially available colorimetric assay (Bio-Rad Protein Assay; Bio-Rad Laboratories, Hercules, CA, U.S.A.).

**Binding studies**

Membranes were prepared from either adipocytes or vascular smooth muscle cells (positive control, kindly provided by Dr Jill Wakefield, Institute of Biomedical and Life Sciences, University of Glasgow, U.K.) and resuspended in radioligand-binding buffer. Duplicate aliquots (150 µl) of the resuspended membranes were then added to glass tubes containing 50 µl of 125I-labelled [Sar¹Ile⁸] AII and either 50 µl of water or losartan (kindly provided by Dr J. Brosnan, Division of Cardiovascular and Medical Sciences, Western Infirmary, University of Glasgow, U.K.) (10 µM final concentration) to define non-specific binding. Equilibrium binding was achieved by incubation at 37 °C for 45 min, and the reaction was terminated by vacuum filtration over 0.3 % (v/v) poly(ethyleneimine)-soaked GF/B filters and washing with ice-cold radioligand-binding buffer supplemented with 0.01 % (w/v) Chaps using a Brandel cell harvester. Receptor-bound ligand was quantified by counting the radioactivity.

**Glucose transport**

Adipocytes were resuspended at 10 % cytocrit. All insulin stimulations were for 30 min, and with 5 min of stimulation remaining, a cocktail of 2-deoxy-d-3[H]glucose, unlabelled 2-deoxy-d-glucose and Krebs–Ringer–Hepes buffer was added, achieving a final concentration of 10 µM 2-deoxy-d-glucose/2.5 µCi/ml (as described in [20]). Each assay condition was matched with an equivalent preparation containing cytochalasin B (10 µM), an effective inhibitor of facilitative glucose transport, and counts subtracted in order to adjust for non-specific association of glucose with the cells. Following incubation, aliquots of cells (50 µl) were removed from each assay condition and centrifuged at 3500 g through an oil layer. The overlying cells were collected and solubilized in Triton X-100, followed by determination of radioactivity using a Beckman scintillation counter. Results were expressed as the fold increase in uptake relative to the basal counts.

**Insulin-mediated suppression of lipolysis**

For these experiments, insulin (0.01–100 nM) and isoproterenol (200 nM) were added to adipocyte suspensions. NEFA release was measured in the un-stimulated state, in response to isoproterenol alone and following increasing insulin concentrations in combination with isoproterenol. To confirm that insulin-mediated suppression of lipolysis is PI 3-kinase dependent, preliminary studies were undertaken with the PI 3-kinase inhibitor wortmannin. To assess the effect of AII on insulin action, adipocytes were incubated with AII (1 µM), insulin and isoproterenol. After 45 min, aliquots of buffer (50 µl) were removed from each individual condition and the amount of fatty acid in the reaction assayed using a commercially available kit (NEFA C, Alpha Laboratories, Eastleigh, Hants, U.K.) according to the manufacturer’s instructions. Each assay was standardized against a 1 mmol/l NEFA standard. The NEFA assay is a two-step enzymic reaction, involving the activity of acyl CoA synthetase, acyl CoA oxidase and peroxidase.

**Statistical analysis**

On the basis of previous data [2], the clinical study of sodium restriction and insulin sensitivity was designed to have 80 % power to detect a 10 % change in insulin sensitivity.
sensitivity. Statistical analysis was performed using Minitab 13.1 (State College, PA, U.S.A.). Data are expressed as the mean ± S.D. (unless otherwise stated) or mean and interquartile range (IQR) when not normally distributed. Data from the two phases of the clinical studies were compared using a paired t test or the Wilcoxon-matched pairs signed rank test where not normally distributed (insulin sensitivity, cholesterol, triacylglycerols, noradrenaline, aldosterone and growth hormone). Comparison of data from the laboratory studies was done using the Mann–Whitney U test. Area under the curve (AUC) was calculated as described previously [21].

RESULTS

Clinical protocol
All 15 recruits completed both phases of the clinical protocol, and experienced no adverse events. Changes in metabolic and clinical variables between the two study phases are shown in Table 1.

There was no significant difference in body mass between phases. Mean urinary sodium for the 24 h prior to the clamp during the placebo phase was 70 ± 45 mmol/24 h, compared with 175 ± 72 mmol/24 h (P < 0.01) (Table 1) during sodium supplementation. Baseline serum sodium was lower after the period of sodium depletion (Table 1). There was no difference in serum potassium between phases, either at baseline or during insulin infusion. There were no statistically significant differences in baseline plasma noradrenaline, serum cortisol, growth hormone, total cholesterol or triacylglycerols between phases (Table 1).

Blood pressure
No significant changes in systolic or diastolic blood pressure were observed between phases (Table 1) when

Table 1 Comparison of body mass, blood pressure and serum biochemistry after 5 days of sodium restricted (deplete) and replete diet
All values are expressed as the mean ± one S.D. or as median and IQR. *P < 0.05 and **P < 0.01 between phases.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sodium deplete</th>
<th>Sodium replete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass (kg)</td>
<td>73 ± 9.1</td>
<td>74 ± 7.8</td>
</tr>
<tr>
<td>Urinary sodium excretion (mmol/24 h)</td>
<td>70 ± 45.1</td>
<td>175 ± 72.1**</td>
</tr>
<tr>
<td>Baseline serum sodium (mmol/l)</td>
<td>139.4 ± 1.72</td>
<td>141.2 ± 1.82*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115 ± 12.3</td>
<td>115 ± 9.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>58 ± 10.7</td>
<td>60 ± 8.2</td>
</tr>
<tr>
<td>Fasting total cholesterol (mmol/l)</td>
<td>3.9 (3.60–4.56)</td>
<td>3.5 (3.28–4.41)</td>
</tr>
<tr>
<td>Fasting triacylglycerols (mmol/l)</td>
<td>0.8 (0.56–0.91)</td>
<td>0.7 (0.53–0.78)</td>
</tr>
<tr>
<td>Growth hormone (m-units/l at 09:00 hours)</td>
<td>0.3 (0.20–0.95)</td>
<td>0.5 (0.20–0.48)</td>
</tr>
<tr>
<td>Noradrenaline (nmol/l)</td>
<td>1.4 (1.20–2.08)</td>
<td>1.6 (1.10–2.08)</td>
</tr>
<tr>
<td>Cortisol (nmol/l at 09:00 hours)</td>
<td>342 ± 135.0</td>
<td>286 ± 91.7</td>
</tr>
</tbody>
</table>

Table 2 Comparison of the components of the circulating RAS after sodium deplete and replete diet
All values are expressed as the mean ± one S.D. or as median and IQR. *P < 0.05 between phases.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sodium deplete</th>
<th>Sodium replete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma renin activity (μunits/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>25.0 ± 10.43</td>
<td>13.4 ± 7.28*</td>
</tr>
<tr>
<td>180</td>
<td>24.9 ± 11.16</td>
<td>12.3 ± 6.58*</td>
</tr>
<tr>
<td>Plasma aldosterone (ng/100 ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>16.0 (8.00–18.00)</td>
<td>6.0 (4.00–10.00)*</td>
</tr>
<tr>
<td>180</td>
<td>9.0 (3.00–19.00)</td>
<td>5.0 (3.00–9.00)*</td>
</tr>
<tr>
<td>Plasma All (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>28.5 ± 17.27</td>
<td>11.0 ± 6.89*</td>
</tr>
<tr>
<td>180</td>
<td>28.3 ± 22.03</td>
<td>12.3 ± 6.85*</td>
</tr>
</tbody>
</table>

Table 3 Comparison of euglycaemic hyperinsulinaemic clamp results on day 5 after sodium deplete and replete diet
All values are expressed as the mean ± one S.D. or as median and IQR. *P < 0.05 between phases. M/I, M value divided by the mean serum insulin concentration during steady state.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sodium deplete</th>
<th>Sodium replete</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin (μunits/ml)</td>
<td>11.6 ± 3.24</td>
<td>11.4 ± 3.02</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/ml)</td>
<td>1.7 ± 0.51</td>
<td>1.6 ± 0.53</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.5 ± 0.33</td>
<td>4.5 ± 0.45</td>
</tr>
<tr>
<td>Insulin sensitivity index (M/I)</td>
<td>0.08 ± 0.033</td>
<td>0.10 ± 0.049*</td>
</tr>
<tr>
<td>Mean steady-state serum insulin (μunits/ml)</td>
<td>147.3 ± 68.16</td>
<td>137.7 ± 67.52</td>
</tr>
</tbody>
</table>

group means were compared. No significant relationship was seen between the degree of salt sensitivity and the change in insulin sensitivity. Similarly, there was no relationship between salt sensitivity and the change in renin, aldosterone or AII between phases (results not shown).

Activation of the RAS
Before each clamp, plasma renin activity, aldosterone and AII were measured after 20 min supine rest to document the degree of activation of the RAS (Table 2). All three variables were significantly greater during the phase of dietary sodium restriction (P < 0.05 comparing renin, AII and aldosterone on sodium deplete and replete diet).

Insulin sensitivity
Fasting insulin, C-peptide and glucose were not significantly different between phases (Table 3). Insulin sensitivity, measured using the euglycaemic hyperinsulinaemic clamp, was compared between phases using non-parametric methodology. The M value was reduced
Insulin sensitivity, measured by the euglycaemic hyperinsulinaemic clamp, was measured after the sodium deplete and replete phases. Insulin sensitivity was significantly reduced during dietary sodium depletion [median M value 12.8 mg·kg of body mass⁻¹·min⁻¹ (IQR 9.60–14.30) sodium replete versus 10.2 mg·kg of body mass⁻¹·min⁻¹ (IQR 9.50–13.85) sodium deplete, \( P < 0.05 \)].

during sodium depletion compared with the sodium-replete phase [median M value during sodium restriction 10.2 mg·kg of body mass⁻¹·min⁻¹ (IQR 9.50–13.85) versus 12.8 mg·kg of body mass⁻¹·min⁻¹ (IQR 9.60–14.30), \( P < 0.05 \) Wilcoxon-matched pairs signed rank test] (Figure 1). This was equivalent to a median fall in insulin sensitivity of 15% (IQR −3.0–+20.4%). In addition, when expressed as the insulin sensitivity index (M value/mean serum insulin during final 40 min of the clamp), there remained a significant difference between phases (Table 3). There was no difference in mean insulin at baseline, or during the final 40 min of the clamp (steady state) (Table 3).

Laboratory studies

All binding to human adipose tissue

Binding studies of radiolabelled AII revealed displacement by losartan of \( 20 \pm 6.8 \) mmol AII/mg of membrane protein (Figure 2), in keeping with previous studies [22]. This technique confirmed the presence and integrity of the receptor and demonstrated specific binding to the AT1 receptor.

All and insulin-stimulated glucose transport

Pre-incubation (Figure 3, upper panel) and co-incubation (Figure 3, lower panel) of adipocytes with AII had no effect on the concentration–response curve of insulin-stimulated glucose transport [comparison of the AUC for insulin-stimulated glucose uptake was seen on pre-incubation or co-incubation of insulin with AII (\( P > 0.05 \) for comparison of AUC between insulin and insulin + AII for both sets of experiments).
insulin-mediated suppression of lipolysis

Co-incubation of AII with insulin resulted in a significant reduction in insulin sensitivity. By comparison of AUC between insulin alone and insulin + AII (P < 0.05), the AUC for insulin alone was taken as 100%, with all other values expressed as a percentage of that value. The presence of AII prevented the suppression of lipolysis by insulin, suggesting that the effect of sodium restriction on insulin sensitivity occurs via an alternative mechanism. Glucose transport was not assessed in these studies, AII reduced insulin-stimulated activation of PI 3-kinase in myocytes, possibly via a losartan-converting-enzyme inhibition [28,29] provide indirect support for this notion.

Previous work in rodent cardiac [7] and vascular smooth muscle [8] cells has identified a mechanism through which AII may reduce insulin action. In these studies, AII reduced insulin-stimulated activation of PI 3-kinase in myocytes, possibly via a losartan-sensitive mechanism. Glucose transport was not assessed in these experiments. In the present study, we used human adipocytes to investigate our hypothesis given the similarities between the signalling cascades in adipocytes and myocytes and the availability of the volume of tissue necessary to undertake these assays. It is acknowledged, however, that we cannot say with certainty that our findings are applicable to skeletal muscle, although previous studies in cultured rodent myoblasts have also shown no effect of AII on insulin-stimulated glucose uptake [30]. It is certainly important to note that skeletal muscle glucose uptake has a far greater contribution to whole-body insulin-stimulated glucose disposal than adipose tissue, making future studies in human muscle cells of considerable interest.
We studied two insulin-activated PI 3-kinase-dependent processes of clinical importance – insulin-stimulated glucose uptake and insulin-mediated suppression of lipolysis – and detected no effect of AII. We used 1 μM AII in the experiments described above to reflect the concentrations shown previously to stimulate intracellular cross-talk in muscle cells [7,8] and focused on cellular end-points (glucose transport and suppression of lipolysis). The only other published studies of AII and insulin action in human adipocytes did not measure glucose uptake, but did demonstrate no effect of AII on insulin-stimulated tyrosine phosphorylation of the insulin receptor [31].

In view of these findings, it is necessary to consider alternative ways via which dietary sodium restriction may reduce insulin sensitivity. In this regard, it has been shown previously that aldosterone modifies the expression of the insulin receptor in monocytes [32], whereas bradykinin may augment insulin-stimulated glucose uptake [33,34]. Further studies in human tissue are required to clarify the role of these hormones. Alternatively, as AII is a vasoactive hormone, it can be hypothesized that its effect on insulin sensitivity may be secondary to abnormal capillary recruitment in insulin-sensitive tissues, such as skeletal muscle [35]. Such an effect would not be evident using the ex vivo adipocyte techniques employed in the present study.

There are limitations in the laboratory aspects of these studies. We were unable to obtain adipocytes from volunteers at the end of each study phase; ethical considerations and the volume of tissue needed to undertake meaningful ex vivo studies prevented this. However, the design of the above experiments did permit us to study the specific effect of AII on insulin-stimulated cellular processes, free from the additional effects of aldosterone and renin. To the best of our knowledge, this is the first time such experiments have been undertaken in human adipose tissue. In addition, we studied adipose tissue obtained from females, while the studies in vivo were undertaken in younger males. We acknowledge that there may be confounding effects of sex hormones on insulin action in adipose tissue; however, while there may be changes in activity of the RAS throughout the female menstrual cycle, there are no published data that suggest fundamental differences in the action of AII between males and females. While we demonstrated activation of the systemic RAS in response to sodium restriction, it is not clear whether there was a similar effect on locally regulated systems, such as adipose tissue. Certainly there are local changes in response to other systemic stimuli, such as RAS blockade and nephrectomy, and further studies are required to characterize the key physiological regulators of these local systems.

The lack of prospective studies measuring insulin resistance and cardiovascular outcomes renders it difficult to assess the clinical importance of a median 15% reduction in insulin sensitivity. However, several longitudinal studies that have measured fasting insulin as a surrogate for insulin resistance have reported a relationship with cardiovascular disease [36,37]. In addition, the findings of the Insulin Resistance and Atherosclerosis Study (‘IRAS’) [38] suggest a continuous relationship between increasing insulin resistance and atheroma of the internal carotid artery. Furthermore, the extent of this change is similar to that noted following therapy with beta-blockers in patients with hypertension [39], a change often considered to be clinically important.

In summary, we have demonstrated a median 15% reduction in insulin sensitivity associated with modest dietary sodium restriction in healthy volunteers. However, although we confirmed the presence of specific AII binding to human adipocyte membranes, we found no impairment of cellular insulin sensitivity by AII in isolated human adipose tissue. We conclude that restriction of sodium reduces insulin sensitivity in healthy subjects, as in patients with Type II diabetes [2], but that this reduction is mediated by mechanisms other than cross-talk between the insulin and AII second messenger systems at the level of post-receptor signalling.

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