Oxyntomodulin ameliorates glucose intolerance in mice fed a high-fat diet

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Submitted 6 September 2007; accepted in final form 30 October 2007

Parlevliet ET, Heijboer AC, Schröder-van der Elst JP, Havekes LM, Romijn JA, Pijl H, Corssmit EP. Oxyntomodulin ameliorates glucose intolerance in mice fed a high-fat diet. Am J Physiol Endocrinol Metab 294: E142–E147, 2008. First published October 30, 2007; doi:10.1152/ajpendo.00576.2007.—We evaluated the acute effects of OXM on glucose metabolism in diet-induced insulin-resistant male C57Bl/6 mice. To determine the effects on glucose tolerance, mice were intraperitoneally injected with OXM (0.75, 2.5, or 7.5 nmol) or vehicle prior to an ip glucose tolerance test. OXM (0.75 nmol/h) or vehicle was infused during a hyperinsulinemic euglycemic clamp to quantify insulin action on glucose production and disposal. OXM dose-dependently improved glucose tolerance as estimated by AUC for glucose (OXM: 7.5 nmol, 1,564 ± 222 mmol·kg⁻¹·min⁻¹ vs. control: 2,226 ± 309 mmol·kg⁻¹·min⁻¹, P < 0.01; 0.75 nmol, 1,828 ± 684, P < 0.01; 0.75 nmol, 2,322 ± 303, P < 0.05; control: 2,790 ± 222 mmol·kg⁻¹·min⁻¹). Insulin levels in response to glucose administration were higher in 7.5 nmol OXM-treated animals compared with controls. In basal clamp conditions, OXM increased EGP (82.2 ± 14.7 vs. 39.9 ± 5.7 μmol·min⁻¹·kg⁻¹, P < 0.001). During insulin infusion, insulin levels were twice as high in OXM-treated mice compared with controls (10.6 ± 2.8 vs. 4.4 ± 2.2 ng/ml, P < 0.01). Consequently, glucose infusion rate (118.6 ± 30.8 vs. 38.8 ± 26.4 μl/h, P < 0.001) and glucose disposal (88.1 ± 13.0 vs. 45.2 ± 6.9 μmol·min⁻¹·kg⁻¹, P < 0.001) were enhanced in mice that received OXM. In addition, glucose production was more suppressed during OXM infusion (35.7 ± 15.5 vs. 15.8 ± 11.4% inhibition, P < 0.05). However, if these data were expressed per unit concentration of circulating insulin, OXM did not affect insulin action on glucose disposal and production. These results indicate that OXM beneficially affects glucose metabolism in diet-induced insulin-resistant C57Bl/6 mice. It ameliorates glucose intolerance, most likely because it elevates glucose-induced plasma insulin concentrations. OXM does not appear to impact on insulin action.

animal model; gut hormone; glucose tolerance; insulin; hyperinsulinemic euglycemic clamp

POSTTRANSLATIONAL PROCESSING OF PROGLUCAGON in the intestine and the central nervous system yields glucagon-like peptide-1 (GLP-1), GLP-2, glicentin, and oxyntomodulin (OXM) (18, 32, 35). OXM contains 37 amino acids, including the complete GLP-1 structure (6, 22). Its anorexigenic effect requires only the terminal part of the molecule (5, 12). A distinct receptor for OXM has not been identified to date. However, OXM can bind to both the glucagon and GLP-1 receptor, which is to be expected on the basis of its molecular structure (6, 22). Its anorexigenic effect requires only the (central) GLP-1 receptor (3). Thus, OXM probably impacts on food intake via neural routes in the ARC, which are similarly engaged by other gut-derived peptides modulating appetite, like GLP-1, ghrelin, and peptide YY3-36 (1, 7, 11, 41, 44).

Gut hormones are important for the regulation of glucose metabolism. We (23, 42) recently showed that ghrelin and peptide YY3-36 affect insulin sensitivity via mechanistic routes that are independent of food intake and body weight. Also, recent evidence (31) suggests that GLP-1 ameliorates insulin resistance and hyperglycemia in diabetic ob/ob mice. Therefore, we speculated that OXM could impact on insulin sensitivity in insulin-resistant mice. The aim of this study was to explore the effects of systemic OXM administration on glucose tolerance and insulin sensitivity in diet-induced insulin-resistant C57Bl/6 mice. To this end, we injected OXM intraperitoneally prior to an intraperitoneal glucose tolerance test (IPGTT) and administered OXM intravenously during a hyperinsulinemic euglycemic clamp in high-fat-fed insulin-resistant C57Bl/6 mice.

METHODS

Animals and Diet

Male C57Bl/6 mice (12 wk old; Charles River, Maastricht, The Netherlands) were housed in a temperature- and humidity-controlled environment and were fed a high-fat diet (44 energy% fat derived from bovine fat; Hope Farms, Woerden, The Netherlands) with free access to water for 16–20 wk to induce insulin resistance (40). All animal experiments were approved by the Animal Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands.

Drugs

OXM (human, mouse, rat; molecular weight = 4,449, 90 g/mol) was purchased from Phoenix Pharmaceuticals (Belmont, CA).

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After 16 wk on diet, mice were fasted for 10 h, with food withdrawn at 2300 on the day prior to the study. At 9:00 AM on the experimental day, mice were given an intraperitoneal injection of OXM dissolved in PBS (0.75, 2.5, or 7.5 nmol) or PBS alone in a volume of 100 μl. The doses were based on a previous report (15, 16) in which 0.75 nmol of OXM reduced food intake in rats. Fifteen minutes after injection, blood was taken, immediately followed by intraperitoneal injection of a 2 g/kg 20% d-glucose solution (t = 0). Additional blood samples were taken via tail bleeding at 5, 15, 30, 45, 60, and 120 min after glucose injection for measurement of plasma glucose and insulin levels.

Hyperinsulinemic Euglycemic Clamp Study

The same cohort of mice was used for the clamp study as for the IPGTT with 4 wk of intermission. Mice were fasted for 16 h, with food withdrawn at 1700 on the day prior to the study. Hyperinsulinemic euglycemic clamp studies started at 9:00 AM and were performed as described earlier (43). During the experiment, mice were sedated with 6.25 mg/kg acepromazine (Alfasan, Woerden, The Netherlands), as described earlier (43). During the experiment, mice were sedated with 6.25 mg/kg midazolam (Roche, Mijdrecht, The Netherlands), and 0.3125 mg/kg fentanyl (Janssen-Cilag, Tilburg, The Netherlands). Vehicle or OXM was administered in a primed (0 and 0.45 nmol, respectively), continuous (0 and 0.75 nmol/h, respectively) intravenous infusion during the whole experiment. First, basal rate of glucose turnover was determined by giving a primed (p) continuous (c) intravenous infusion of [14C]glucose (p: 0.7 μCi, c: 1.2 μCi/h; GE Healthcare, Little Chalfont, UK) for 60 min. Subsequently, insulin was administered in a primed (4.5 mU), continuous (6.8 mU/h) intravenous infusion for 90 min to attain steady-state circulating insulin levels of ~4 ng/ml. A variable intravenous infusion of a 12.5% d-glucose solution was used to maintain euglycemia as determined at 10-min intervals via tail bleeding (<3 μl, Accu-chek, Sensor Comfort; Roche Diagnostics, Mannheim, Germany). Blood samples (60 ml) were taken during the basal period (after 50 and 60 min) and during the clamp period (after 70, 80, and 90 min) to determine plasma concentrations of glucose, nonesterified fatty acids (NEFA), insulin, and plasma [14C]glucose-specific activities. To estimate insulin-stimulated glucose uptake in individual tissues, 2-deoxy-d-[3H]glucose (2-[3H]DG; Amersham, Little Chalfont, UK) was administered as a bolus (2 μCi) 40 min before the end of the clamp. At the end of the clamp, mice were killed and skeletal muscle (hindlimb) and white adipose tissue (abdomen) were isolated and immediately frozen in liquid nitrogen for subsequent analysis.

Analytical Procedures

Plasma levels of glucose and NEFA were determined using commercially available kits (Instruchemie, Delfzijl, The Netherlands). Plasma insulin concentrations were measured by a mouse insulin ELISA (Mercodia, Uppsala, Sweden). Total plasma [14C]glucose was determined in 7.5 μl of plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water, as described earlier (21).

Tissue Analysis

For determination of tissue 2-[3H]DG uptake, the homogenate of muscle and adipose tissue was boiled and the supernatant subjected to an ion exchange column to separate 2-[3H]DG-6-phosphate from 2-[3H]DG, as described previously (39).

Calculations

Areas under the curve (AUC) for glucose (AUCglucose) and insulin (AUCinsulin) were calculated using the trapezoidal rule. The turnover rate of glucose (μmol·min⁻¹·kg⁻¹) was calculated during the basal period and under steady-state clamp conditions as the rate of tracer infusion (dpm/min) divided by the plasma-specific activity of [14C]glucose (dpm/μmol). The ratio was corrected for body weight. Endogenous glucose production (EGP) was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate. The whole body insulin sensitivity index was calculated as the ratio of glucose disposal to plasma insulin levels during hyperinsulinemic conditions. The hepatic insulin sensitivity index was calculated as the ratio of the relative suppression of EGP during the hyperinsulinemic condition to the change in plasma insulin levels from basal to hyperinsulinemic conditions. Tissue-specific glucose uptake was calculated from tissue 2-[3H]DG of the dosage per gram of tissue, as previously described (39).

Statistical Analysis

Differences between groups were determined with the Kruskal-Wallis nonparametric test for k-independent samples. When significant differences were found, the Mann-Whitney nonparametric test was used as a post hoc test to determine differences between two independent groups. A P value of <0.05 was considered statistically significant. Data are presented as means ± SD.

RESULTS

Glucose Tolerance Test

OXM significantly improved glucose tolerance in a dose-dependent way, as evidenced by a reduction of the area under the curve of glucose concentrations (AUCglucose: OXM, 7.5 nmol: 1.564 ± 460 mmol·l⁻¹·120 min, P < 0.01; 2.5 nmol: 1.828 ± 684 mmol·l⁻¹·120 min, P < 0.01; 0.75 nmol: 2.322 ± 303 mmol·l⁻¹·120 min, P < 0.05; control: 2.790 ± 222 mmol·l⁻¹·120 min; Fig. 1, A and B). Insulin levels in response to glucose administration were significantly increased in animals receiving 7.5 nmol of OXM compared with control animals (AUCinsulin: 343 ± 113 vs. 231 ± 39 ng·ml⁻¹·120 min, P < 0.05; Fig. 2, A and B), but not in animals treated with 2.5 or 0.75 nmol of OXM.

Hyperinsulinemic Euglycemic Clamp

Plasma parameters and body weight. Body weight, plasma glucose, insulin, and NEFA concentrations in basal and hyperinsulinemic conditions are shown in Table 1. Basal glucose and insulin levels were significantly higher and basal NEFA levels significantly lower in mice that received OXM compared with control mice. In hyperinsulinemic steady-state clamp conditions, insulin levels remained significantly higher and plasma NEFA levels significantly lower in OXM-treated mice compared with control mice.

Glucose turnover and tissue-specific glucose uptake. In basal conditions, EGP was significantly higher in the OXM-treated animals compared with control animals (82.2 ± 14.7 vs. 39.9 ± 5.7 μmol·min⁻¹·kg⁻¹, P < 0.001). The rate of glucose infusion required to maintain euglycemia during insulin infusion was significantly higher in OXM-treated mice than in control mice (118.6 ± 30.8 vs. 38.8 ± 26.4 μl/h, respectively, P < 0.001). This was because glucose disposal was significantly enhanced (88.1 ± 13.0 vs. 45.2 ± 6.9 μmol·min⁻¹·kg⁻¹ in controls, P < 0.001; Fig. 3A) and glucose production more suppressed (35.7 ± 15.5 vs. 15.8 ± 11.4% inhibition in controls, P < 0.05; Fig. 4A) during insulin infusion in OXM-treated animals. Accordingly, insulin-medi-
ated 2-[3H]DG uptake in adipose and muscle tissue were significantly higher in OXM-treated mice (adipose tissue: 1.3 ± 0.8 vs. 0.5 ± 0.2 μmol/g tissue in controls, P < 0.01; muscle: 5.2 ± 2.9 vs. 2.2 ± 1.1 μmol/g tissue in controls, P < 0.05). However, the plasma insulin concentrations attained by insulin infusion were significantly higher in OXM-treated mice compared with controls (10.6 ± 2.8 vs. 4.4 ± 2.2 ng/ml, P < 0.01). Therefore, we also present the data per unit of circulating insulin concentration. The whole body and hepatic insulin sensitivity indexes were not different between groups [whole body insulin sensitivity index: 8.9 ± 2.6 vs. 12.2 ± 5.2 μmol·min⁻¹·kg⁻¹·ng insulin⁻¹·ml⁻¹, not significant (NS); hepatic insulin sensitivity index: 3.5 ± 1.5 vs. 3.3 ± 1.8% inhibition from basal per ng insulin/ml, NS; Figs. 3B and 4B, respectively].

**DISCUSSION**

This is the first study to show that systemic administration of OXM beneficially affects glucose metabolism in diet-induced insulin-resistant C57Bl/6 mice. A single intraperitoneal injection of OXM dose-dependently ameliorates glucose intolerance in these animals. The plasma insulin concentrations in response to glucose administration were higher in OXM-treated animals, particularly after the highest dose. In apparent contrast, intravenous OXM administration clearly enhanced EGP in fasted animals. During the euglycemic clamp, intravenous OXM stimulated glucose uptake and curtailed EGP, suggesting that it improved insulin action. However, circulating insulin levels were more than double during insulin infusion in OXM-treated mice, complicating the interpretation of the data. Indeed, per unit of ambient insulin concentration, glucose uptake and EGP were not different in OXM and placebo-treated animals. In aggregate, our data indicate that systemic OXM administration improves glucose tolerance in insulin-resistant C57Bl/6 mice, primarily because it elevates plasma insulin levels. Minor effects on insulin action cannot be excluded. In the fasting condition, systemic OXM injection stimulates EGP.

**Table 1. Body weight and plasma parameters under basal and hyperinsulinemic conditions in mice that received continuous intravenous infusion of vehicle (n = 8) or OXM (n = 7)**

<table>
<thead>
<tr>
<th>Basal</th>
<th>Hyperinsulinemic</th>
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<tr>
<td></td>
<td>Control</td>
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<tr>
<td>Body weight, g</td>
<td>37.1 ± 3.2</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.9 ± 0.8</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>2.0 ± 1.4</td>
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<tr>
<td>NEFA, mmol/l</td>
<td>0.6 ± 0.2</td>
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Values are ± means SD. OXM, oxyntomodulin; NEFA, nonesterified fatty acids. *P < 0.05 vs. control. **P < 0.01 vs. control. ***P < 0.001 vs. control.
OXM dose-dependently improved glucose tolerance in mice maintained on a high-fat diet. Postload plasma glucose concentrations are the result of the concerted effects of insulin secretion, clearance, and action on glucose metabolism. Insulin levels in response to glucose administration were significantly higher in OXM-treated animals, suggesting that OXM stimulates insulin secretion and/or inhibits insulin clearance from the circulation. Indeed, OXM has been shown (26) to stimulate glucose-induced insulin release in perfused rat pancreas, which may be mediated by binding of OXM to the GLP-1 and/or glucagon receptor in β-cells. A unique receptor for OXM has not been identified to date. However, OXM was shown (6, 22) to bind and activate GLP-1 and glucagon receptors, albeit with lower affinity than the cognate ligands. β-cells express GLP-1 as well as glucagon receptors (33, 34), and both ligands reinforce glucose-induced insulin secretion (24, 25, 28, 36). Thus, OXM most likely triggered insulin secretion by simultaneous activation of GLP-1 and glucagon receptors on β-cells (17). However, interaction with (an)other receptor(s) cannot be excluded, because OXM was shown (38) to induce contraction of antral smooth muscle cells in an in vitro model in which GLP-1 and glucagon had no effect at all.

GLP-1 retards insulin clearance from the circulation in mice (2), which suggests that OXM may also slow insulin clearance to elevate glucose-induced plasma insulin levels. This inference is strongly supported by our finding that OXM administration more than doubled plasma insulin concentrations in the face of insulin infusion rates similar to those in placebo-treated animals during the hyperinsulinemic clamp. In any case, our data clearly suggest that the beneficial effect of OXM on glucose tolerance is entirely due to elevation of postload plasma insulin concentrations, because OXM did not significantly impact on insulin action as quantified by hyperinsulinemic euglycemic clamp.

OXM inhibits food intake in rats, mice, and humans (14–16). Other gut peptides that similarly decrease appetite also modulate insulin action and glucose metabolism (23, 31, 42). In fact, many peptides involved in the control of feeding simultaneously regulate metabolism, illustrating the intuitively obvious notion that food intake and metabolism share common regulatory pathways (5, 37, 45). Therefore, we speculated that OXM could also impact on insulin action. However, although OXM dose-dependently improved glucose tolerance, it did not appear to affect insulin action, as glucose disposal and EGP on a per-unit-of-circulating-insulin-concentration basis were not different in OXM vs. placebo-infused animals during the euglycemic clamp. Correction of glucose production and uptake rates for plasma insulin levels is rather complex, because insulin action does not linearly correlate with plasma insulin concentrations (9). Thus, glucose production and disposal expressed per unit of circulating insulin concentration may not be an optimal measurement of insulin’s capacity to impact on these metabolic processes. However, we believe it is the best measurement we have given the fact that circulating insulin levels were almost twofold higher in OXM-treated animals during the clamp. Therefore, we cannot exclude minor effects of OXM on insulin sensitivity also in light of the data from the IPGTT showing that the two lowest doses of OXM did improve glucose tolerance without increased insulin levels.

Fig. 3. Hyperinsulinemic glucose disposal (A) and whole body insulin sensitivity index (B) in mice that received continuous intravenous infusion of vehicle (n = 8) or OXM (n = 7) during a hyperinsulinemic euglycemic clamp. Values represent means ± SD. *P < 0.001 vs. control.

Fig. 4. Inhibition of endogenous glucose production (A) and hepatic insulin sensitivity index (B) in mice that received continuous intravenous infusion of vehicle (n = 8) or OXM (n = 7) during a hyperinsulinemic euglycemic clamp. Values represent means ± SD. *P < 0.05 vs. control.
The failure of OXM to deeply impact on insulin action may have to do with its affinities for both the GLP-1 and the glucagon receptors. Although GLP-1 and glucagon similarly stimulate insulin secretion, these peptides generally relay opposing metabolic messages. GLP-1 inhibits food intake (41) and simultaneously improves insulin action in diabetic mice (31), whereas glucagon antagonizes insulin action [it stimulates EGP and indirectly inhibits glucose disposal by stimulating lipolysis (13, 27)]. Thus, simultaneous activation of both GLP-1 and glucagon receptors by systemic administration of OXM is unlikely to significantly impact on insulin action.

Our findings suggest that OXM may be the base of new pharmacological tools to ameliorate glucose intolerance in insulin-resistant subjects. The mice examined in our studies were maintained on a high-fat diet, which invariably induces obesity and insulin resistance in the C57Bl/6 strain (40). Thus, the fact that OXM can improve glucose tolerance in these animals indicates that this peptide (or any novel peptide-mimetic) may be useful in the treatment of obesity and type 2 diabetes, like other proglucagon derived peptides (10). However, OXM enhanced glucose production in the basal condition, probably by virtue of its capacity to activate the glucagon receptor in the liver (4, 6). Obviously, stimulation of glucose production is an undesirable quality of drugs used to treat insulin resistance that requires careful evaluation. At the end of the day, if it turns out to be a consistent metabolic effect of treatment with OXM (mimetics), excessive glucose production clearly limits the clinical applicability of this peptide.

In conclusion, OXM beneficially affects glucose metabolism in diet-induced insulin-resistant C57Bl/6 mice. It improves glucose tolerance, enhances glucagon disposal by peripheral tissues, and reduces glucose production primarily by elevating glucagon-induced plasma insulin levels.

GRANTS
This study was supported by the Dutch Scientific Research Council projects 907-00-002 (to E. P. M. Corssmit) and 980-10-017 (to H. Pijl).

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