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Published in:
American Journal of Physiology - Endocrinology and Metabolism

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1996

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Effects of glucagon-like peptide-I on glucose turnover in rats

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Department of Animal Physiology, University of Groningen, 9750A Haren, The Netherlands; Department of Medicine, Lund University S-205 02, Malmö, Sweden; and Department of Medical Physiology, Copenhagen University, DK-2200 Copenhagen, Denmark

Van Dijk, Gertjan, Stefan Lindskog, Jens J. Holst, Anton B. Steffens, and Bo Ahren. Effects of glucagon-like peptide-I on glucose turnover in rats. Am. J. Physiol. 270 (Endocrinol. Metab. 33): E1015–E1021, 1996.—The influences of glucagon-like peptide-I (7–36) amide (GLP-I, 15 pmol·kg⁻¹·min⁻¹) on glucose turnover were studied in freely moving Wistar rats. In fed rats, GLP-I reduced plasma glucose (from 7.3 ± 0.2 to 5.6 ± 0.3 mmol/l; P = 0.017), increased plasma insulin (from 20 ± 3 to 89 ± 11 mU/l; P = 0.002), and reduced plasma glucagon (from 41 ± 1 to 35 ± 2 pg/ml; P = 0.009), and glucose appearance rate (Rₚ; from 3.9 ± 0.2 to 1.7 ± 0.7 pmol·min⁻¹·100 g⁻¹ after 30 min; P = 0.049) without affecting glucose disappearance rate (Rₜ). The glucose clearance rate (MCR) was increased (P = 0.048). In 48-h-fasted rats, GLP-I reduced plasma glucose (from 5.0 ± 0.2 to 4.4 ± 0.3 mmol/l; P = 0.035) and increased plasma insulin (from 4 ± 1 to 25 ± 10 mU/l; P = 0.042) and plasma glucagon (from 43 ± 3 to 61 ± 7 pg/ml; P = 0.046). Rₚ and Rₜ were not significantly affected, although Rₚ was lower than Rₜ after 15–30 min (P = 0.005) and MCR was increased (P = 0.049). Thus GLP-I reduces Rₜ in fed rats and increases MCR in fed and fasted rats. The reduced Rₚ seems mediated by an increased insulin-to-glucagon ratio, the increased glucose clearance seems dependent on insulin and a peripheral effect of GLP-I.

The mechanism underlying the reduced circulating glucose levels by GLP-I has not been established. In overnight-fasted human subjects, infusion of GLP-I reduces hepatic glucose delivery, which most likely is a consequence of increased insulin and decreased glucagon secretion (16). In addition, GLP-I increases the metabolic clearance of glucose, presumably because of increased insulin levels (16). There is also evidence, however, that GLP-I reduces circulating glucose levels by a peripheral insulin independent action, because the peptide increased glucose utilization during a hyperglycemic hyperinsulinemic clamp in type 1 diabetic patients (12). Furthermore, GLP-I might also reduce the glucose levels by potentiating the insulin-independent glucose disposal, as has been shown in fasted healthy human subjects (5). This is in agreement with our previous observation that the peptide reduced circulating glucose levels in mice which had been given 2,5-anhydro-D-mannitol, a compound known to inhibit glycogenolysis, gluconeogenesis, and insulin secretion (4). Alternatively, the effects described above might be explained by an inhibitory action of GLP-I on hepatic glucose delivery through a potentiation of the previously described glucose-dependent inhibition of hepatic glucose delivery (19). The differing results indicate that the relative contribution to the glucose-lowering effect of GLP-I by reduction of hepatic glucose delivery vs. stimulation of peripheral glucose disposal is still not established. Neither is it known whether the mechanism of the glucose-lowering effect of GLP-I is different under fasting and fed conditions.

To study further the mechanism of the glucose-lowering effect of GLP-I, we examined the influence of the peptide on hepatic glucose delivery and peripheral glucose disposal by use of the glucose tracer technique in rats under both fed and fasting (48 h) conditions.

MATERIALS AND METHODS

Animals. Male Wistar rats, weighing 290–320 g at the beginning of the present experiment, were used. The animals were individually housed in Plexiglas cages (25 × 25 × 30 cm) at room temperature (20 ± 2°C) and had continuous access to food (Hope farm chow) and water unless otherwise stated. The rats were maintained on a 12:12-h light-dark cycle (0700–1900 light), and they were handled and weighed every day. Under halothane anesthesia and 3 wk before the experiments, all rats were provided with a nonocclusive catheter in the abdominal artery according to techniques described elsewhere (29). This catheter allows frequent arterial blood sampling in freely moving undisturbed rats. For venous infusion of tritiated glucose and/or GLP-I solutions, rats were also provided with a silicon catheter that was inserted in the heart via the right jugular vein (28). Before experiments, catheters were filled with polyvinylpyrrolidone (mol wt 2,500). Patency of the aortic and venous catheters was checked every 3 and 7 days, respectively.

Experimental procedures. All experiments were performed in the light period between 1100 and 1400 in the home cage of the animals. Food was removed from the cage 2 or 48 h before the start of the experiment. One hour before blood sampling, venous and arterial catheters were connected to polyethylene tubes (length 0.4 m, OD 1.25 mm, ID 0.75 mm) filled with citrated saline. Forty-five minutes before the first blood
sample, a priming dose of 2.1 μCi D-[3-3H]glucose (NET 331, New England Nuclear, Boston, MA), followed by a constant-rate infusion of 93 nCi/min, was delivered intravenously. After withdrawal of each blood sample, an equal amount of citrated blood (10%) was given to avoid diminution of the blood volume. Donor blood was obtained from undisturbed donor rats with permanent heart catheters. In the intervals between withdrawal of each blood sample, the tip of the aortic blood volume. Donor blood was obtained from undisturbed New England Nuclear, Boston, MA), followed by a constant-titrated blood

After withdrawal of each blood sample, an equal amount of sample, a priming dose of 2.1 μCi D-[3-3H]glucose (NET 331, El016) was administered as an intravenous bolus injection in rats and found to augment glucose-stimulated insulin secretion and at the same time be metabolized within 8 min (14). During infusion of GLP I or vehicle, blood samples were taken at t = -30 min, -15 min, and -30 s. The dosage of GLP-I was chosen to deliver 15 pmol·min⁻¹·kg⁻¹. This dose was selected on the basis of a previous study in rats in which a dose of ~125 pmol/kg of the similar peptide GLP-I-(7-37) was administered as an intravenous bolus injection in rats and found to augment glucose-stimulated insulin secretion and at the same time be metabolized within 8 min (14). During infusion of GLP I or vehicle, blood samples were taken at t = 15, 30, 45, 60, and 75 min.

Chemical determinations. Blood samples were immediately transferred to chilled (0°C) centrifuge tubes containing EDTA (0.01%), heparin (500 IU/ml), and aprotinin (trypsin inhibitor, 500 kalikrein inhibitor units/ml) and were centrifuged for 15 min at 5,000 rpm at 4°C. The supernatant was stored at -70°C until analyzed. Determination of plasma glucose concentrations was enzymatically performed in triple 10 μl samples. Rat-specific plasma immunoreactive insulin and glucagon were determined by means of radioimmunoassay kits. Guinea pig serum (for insulin M8309 and for glucagon K5563, both from Novo Nordisk, Bagsvaerd, Denmark) served as antisera. A second antibody binding to donkey anti-rabbit antibody-coated celluloanse particles (SAC-CEL ASAC 1; Immuno Diagnostics, UK) was performed for glucagon analysis. Plasma concentrations of GLP-I were measured with a radioimmunoassay after extraction with ethanol, as previously described (24). The antiserum used (code no. 89390) is highly specific for GLP-I of intestinal origin. Analytic procedures for [3H]glucose were performed as previously described (30).

Calculations and statistics. Glucose Rₜ and Rₐ were calculated using Steele's equations (27), which allow determinations in steady-state as well as non-steady-state conditions. The volume of distribution in which rapid changes in glucose concentration and specific activity of [3H]glucose take place was set to 188 ml/kg (25). Rₜ and Rₐ values in Figs. 2, 4, and 5 are demonstrated between the time points for the samples used for their calculations. For example, when samples taken at t = 15 and t = 30 min were used for the calculation, the Rₜ and Rₐ are shown at t = 22.5 min. The clearance of glucose was calculated by glucose Rₜ divided by plasma glucose concentrations. All results are presented as means ± SE. For statistical evaluation of the data, Student's paired and unpaired t-tests were used.

RESULTS

Fed rats. On the experimental day, the ad libitum-fed rats receiving the GLP-I solution (n = 5) and vehicle (n = 6) weighed 357 ± 8 and 353 ± 5 g, respectively. Table 1 shows the baseline plasma glucose, insulin, and glucagon levels as well as the Rₜ, Rₐ, and the clearance rate of glucose. Fig. 1 shows the plasma levels of glucose, insulin, and glucagon before and during infusion of GLP-I (n = 5) or vehicle (n = 6) in the fed rats. Intravenous infusion of GLP-I reduced the plasma glucose levels from 7.3 ± 0.2 to 5.6 ± 0.3 mmol/l at 30 min, i.e., by 1.7 ± 0.5 mmol/l (P = 0.017). Thereafter the glucose levels gradually returned toward the preinfusion levels. Furthermore, GLP-I markedly, although transiently, increased plasma insulin levels from 20 ± 8 to 89 ± 11 μU/ml after 15 min of infusion, i.e., by 69 ± 10 μU/ml (P = 0.002). After 15 min of infusion, the plasma insulin levels returned to baseline despite the ongoing GLP-I infusion. The plasma insulin levels did not drop below those in the controls, even though the plasma glucose levels were lower. Plasma glucagon levels were constantly reduced during the GLP-I infusion, being 44 ± 1 pg/ml before GLP-I infusion and 35 ± 2 pg/ml after 45 min of peptide infusion (P = 0.009). After 15 min of GLP-I infusion, the plasma insulin-to-plasma glucagon ratio was 2.5 ± 0.4 μU/pg, whereas the corresponding ratio in saline-infused controls was 0.39 ± 0.03 μU/pg (P = 0.009). In the vehicle-infused control rats, no significant changes were observed in plasma glucose, insulin, or glucagon levels.

Table 1. Baseline levels of plasma insulin, glucagon, glucose, and GLP-I, the glucose Rₜ and Rₐ, and the MCR in freely fed and 48 h fasted rats

<table>
<thead>
<tr>
<th></th>
<th>Fed Rats (n = 11)</th>
<th>Fasted Rats (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, μU/ml</td>
<td>23.0 ± 1.6</td>
<td>3.9 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>46 ± 1</td>
<td>44 ± 2</td>
<td>0.96 (NS)</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>7.2 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GLP-I, pmol/l</td>
<td>18.5 ± 1.7</td>
<td>12.6 ± 1.3</td>
<td>0.013</td>
</tr>
<tr>
<td>Glucose Rₜ, pmol·min⁻¹</td>
<td>3.8 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose Rₐ, pmol·min⁻¹</td>
<td>3.7 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCR, ml·min⁻¹·100 g⁻¹</td>
<td>0.51 ± 0.01</td>
<td>0.27 ± 0.02</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE. GLP-I, glucagon-like peptide-I. Glucose rates of appearance (Rₜ) and disappearance (Rₐ) and the glucose clearance rate (MCR) were calculated after 30–45 min of infusion of D-[3-3H]glucose. P indicates the probability level of random difference between the 2 groups; NS indicates no significant difference (P > 0.050).
Thereafter, plasma glucose levels gradually returned to baseline values. Similarly to the fed rats, GLP-I induced a transient increase in plasma insulin levels in the fasted rats, from $4 \pm 1$ to $25 \pm 10 \text{mU/l}$ after 15 min of infusion, i.e., by $21 \pm 8 \text{mU/l}$ ($P = 0.042$). Thereafter, the insulin values returned to the low baseline levels despite the ongoing GLP-I infusion. As in the fed rats, plasma insulin levels never dropped below control values, yet plasma glucose levels were lower in GLP-I-than in vehicle-infiltrated rats. In contrast to what was observed in the fed rats, plasma glucagon levels transiently increased during the GLP-I infusion in the 48-h fasted rats, from $43 \pm 3$ to $61 \pm 7 \text{pg/ml}$, i.e., by $18 \pm 6 \text{pg/ml}$ after 30 min of infusion ($P = 0.046$). The peak glucagon values coincided with the nadir glucose value; thereafter glucagon levels returned to preinfusion values. After 15 min of GLP-I infusion, the plasma insulin-to-plasma glucagon ratio was $0.49 \pm 0.18 \text{U/}
ug$, whereas the corresponding ratio in vehicle-infused controls was $0.06 \pm 0.02 \text{U/pg}$ ($P = 0.041$). However, after 30 min of infusion, the plasma insulin-to-plasma glucagon ratio had returned to $0.11 \pm 0.04 \text{U/pg}$ in the GLP-I-infused animals vs. $0.06 \pm 0.02 \text{U/pg}$ in the controls (NS). In the vehicle-infused control rats, no significant changes were observed in plasma glucose, insulin, or glucagon levels.

Figure 4 shows the glucose $R_a$ and $R_d$ during infusion of GLP-I and vehicle in the 48-h-fasted rats. Neither the glucose $R_a$ nor the glucose $R_d$ was significantly affected by GLP-I. $R_a$ was significantly lower than $R_d$ at the interval of 15–30 min after start of GLP-I infusion ($0.83 \pm 0.15 \text{vs. } 1.63 \pm 0.15 \text{mMol} \cdot \text{min}^{-1} \cdot \text{100 g}^{-1}$, $P =$...
Fig. 3. Plasma glucose (A), insulin (B), and glucagon concentrations (C) in rats fasted for 48 h and infused intravenously with GLP-I (n = 5; 15 pmol·kg⁻¹·min⁻¹; •) or vehicle (n = 5; ○). Infusions are as described in Fig. 1. Values are means ± SE.

0.005; Fig. 5), which was at the time point when plasma glucose values were lowered by GLP-I (see Fig. 3). The glucose clearance rate, i.e., glucose Rₜ-to-plasma glucose ratio, was significantly higher after 30 min of infusion with GLP-I, 0.38 ± 0.04 ml·min⁻¹·100 g⁻¹, than during infusion of vehicle, 0.26 ± 0.03 ml·min⁻¹·100 g⁻¹ (P = 0.049). Vehicle infusion did not significantly alter glucose Rₜ, glucose Rₜ, or the clearance of glucose in the fasted rats.

Plasma GLP-I levels. Table 1 and Fig. 6 show plasma GLP-I levels before and during infusion of GLP-I and vehicle in freely fed and 48-h-fasted rats. The baseline plasma GLP-I levels were significantly higher in fed rats (18.5 ± 1.7 pmol/l) than in 48-h-fasted rats (12.6 ± 1.3 pmol/l, P = 0.015). The plasma GLP-I levels in fed and fasted rats during GLP-I infusions were not significantly different.

DISCUSSION

In the present study, we found that GLP-I transiently reduces the Rₜ of glucose by 31% in freely fed rats, without affecting the Rₜ of glucose. This resulted in a transient reduction of plasma glucose levels by 1.7 mmol/l (Fig. 1). The clearance rate of glucose, i.e., the rate by which each circulating millimole of glucose is cleared, was increased during GLP-I infusion, because glucose Rₜ remained unaffected whereas plasma glucose levels decreased. Furthermore, the study demonstrates that the peptide increases plasma insulin and reduces plasma glucagon in fed rats. We interpret these findings to indicate that GLP-I in fed rats markedly increases the insulin-to-glucagon ratio, which reduces hepatic glucose output. The insulinotropic action of GLP-I vanished after 30 min, and, as a result, the low glucose Rₜ increased again toward control values. Thus, despite levels of plasma glucagon constantly remaining low, GLP-I only transiently influenced glucose Rₜ. At the same time, GLP-I increases the efficacy of peripheral glucose uptake, which might be an effect mediated by insulin or directly stimulated by GLP-I.

Our results confirm the previously reported study in overnight-fasted humans, in which GLP-I reduced the
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R_{a} and lowered circulating glucose without affecting the R_{d} (16). Our result, that GLP-I increased the glucose clearance, also confirms the studies by Gutniak et al. (12), showing that GLP-I increases insulin sensitivity during a hyperglycemic clamp in type 1 diabetics, and by D’Alessio et al. (5), showing that GLP-I potentiates insulin-independent glucose disposal. Finally, the results corroborate our recent studies in mice, demonstrating that GLP-I lowers plasma glucose both in insulin-deficient alloxan-diabetic mice (2), and after inhibition of liver gluconeogenesis, liver glycogenolysis, and insulin secretion from B cells by 2,5-anhydro-D-mannitol in normal mice (4). Therefore, we conclude that GLP-I reduces glucose levels by a hepatic, but possibly also by a peripheral effect, the hepatic effect being the result of an increased insulin-to-glucagon ratio.

In the present study, infusion of GLP-I led to increased plasma insulin levels and reduced plasma glucose levels in both the fed and the fasted state. In the fasted rats, no significant differences were observed in absolute values of R_{a} or R_{d} during the intravenous infusion with GLP-I. However, at 15–30 min after start of the infusion, R_{a} was significantly higher than R_{d}, which would suggest a change in the R_{a}-R_{d} balance, indicative of a stimulated clearance of glucose. The main mechanism underlying the glucose-reducing effect of GLP-I under fasting conditions is therefore probably a stimulated clearance of glucose. The fact that glucose R_{a} was not altered during GLP-I infusion in fasted rats is probably due to the fact that the increase in the ratio of plasma insulin to plasma glucagon was low in these rats. During GLP-I infusion in fasted rats, plasma glucagon levels were not reduced. This was not expected, because GLP-I has previously been shown to inhibit glucagon secretion in humans (12) as well as in experimental animals, including rodents (9, 23). It is likely that the hypoglycemic action of GLP-I under the 48-h-fasting condition led to an increased activation of the sympathoadrenal system (13), which may have counteracted the direct inhibitory action of GLP-I on glucagon secretion. Taken together, the results of the present study indicate that the glucose-lowering effect of GLP-I in fasted rats is mediated by stimulated glucose clearance rate and that the reduced R_{a}, which is seen only under fed conditions, is not directly mediated by GLP-I. Rather, the reduced R_{a} seems to be mediated by the increased insulin-to-glucagon ratio in plasma, which was markedly increased by GLP-I under fed conditions but only mildly increased during fasting conditions. It cannot be excluded, however, that GLP-I in addition to its effects via pancreatic hormone secretion also directly reduces R_{a} and that this effect was simply counteracted by the rising plasma glucagon levels due to hypoglycemia under fasting conditions. Therefore more direct studies of the effects on GLP-I on liver cell glucose metabolism are required.

Insulin secretion was clearly and markedly stimulated by GLP-I, although this effect was more pronounced under fed than under fasted conditions. This could not be attributed to differences in concentrations of GLP-I in the circulation, because the infusion of GLP-I led to similar levels of plasma GLP-I in fed and fasted rats. Thus this observation may reinforce the idea that the insulinotropic action of GLP-I is glucose dependent (3, 6, 10, 21). In particular, a recent study in the mouse showed a more marked insulinotropic action of GLP-I under fed conditions than during fasting (1). The finding in the present study, that the increased plasma insulin levels returned to preinfusion values already during the ongoing infusion of the peptide, has previously been demonstrated in humans as well (21). This phenomenon might be explained by the gradual lowering of plasma glucose levels, which weakens the insulin secretory action of the peptide. However, because the plasma glucose levels, at least under fed conditions, were still above levels required for the insulinotropic action of GLP-I, other explanations are also possible. For example, it may be speculated that GLP-I has induced a desensitization on the B cells during its infusion, as has been suggested from studies on the effect of GLP-I on adenosine 3′,5′-cyclic monophosphate production in cultured cell lines transfected to express the GLP-I receptor (31). Whether desensitization occurs of GLP-I receptors located on B cells in vivo remains to be studied.

A question that still remains to be solved is whether the effects on glucose turnover observed during GLP-I infusion, i.e., reduced R_{a} in fed rats and increased glucose clearance rate in both fed and fasted animals, are solely mediated by the increased insulin-to-glucagon ratio or whether a direct action of GLP-I contributes to these phenomena. The increased glucose clear...

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Fig. 6. Plasma GLP-I concentrations before and during intravenous infusion of GLP-I (15 pmol kg⁻¹ min⁻¹; •) or vehicle (○) in freely fed rats (A) and in rats fasted for 48 h (B). Infusions started at time 0. Values are means ± SE for 5–6 rats in each group.
Glucose output and the stimulation of the peripheral effect of GLP-I. Because diabetes is accompanied by insufficient insulin and reduced glucagon secretion, whereas the stimulation of the increased clearance rate of glucose, whereas, under fasting conditions, the glucose-lowering effect of GLP-I is mediated mainly by increasing the glucose clearance rate. Furthermore, our study presents evidence that the reduced insulin secretory response induced by GLP-I is secondary to increased glucose clearance. However, the exact mechanism underlying the increased glucose clearance during GLP-I infusion remains to be established.

In conclusion, we have demonstrated that the mechanism of the plasma glucose-reducing effect of GLP-I in the rat is dependent on the nutritional status. Thus, in the freely fed state, the peptide reduces plasma glucose by reducing hepatic glucose delivery and increasing the clearance rate of glucose, whereas, under fasting conditions, the glucose-lowering effect of GLP-I is mediated mainly by increasing the glucose clearance rate. Furthermore, our study presents evidence that the reduced glucose clearance induced by GLP-I is secondary to increased insulin and reduced glucagon secretion, whereas the stimulation of the increased clearance rate of glucose seems to be at least partially secondary to a direct peripheral effect of GLP-I. Because diabetes is accompanied by inappropriately elevated hepatic glucose production and reduced glucose clearance (7), the inhibition of hepatic glucose output and the stimulation of the peripheral glucose uptake by GLP-I, together with its effects to stimulate insulin secretion and inhibit glucagon secretion, reinforce the need for further studies to explore the use of this substance or its analogues in the treatment of diabetes.

This study was supported by the Swedish Medical Research Council (Grants 14X-6894 and 12X-712); the Albert Pahlsson, Ernhold Lundström, Novo Nordisk, and Crafoord Foundations; The Swedish Diabetes Association; Malmö University Hospital; the Faculty of Medicine; Lund University; and the Dutch Scientific Organization.

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Received 13 September 1995; accepted in final form 5 January 1996.

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