Thiazolidinediones and the renal and hormonal response to water immersion-induced volume expansion in type 2 diabetes mellitus

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Thiazolidinediones and the renal and hormonal response to water immersion-induced volume expansion in type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 294: E733–E739, 2008. First published January 29, 2008; doi:10.1152/ajpendo.00583.2007.—Thiazolidinediones cause sodium retention and edema by a direct effect on the kidneys. The aim of this study was to use the technique of head-out water immersion to investigate the effects of rosiglitazone on sodium and volume homeostasis in subjects with type 2 diabetes mellitus. The volume expansion response to water immersion was compared with the response on a non-immersion control day in 12 nondiabetic male subjects and 8 diet-controlled male type 2 diabetic subjects with hourly blood and urine sampling over a 4-h period. This was repeated after both groups had taken 4 mg of rosiglitazone daily for 7 days. Immersion produced a natriuresis in both groups (P < 0.001). An impairment of this natriuresis was seen in the diabetic subjects (P = 0.006). However, when rosiglitazone was taken, there was no significant difference in immersion-induced natriuresis compared with nondiabetic controls (P = 0.2). There was an immersion-induced rise in atrial natriuretic peptide (ANP) and urinary cyclic guanosine monophosphate (cGMP), in the healthy subjects (ANP P = 0.001, cGMP P = 0.043), which was not seen in the diabetic subjects (ANP P = 0.51, cGMP P = 0.74). Rosiglitazone restored the immersion-induced increase in cGMP excretion and rise of ANP in the diabetic group (ANP P = 0.048, cGMP P = 0.009). This study confirms that type 2 diabetic subjects have an impaired natriuretic response to acute volume expansion, which appears to be enhanced rather than diminished by rosiglitazone. This may be related to its effects in increasing natriuretic peptides and restoring the impaired cGMP excretion to volume expansion.

Rosiglitazone; atrial natriuretic peptide; renin; aldosterone; cyclic guanosine monophosphate.

THIAZOLIDINEDIONES (TZDs) act as agonists for peroxisome proliferator-activated receptor-γ (PPARγ), and these insulin-sensitizing drugs, which include rosiglitazone and pioglitazone, are effective and increasingly used to treat patients with type 2 diabetes (5, 28). In addition to lowering blood glucose, these drugs benefit cardiovascular parameters such as blood pressure and endothelial function (24, 29). Although these properties have been promoted with the suggestion that they might reduce cardiovascular risk (5), recent concerns have emerged regarding their cardiovascular safety, in particular the possible increased risk of myocardial infarction and death from cardiovascular causes (20).

One of the properties of these drugs that may lead to adverse cardiovascular outcomes is their propensity to cause fluid retention, which may precipitate or exacerbate heart failure in susceptible individuals (19, 26). There is now evidence to show that TZDs decrease urinary sodium excretion (31) and that stimulation of PPARγ in the kidney causes distal tubular collecting duct sodium retention in human and mouse (31, 32). Elegant murine studies using transgenic models have shown that PPARγ agonists in the renal collecting duct cause sodium retention by activation of epithelial sodium channel (ENaC) transport (32).

Animal and human studies suggest that PPARγ may function as a physiological regulator of the sodium transport process in the distal nephron and may be important in modulating extreme changes in sodium intake or volume change (32). We hypothesized that TZDs would alter the renal physiology of sodium transport and might alter the response to acute volume expansion produced by water immersion in normal volunteers and type 2 diabetic subjects.

Immersion to the neck in water causes a 16% increase in plasma volume and a redistribution of 700 ml of blood centrally to the thoracic cavity produced by the hydrostatic pressure gradient (1, 13). The consequent central volume expansion brings about a diuresis and natriuresis (8), suppression of the renin-angiotension-aldosterone system (9), and increases in atrial natriuretic peptide (ANP) (9), which acts via cyclic guanosine monophosphate (cGMP) to involve the nitric oxide system and produce natriuresis and vasodilatation.

Animal studies of sodium balance during rosiglitazone treatment suggest that sodium retention by the collecting duct peaks by day 6 and that balance returns to normal by day 9 (32), and we hypothesise that “escape” mechanisms come into play (3, 33). Our studies on the effects of rosiglitazone on volume expansion in normal subjects and type 2 diabetes were carried out after 7 days’ treatment and reflect the acute homeostatic response. We used the technique of head-out water immersion to cause volume expansion to study the renal and hormonal effects of rosiglitazone treatment.

MATERIALS AND METHODS

Subjects

We recruited eight subjects with type 2 diabetes mellitus (which had previously been diagnosed either by a fasting plasma glucose of >7 mmol/l or a 2-h plasma glucose of >11.1 mmol/l on a standard 75-g oral glucose tolerance test) and 12 healthy nondiabetic control subjects. All subjects recruited were male, and both the healthy and diabetic groups were matched for age and body mass index (BMI). The diabetic subjects were diet treated, had good glycaemic control and had previously been diagnosed either by a fasting plasma glucose of >7 mmol/l or a 2-h plasma glucose of >11.1 mmol/l on a standard 75-g oral glucose tolerance test. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
(defined as an Hb A1c of <7%) and no glycosuria. Neither the control or diabetic subjects were taking medication or had any evidence of clinical cardiovascular disease, hypertension (defined as a blood pressure of above 140/90 mmHg in healthy nondiabetic control subjects and above 140/80 in diabetic subjects), microalbuminuria, or autonomic neuropathy.

Ethical permission was obtained from the Local Ethics Committee in Warwickshire, UK, and all subjects gave informed consent prior to commencing the research.

**Head-Out Water Immersion**

Subjects were asked to maintain their own diet but to make a record for 3 days prior to the first experiment with a view to repeating the same diet before each of the other three experiments, thereby reducing within-subject variation and enabling individuals to act as their own controls. No attempt was made to provide a regulated sodium intake, because we wished to study the subjects on their normal diets. Subjects refrained from alcohol and caffeine for 24 h prior to each study. Four sets of studies were conducted: 1) healthy control subjects, 2) healthy control subjects after 4 mg of rosiglitazone for 7 days, 3) diabetic subjects, and 4) diabetic subjects after they had taken 4 mg of rosiglitazone every morning for 7 days prior to the study (with the last dose taken on the morning of the experiment).

Each set of studies consisted of two experiments, an immersion day, and a dry, nonimmersion control day. An immersion spa (Aquatech, Newbury, UK) was used for experiments with identical seating arrangements in and out of the pool. At least 7 days separated individual experiments, which were done in random order.

After an overnight fast, subjects drank 400 ml of water at 0730 and then voided. For the experiments in which rosiglitazone was used, the subjects took their 4-mg dose with this 400-ml water load. At 0815, an intravenous cannula was sited into an antecubital fossa. Subjects then drank a further 200 ml of water at 0830, when they again voided. At the end of hour 1 (0930), 20 ml of blood was drawn, and the subjects emptied their bladders. The urine was collected, measured for volume, and aliquotted. This was repeated hourly until the end of the experiment, at hour 6 (1430). Blood pressure was measured by conventional sphygmomanometry at hourly intervals throughout the study. The subjects were immersed on the immersion days for 4 h between 0930 and 1330 (hour 2 to hour 5), with a “preimmersion control hour” from 0830 to 0930 (hour 1), when subjects sat outside the spa and a “postimmersion control hour”, from 1330 to 1430 (hour 6), when subjects also sat outside the spa. During water immersion, subjects sat upright in the immersion spa with the level of water adjusted to the suprasternal notch, and the water temperature was kept at 34–35°C. On the dry, “nonimmersion” control days, the experiments were identical, lasting 6 h, the only exception being that the subjects did not enter the spa.

All subjects involved in this study had their blood glucose levels checked on arrival at the laboratory by a One Touch II glucose meter (Johnson and Johnson). No attempt was made to clamp the glucose level, as this would involve using an exogenous insulin infusion, which itself could cause sodium retention.

**Assays**

The measurement system for urinary sodium concentration included the Ion-Selective Electrode, a reference electrode, and electronic circuits to measure and process the electromotive force to give the test ion concentration (Roche Hitachi Modular P800 chemical analyzer, Roche Diagnostics). The interassay coefficient of variation for urinary sodium was 1.9%. The hematology samples were analyzed using the SF 3000 hematology analyzer (TOA Medical Electronics/Sysmex). Glucose was measured photometrically by an enzymatic colorimetric assay with a coefficient of variation of 1.6%. Insulin was measured using radioimmunoassay (DRG Instruments) with a coefficient of variation of 5.6–9.8%. Direct renin was analyzed using an automated immunochemiluminescence assay, referenced to the WHO standard (NIBSC code 68/356) and performed on the automated Nichols Advantage system (Quest Diagnostics Laboratories, Heston, Middlesex, UK). Aldosterone was measured using a highly sensitive and specific radioimmunoassay (Quest Diagnostics Laboratories). The interassay precision for the direct renin assay is 6.9–14.7% and for the aldosterone in 7.7–16%. ANP was assayed using an extraction method followed by radioimmunoassay (23), with an interassay coefficient of variation of 8% at a level of 25 pg/ml. Urinary cGMP was analyzed using a 3-h competitive enzyme immunoassay (R&D systems), and the interassay coefficients of variation were 3.5% at a level of 16.9 pmol/ml and 5% at a level of 359 pmol/ml.

**Statistical Analysis**

The size of the sample for these volume expansion manoeuvres was based on published literature in animals and man (1, 8–10, 13, 22, 25). Data do not exist to allow a meaningful calculation of power for these proof-of-concept studies. SPSS 11.0 for Windows was used as the statistical package for all analyses. Data were first subjected to the Shapiro-Wilks test of normality, following which nonparametric data were subjected to logarithmic conversion. For comparison between groups at specific time points normalized data then underwent paired or unpaired t-testing, whereas those that failed to normalize with log transformation underwent nonparametric assessment using the Wilcoxon paired or the Mann-Whitney unpaired tests. When comparison of sequential time data over the immersion hours (hours 2–5) either within or between groups was required, an analysis of variance with repeated measures design was employed. The parametric data are expressed as means ± SE and, if nonparametric, are expressed as medians followed by interquartile (IQ) ranges in parentheses. Statistical significance was taken at the 5% level (P < 0.05).

**RESULTS**

**Subjects**

The baseline characteristics of the subjects are summarised in table 1. The control and diabetic groups were matched for age, BMI, and blood pressure. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula HOMA-IR = fasting insulin (µIU/ml) × fasting glucose (mmol/l)/22.5 (16). As expected, the diabetic subjects had a higher fasting glucose and higher HOMA-IR than the control subjects (P < 0.05 for all). However, there were no differences in fasting glucose, insulin levels, or HOMA-IR when the subjects were treated with rosiglitazone compared to without.

**Nonimmersion (Dry) Days**

During the dry, “nonimmersion” studies, there were no significant changes throughout the 6 h in sodium excretion, direct renin and aldosterone levels, ANP levels, and urinary cGMP excretion (all in Table 2). Hematocrit (Hct) was used as a measure of hemodilution. Although the rosiglitazone-induced hemodilution did not reach statistical significance, there was a clear trend toward hemodilution in both the healthy (Hct off meds 45.1 ± 0.58% vs. Hct on rosiglitazone 43.7 ± 1.04%) and diabetic subjects (Hct off meds 43.9 ± 0.81% vs. Hct on rosiglitazone 42.2 ± 0.82%), with administration of the rosiglitazone.
groups when compared the dry experiments (natriuresis on immersion in both the control and diabetic P 0.001; Fig. 1). The diabetic subjects had a lower urinary sodium excretion, in response to the water immersion, compared with the healthy controls (HOMA-IR on morning of experiment, mmol/l (mean ± SE) 0.71, healthy on rosi 3.4 0.61 vs. 5.70 0.61, diabetic on rosi 2.30 (1.43–2.69) 4.13 (2.30–5.13)

Immersion Studies

Hemodynamic response. There were no significant differences in systolic or diastolic blood pressures with addition of the rosiglitazone or on immersion.

Renal response. There was a highly significant diuresis on immersion in all groups (dry vs. immersed mean urine output over the 4 immersion hours in ml/min: healthy off meds 3.4 ± 0.30 vs. 7.0 ± 0.61, healthy on rosi 3.4 ± 0.61 vs. 6.0 ± 0.84, diabetic off meds 2.6 ± 0.18 vs. 5.70 ± 0.61, diabetic on rosi 6.3 ± 0.71, P < 0.001 for all). There was a significant natriuresis on immersion in both the control and diabetic groups when compared the dry experiments (P < 0.001; Fig. 1). The diabetic subjects had a lower urinary sodium excretion, in response to the water immersion, compared with the healthy controls (P = 0.006; Fig. 1A); however, this was no longer the case if the diabetic subjects were taking rosiglitazone (P = 0.20; Fig. 1B).

Hormonal response. Control subjects demonstrated a significant immersion-induced suppression of renin and aldosterone (P < 0.01; Tables 2 and 3). The suppression of renin was no longer statistically significant if the healthy subjects were immersed while taking rosiglitazone (P = 0.38); however, the aldosterone suppression remained significant (P = 0.001). There was a trend toward immersion-induced suppression of renin with water immersion in the diabetic subjects both with and without rosiglitazone administration; however, this appeared variable and did not reach overall statistical significance (P = 0.12 and P = 0.17, respectively). There was suppression of aldosterone with water immersion in the diabetic subjects (P < 0.01; Tables 2 and 3), and this appeared to be similar if they were taking rosiglitazone but was not overall statistically significant (P = 0.22).

The control subjects demonstrated a significant rise in ANP with the addition of rosiglitazone in the dry seated experiments. Results are expressed as median (IQ).

Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Type 2 Diabetic Subjects</th>
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<tbody>
<tr>
<td>Age, yr (mean ± SE)</td>
<td>52.2 ±4.6</td>
<td>54.4±4.2</td>
</tr>
<tr>
<td>BMI, kg/m² (mean ± SE)</td>
<td>29.2 ±1.57</td>
<td>29.7±1.59</td>
</tr>
<tr>
<td>Systolic BP, mmHg (mean ± SE)</td>
<td>127.8±2.6</td>
<td>129.8±2.2</td>
</tr>
<tr>
<td>Diastolic BP, mmHg (mean ± SE)</td>
<td>72.5±1.9</td>
<td>69.4±1.5</td>
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</tbody>
</table>

Table 2. Hormonal responses on dry nonimmersion day

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Type 2 Diabetic Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose on morning of experiment, mmol/l (mean ± SE)</td>
<td>3.8±0.22</td>
<td>6.7±0.41</td>
</tr>
<tr>
<td>Immersed</td>
<td>4.0±0.17</td>
<td>6.8±0.24</td>
</tr>
<tr>
<td>Dry, on rosiglitazone</td>
<td>4.1±0.31</td>
<td>6.7±0.35</td>
</tr>
<tr>
<td>Immersed, on rosiglitazone</td>
<td>4.2±0.35</td>
<td>6.8±0.48</td>
</tr>
<tr>
<td>Fasting insulin on morning of experiment, μIU/ml (median + IQ ranges)</td>
<td>11.6 (8.8–15.2)</td>
<td>11.5 (8.2–19.2)</td>
</tr>
<tr>
<td>Immersed</td>
<td>11.8 (7.8–16.7)</td>
<td>14.6 (11.4–19.6)</td>
</tr>
<tr>
<td>Dry, on rosiglitazone</td>
<td>15.4 (8.4–18.8)</td>
<td>11.2 (9.9–18.7)</td>
</tr>
<tr>
<td>Immersed, on rosiglitazone</td>
<td>14.1 (7.6–16.0)</td>
<td>13.1 (8.6–14.8)</td>
</tr>
<tr>
<td>HOMA-IR on morning of experiment (median + IQ ranges)</td>
<td>2.27 (1.25–2.75)</td>
<td>4.39 (3.60–5.84)</td>
</tr>
<tr>
<td>Immersed</td>
<td>2.17 (1.71–3.76)</td>
<td>2.92 (2.73–6.05)</td>
</tr>
<tr>
<td>Dry</td>
<td>2.30 (1.49–2.9)</td>
<td>4.13 (2.30–5.13)</td>
</tr>
</tbody>
</table>

No difference in age, BMI, or blood pressure (BP) between groups. IQ, interquartile range; HOMA-IR, homeostasis model assessment of insulin resistance. Diabetic subjects have higher fasting glucose and HOMA-IR than healthy controls (P < 0.05 for all).
(P = 0.045; Fig. 2, A and C). The control subjects also demonstrated a significant rise in ANP in response to water immersion (P < 0.001; Fig. 2A) in contrast to the diabetic subjects where there was no immersion-induced rise in ANP (P = 0.51; Fig. 2B). However, a significant ANP rise with water immersion was seen in diabetic subjects taking rosiglitazone (P = 0.048; Fig. 2D). The cGMP response demonstrated a similar pattern, with a significant rise in cGMP seen in the healthy subjects with addition of rosiglitazone in the dry seated experiment (P = 0.048; Tables 2 and 3) and in the healthy controls with water immersion (P = 0.043; Tables 2 and 3), but with nonimmersion-induced increase in cGMP in the diabetic subjects (P = 0.742; Tables 2 and 3), unless they were immersed on rosiglitazone (P = 0.009; Tables 2 and 3).

**DISCUSSION**

We used acute volume expansion produced by immersing subjects in water to study the effects of this dynamic stimulus on the renal and hormonal response in normal subjects and type 2 diabetic subjects. We confirm that in type 2 diabetes there is an impaired natriuretic response, diminished ANP, and blunted cGMP response to volume expansion. Short-term treatment for 7 days with rosiglitazone induces a significant increase in ANP and cGMP in the healthy control subjects and paradoxically appears to restore both the natriuretic and ANP response to volume expansion in the type 2 diabetic subjects.

The impaired natriuretic response to water immersion seen in type 2 diabetic subjects is similar to the response described in type 1 diabetic subjects (22) and confirms observations made producing volume expansion by water immersion or saline infusion by others (4, 6). Subjects with diabetes have been shown to have a higher exchangeable body sodium (4, 30), and it has been suggested that this propensity to retain sodium may contribute to the pathophysiology of the high prevalence of hypertension in diabetes (22, 30). Hyperinsulinemia has been suggested to play a significant role in sodium retention (17).

A recent study demonstrated that mice with collecting duct-specific deletion of PPARγ receptors are resistant to the in vitro...
creases in body weight, plasma volume expansion, and sodium retention seen in control mice treated with rosiglitazone for 9 days (32). This study clearly demonstrates a renal cause for the edema caused by rosiglitazone and demonstrates increased activity of ENaC in the distal collecting duct (32). Of more interest is the fact that, although all the mice treated with rosiglitazone reduced urinary sodium excretion, this retention was maximal by day 6 but was returning toward normal by day 9. This suggests that in the normal mammalian kidney there exists an “escape” phenomenon from the basal sodium-retaining effects of rosiglitazone similar to that seen with mineralocorticoid excess (3, 33). It is suggested that ANP is involved in this escape mechanism (3, 33), particularly in subjects with low renin states. Our studies, in both normal and type 2 diabetic subjects, suggest a significant rise in ANP and cGMP on rosiglitazone, confirming previous studies (14). This hypothesis that ANP mediates the “escape” from rosiglitazone-induced sodium retention could explain the findings of another study in humans, examining the effects of TZDs on sodium excretion after 6 wk, which demonstrated that pioglitazone caused urinary sodium retention in subjects when on a low-salt diet but not on a high-salt diet (31). Those on the high-salt diet would have already suppressed the renin-angiotensin-aldosterone system (RAAS) and increased ANP to correct the effect and been unable to produce further significant responses.

In the type 2 diabetic subjects we studied, there was an impaired ANP response to immersion, which is consistent with previous published data suggesting that ANP release is resistant to changes in plasma volume in type 2 diabetes mellitus (6, 18, 25). The impaired ANP response to immersion was restored by rosiglitazone, which may form part of the explanation of the paradox that rosiglitazone enhances rather than reduces the natriuresis during volume expansion. Similar observations to our immersion studies have been made with Zucker rats that constitute an animal model of type 2 diabetes. These animals, when treated with rosiglitazone, show a more rapid excretion of an NaCl load (a volume expansion stimulus) compared with control animals (15). If the hypothesis is that the initial sodium retention leads to increased ANP levels or sensitivity, then during further volume expansion or salt loading there could be an enhanced natriuretic response.

Although we believe that it is likely that our results are due to differences in the hormonal mediators of natriuresis, an alternative explanation could be a difference in baroreceptor reflexes between the groups. We feel this to be less probable since we failed to demonstrate any reduction in the natriuresis in diabetic patients with autonomic neuropathy (21), and others have consistently shown that the natriuresis persists in denervated animals and man (7).
Limitations of this study are its size, and these detailed physiological studies in humans need to be considered as proof of concept with their strength derived from the within-subject comparisons from the powerful physiological stimulus of water immersion. The numbers we have studied are similar to those in the published literature using volume expansion techniques in humans or animals (1, 8–10, 13, 15, 22, 25, 31, 32). Another limitation of the study is the short duration of 7 days on rosiglitazone before being studied. Although it is known that the full effects of the drug on insulin resistance can take months, we chose a 7-day exposure because it was felt that it would not be ethical to ask the normal controls to take the drug for longer and because the animal studies suggested that renal effects would be apparent at this stage (15, 32). Also the trend toward hemodilution seen in the rosiglitazone-treated subjects in our experiments would suggest that there was an effect of the drug seen after 7 days. We also decided to study well-controlled type 2 diabetes subjects who were, as far as we could judge, clinically free from complications or any comorbidity or treatment. Studies have shown that abnormalities of tubular sodium handling occur even in subjects with the metabolic syndrome (2) and certainly in uncomplicated type 2 diabetes (30). Subjects were studied under fasting conditions with blood glucose levels below the renal threshold to avoid the need for insulin clamping, which would have introduced further variables.

It is now certain that TZDs cause renal sodium retention (32), and the recent concerns over the potential cardiovascular risk of these commonly used drugs highlights the need for a fuller understanding of the pathophysiology of sodium retention and the mechanisms that could protect against it (20). We would propose the existence of an “escape” phenomenon from their sodium-retaining effects, mediated by a rise in ANP, which we demonstrated during a volume expansion manoeuvre. This may provide a protective homeostatic mechanism against fluid retention. Clinical risk factors for rosiglitazone-induced edema include insulin treatment, renal dysfunction, and cardiac disease (12, 19). Furthermore, the presence of the common proline-to-alanine substitution at codon 12 (Pro12Ala) of exon B in the PPARγ gene provides a pharmacogenetic risk factor for TZD-induced edema (11). Further research is required to see whether the natriuretic response to ANP or its interaction with the RAAS is compromised in individuals who would be more at risk of fluid retention. It is of interest, for example, that ACE inhibitors are found to be less effective in patients with the Pro12Ala polymorphism (27), as this may imply a lower state of activation of the RAAS in this population and consequent increased propensity to develop edema when treated with TZDs. It is of is also interest that Black and Asian ethnic groups characterized as having low-renin volume mechanisms have been reported to show a higher incidence of sodium retention (2) and therefore may be more susceptible to TZD-induced edema. Further studies to understand the “escape” mechanism in these various groups at greater risk is clearly warranted.

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DISCLOSURES

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