Free fatty acids normalize a rosiglitazone-induced visfatin release

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Free fatty acids normalize a rosiglitazone-induced visfatin release. Am J Physiol Endocrinol Metab 291: E885–E890, 2006. First published May 30, 2006; doi:10.1152/ajpendo.00109.2006.—The detrimental effect of elevated free fatty acids (FFAs) on insulin sensitivity can be improved by thiazolidinediones (TZDs) in patients with type 2 diabetes mellitus. It is unknown whether this salutary action of TZD is associated with altered release of the insulin-mimetic adipocytokine visfatin. In this study, we investigated whether visits concentrations are altered by FFA and TZD treatment. In a randomized, double-blind, placebo-controlled, parallel-group study 16 healthy volunteers received an infusion of triglycerides/heparin to increase plasma FFA after 3 wk of treatment with rosiglitazone (8 mg/day, n = 8) or placebo (n = 8), and circulating plasma visfatin was measured. As a corollary, human adipocytes were incubated with synthetic fatty acids and rosiglitazone to assess visfatin release in vitro. The results were that rosiglitazone treatment increased systemic plasma visfatin concentrations from 0.6 ± 0.1 to 1.7 ± 0.2 ng/ml (P < 0.01). Lipid infusion caused a marked elevation of plasma FFA but had no effect on circulating visfatin in controls. In contrast, elevated visfatin concentrations in subjects receiving rosiglitazone were normalized by lipid infusion. In isolated adipocytes, visfatin was released into supernatant medium by acute addition and long-term treatment of rosiglitazone. This secretion was blocked by synthetic fatty acids and by inhibition of phosphatidylinositol 3-kinase or Akt. In conclusion, release of the insulin-mimetic visfatin may represent a major mechanism of metabolic TZD action. The presence of FFA antagonizes this action, which may have implications for visfatin bioactivity.

Thiazolidinediones (TZDs), which act via stimulation of peroxisome proliferator-activated receptor-γ (PPARγ)-dependent and -independent mechanisms (4), enhance the flux of free fatty acids (FFAs) into the tissue and facilitate their enzymatic degradation (35). TZDs have been shown (35) to improve insulin sensitivity in patients with type 2 diabetes mellitus and insulin resistance. FFAs directly impair insulin sensitivity and are critically involved in the pathophysiology of diabetes mellitus (32). Although reduction of FFA concentrations has therefore been proposed as a therapeutic goal in these subjects, the contribution of TZDs on these mediators of insulin resistance is poorly characterized.

Recently, the adipocytokine visfatin has been demonstrated (12) to act as an insulin-mimetic hormone by direct activation of the insulin receptor. The observation that visfatin expression and release is increased in obese subjects (3) and plasma concentrations increased in patients with type 2 diabetes mellitus (7) indicates that visfatin release may represent a nutrient sensor reaction. Consequently, interventions affecting insulin sensitivity, such as altered FFA concentrations or treatment with PPARγ agonists, could influence circulating concentrations of visfatin. On the other hand, changes in visfatin concentrations may synergistically sensitize tissue for insulin, an action described for PPARγ agonists. We have therefore hypothesized that rosiglitazone and FFA can alter visfatin plasma concentrations and have tested this assumption in a double-blind, randomized, placebo-controlled trial. Additionally, human adipocytes were isolated and exposed to rosiglitazone and FFAs to detect dose or time dependencies of visfatin release and to assess signaling pathways involved.

Materials and Methods

Study population. The study protocol was approved by the Ethics Committee of the University of Vienna and conforms with the principles of the Declaration of Helsinki, including present revisions and the Good Clinical Practice guidelines.

Sixteen healthy male subjects from whom informed consent was obtained before enrollment were included in this double-blind, randomized, placebo-controlled, parallel-group study. Subject characteristics are shown in Table 1. All of the subjects claimed not to have ingested any prescribed medications or over-the-counter drugs from 2 wk before screening until the study was complete. All subjects were given a complete health examination (including physical examination, ECG, and laboratory screening) ≤14 days before the first study day. Subjects were studied after overnight fasting. Studies were conducted in a quiet room with an ambient temperature of 22°C with complete resuscitation facilities.

Experimental setup. Rosiglitazone (8 mg/day, n = 8) or placebo (n = 8) were administered in single daily oral doses for 3 wk. On the last trial day, an intravenous infusion of triglycerides/heparin was administered for 360 min to increase circulating FFA concentrations. Venous blood samples for determination of insulin, glucose, visfatin, and FFA plasma concentrations were drawn before and at timed intervals during triglyceride/heparin infusion. Blood for measurement of FFAs was collected into vials containing tetrahydrolipstatin to avoid in vitro lipolysis, which could have resulted in artificially high FFA concentrations (21). Plasma was separated after centrifugation and stored at −80°C until further analysis. The intravenous infusion of the lipid emulsion (20% Intralipid, 1.5 ml/min; Pharmacia Upjohn, Vienna, Austria) plus heparin (bolus: 200 IU; constant infusion rate: 0.2 IU·kg⁻¹·min⁻¹; Baxter, Vienna, Austria) was administered to achieve systemic FFA concentrations typical for severely insulin-resistant subjects (30, 38). Heparin was added to enhance the breakdown of triglycerides to FFAs in plasma (30, 39).

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Table 1. Subject characteristics before and after 3-wk treatment with placebo or rosiglitazone

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Rosiglitazone</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>28 ± 4</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.0 ± 2.6</td>
<td>25.5 ± 1.6</td>
</tr>
<tr>
<td>Viscatin, ng/ml</td>
<td>Before After</td>
<td>Before After</td>
</tr>
<tr>
<td>Insulin, μU/l</td>
<td>0.5 ± 0.0</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>FFA, μmol/l</td>
<td>337 ± 177</td>
<td>278 ± 80*</td>
</tr>
</tbody>
</table>

Data are given as means ± SD; n = 8/group. BMI, body mass index; FFA, free fatty acids. *P < 0.05 vs. before treatment; †P < 0.05 between groups.

Biochemical assays. Venous blood glucose concentrations were determined by routine laboratory methods. Plasma concentrations of FFAs were measured enzymatically, as described previously (31). Insulin concentrations were determined using a double-antibody RIA (Diagnostics Systems Laboratories, Webster, TX). Viscatin was measured with an ELISA assay from Phoenix Peptides (Karlsruhe, Germany). For insulin, results were expressed as means ± SD while for visfatin and FFAs, results were expressed as means ± SE unless indicated otherwise.

Statistical analysis. Because of the skewed distribution of the parameters under study, nonparametric tests were used. Between-group comparisons were performed using the Mann-Whitney U-test. Within groups, effects were tested by Friedman’s ANOVA and the Wilcoxon signed-rank test, respectively. Pearson’s correlation was used for calculation of associations between variables. All calculations were performed using the Statistica software package (release 5.1: StatSoft, Tulsa, OK). P ≤ 0.05 was considered significant. Values are expressed as means ± SD unless indicated otherwise.

RESULTS

Effect of rosiglitazone treatment. Outcome parameters were comparable between groups before treatment with rosiglitazone or placebo (Table 1). Rosiglitazone treatment for 3 wk increased mean circulating visfatin by 283% (P < 0.01) and reduced mean FFA concentrations by 50% (P < 0.01; Table 1). Fasting insulin was not affected by rosiglitazone treatment. Infusion of triglycerides/heparin increased mean FFA concentrations by 1,700% (Fig. 1) and circulating insulin to a maximum of 31.9 ± 20.3 μU/l (P < 0.05) during placebo conditions but had no effect on plasma visfatin concentrations. The increase of systemic FFAs by infusion of triglycerides/heparin was smaller in subjects receiving rosiglitazone (P < 0.05 vs. placebo). However, the rosiglitazone-induced elevation of circulating visfatin was reduced to baseline during triglyceride/heparin infusion to concentrations seen in controls (Fig. 1). No correlations were detectable between body mass index (BMI) and changes of visfatin induced by rosiglitazone treatment. Furthermore, no association between changes of visfatin during FFA infusion and BMI was demonstrable in this group.

In subjects receiving placebo, there was no relationship between BMI and changes in visfatin over 3 wk or during FFA infusion.

Isolated adipocytes. Incubation of adipocytes with rosiglitazone caused a time- and concentration-dependent visfatin release into the supernatant medium. Rosiglitazone concentrations of 600 ng/ml significantly increased supernatant visfatin concentrations by ~13% after 18 h (P = not significant) and by 405% after 24 h (P < 0.05; Fig. 2). Incubation of adipocytes with FFA mix had no effect on supernatant visfatin (data not shown), but coinubation of FFA mix with rosiglitazone completely prevented the rosiglitazone-induced visfatin release (P < 0.05; Fig. 3). Consistently, addition of FFA mix to adipocytes preincubated for 20 h with rosiglitazone abrogated the continued visfatin release (Fig. 3). To confirm signal transduction by rosiglitazone, protein expression of p85 (for PI 3-kinase) and phospho-Akt for Akt was assessed by Western
Blotting. Expression of p85 and phospho-Akt were upregulated 1.7- and 4.5-fold by rosiglitazone, respectively. This increase was mitigated by the inhibition of PI 3-kinase 1.3-fold and by Akt inhibition 2.3-fold in coincubation experiments. Inhibitors alone had no effect on basal p85 and phospho-Akt expression. Likewise, inhibitors of PI 3-kinase or Akt had no effect alone (data not shown) but mitigated the rosiglitazone-induced visfatin release during coincubation (Fig. 4). Again, incubation of PI 3-kinase or Akt inhibitors after 20 h of rosiglitazone preincubation reduced visfatin release into the supernatant medium (Fig. 4). Blockade of FFA mix oxidation with 3-hydroxybutyrate had no effect on the action of rosiglitazone but partially prevented the inhibitory effect of FFA mix on rosiglitazone-induced visfatin secretion during coincubation (Fig. 4).

DISCUSSION

This study demonstrates that rosiglitazone treatment increases circulating visfatin concentrations in healthy humans and induces a release of visfatin from isolated adipocytes into the supernatant medium. This effect is counteracted by FFA and can be influenced in vitro by antioxidant strategies. Furthermore, visfatin secretion from adipocytes by rosiglitazone involves activation of PI 3-kinase and Akt.

FFA elevation alone had no effect on resting visfatin concentrations, and the inhibitory action of FFAs was only detectable during rosiglitazone-increased visfatin release. This is consistent with recent results (29) where plasma visfatin and adipocyte visfatin mRNA was unchanged by FFA infusion in healthy subjects. This secretion pattern suggests a potential nutritional sensor role for visfatin, as also demonstrated for other adipocytokines like leptin, adiponectin, and resistin (18). On the other hand, the effects of the acute and excessive elevation of FFAs in healthy subjects might differ from the insulin resistance pattern observed in type 2 diabetes (10). For instance, animal studies (6, 15, 19, 20, 26, 33, 34, 40) investigating the effect of TZD on lipid infusion or high-fat diet-induced insulin resistance have produced variable results. Interestingly, FFA-induced insulin resistance as assessed by a euglycemic clamp is not improved by pretreatment with rosiglitazone (10). Thus the systemic effect of rosiglitazone on plasma visfatin concentrations is not likely the result of altered insulin sensitivity, and the changes observed occurred in the absence of altered circulating insulin concentrations in healthy subjects.

The marked effect of TZD treatment on adipocyte visfatin secretion is also in good agreement with previous data (8)
demonstrating that rosiglitazone increases visfatin mRNA expression in animal adipocyte deposits. Furthermore, the smaller increases of circulating FFAs in subjects receiving rosiglitazone compared with placebo are similar to results obtained by others (10, 27). Consequently, the action of TZD on FFA handling as paralleled by release of the insulin-mimetic adipocytokine visfatin might contribute to the anti-diabetic drug effect. This may be further influenced by simultaneous changes in other adipocytokines, such as adiponectin (23). However, circulating visfatin concentrations in healthy subjects were much lower than in patients with diabetes mellitus type 2 (7), even when stimulated with rosiglitazone for 3 wk. In a recent study, 3-wk treatment of pioglitazone in patients with type 2 diabetes had no effect on visfatin mRNA expression or systemic release (14). Because visfatin mRNA expression and circulating levels are increased in patients with type 2 diabetes, it is unclear whether this condition overlaps a possible TZD effect as detected in our young and healthy cohort (3, 7, 14). Additionally, the increase compared with levels in diabetic patients appeared to be small (14). Subjects with type 2 diabetes under study in this open-label trial had comorbidity and were of a higher age, which might have influenced the result. In our study, healthy young males were investigated to exclude a potential influence of comorbidity or metabolic derangements. Although several results from clinical studies (13) suggest differences between rosiglitazone and pioglitazone...
zone, cell culture experiments did not confirm major differences regarding PPAR activation (22, 24, 28). However, the agonistic properties on these receptors and other actions might be more augmented for some TZDs (2, 11, 16). It is presently unclear whether the concentrations of visfatin achieved are able to cause pharmacological actions. Furthermore, the systemic anti-diabetic mechanisms of TZDs are not fully characterized yet, and the source of plasma visfatin is unclear. It is also unknown whether adipocytes are particularly sensitive to TZDs to release visfatin because tissue differences may exist. Our results demonstrate that the effect of rosiglitazone on visfatin release from adipocytes depends on the PI 3-kinase/Akt pathway and confirm previous data on TZD-induced activation of these signal transduction proteins (36). The concentration and time required for visfatin release during rosiglitazone incubation indicates that upregulation of protein synthesis might represent an additional underlying mechanism of elevated visfatin plasma concentrations in response to TZDs. Enhanced visfatin release after 24 h of rosiglitazone incubation in vitro could be related to cell-specific mechanisms of TZD signal transduction. This would be compatible with data demonstrating increased protein release from isolated skeletal muscle cells upon continued exposure to pioglitazone or rosiglitazone, demonstrating increased protein release from isolated skeletal muscle cells upon continued exposure to pioglitazone or rosiglitazone (22). The increase of circulating visfatin in vivo after 3 wk of systemic TZD treatment may be due to the fact that not adipocytes alone but also other cells such as macrophages can release visfatin (9). The net contribution of adipocytes and other cells for this finding, however, is speculative and cannot be derived from the present study.

Visfatin gene expression and translational regulation data from humans are limited at present. However, visfatin secretion is apparently influenced by oxidation products of FFAs from humans are limited at present. However, visfatin secretion is apparently influenced by oxidation products of FFAs that mimics the effects of insulin. Plasma visfatin concentrations and fat acid oxidation (37), partially reversed the inhibitory effect of FFAs on visfatin release by human adipocytes. The increase of circulating visfatin in vivo after 3 wk of systemic TZD treatment may be due to the fact that not adipocytes alone but also other cells such as macrophages can release visfatin (9). The net contribution of adipocytes and other cells for this finding, however, is speculative and cannot be derived from the present study.

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In summary, basal visfatin release is enhanced in vitro and in healthy subjects by rosiglitazone treatment, which may contribute to its anti-diabetic pharmacological action. This effect is acutely reduced by elevation of FFAs, which suggests a potential nutrient sensor to be involved in adipocytokine release. Visfatin bioactivity may therefore be modulated by food intake.

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