Effect of GLP-1 on D-glucose transport, lipolysis and lipogenesis in adipocytes of obese subjects

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Received October 12, 2005; Accepted December 5, 2005

Abstract. GLP-1 has anorectic properties and regulates fuel homeostasis through both its insulinotropic and insulinotrophic actions and effects in extrapancreatic tissue. This study is aimed at characterizing the response to GLP-1 of adipocytes from obese patients, in terms of D-glucose transport and lipid metabolism, in comparison with data from normal subjects. Adipocytes were obtained by enzymatic digestion from the abdominal fat tissue of 25 morbidly obese patients and 8 normal subjects undergoing bariatric or inguinal hernia surgery, respectively. Basal GLUT4 expression, D-glucose transport, glycerol release and lipogenesis were measured in cells treated, when required, with 10^{-12}-10^{-9} M GLP-1, insulin, glucagon and the GLP-1 structurally related peptides, exendin-4 and exendin-9. In obese patients, versus normal subjects, a trend towards lower values was found in GLUT4 protein or mRNA, although the differences were not statistically significant; insulin-stimulated glucose uptake was higher and cells did not respond to GLP-1, while both exendins (10^{-10} and 10^{-9} M) exerted an inhibitory action; basal lipolysis was higher and so was the effect of GLP-1 and glucagon, whereas insulin abolished the lipolytic action of all peptides; both basal lipogenesis and the response to insulin were higher while GLP-1 and exendin-4 were ineffective. These results document the analogies and dissimilarities between the response to GLP-1, exendin-4 and exendin-9, as well as to insulin and glucagon, relative to glucose transport and lipid metabolism of fat tissue from obese patients versus normal subjects, the reduced lipogenic effect and enhanced lipolytic action of GLP-1 being, perhaps, adequate for its therapeutic use in obesity.

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Key words: GLP-1, glucose transport, GLUT4, lipid metabolism, obesity

Introduction

GLP-1, apart from its proven insulinomimetic properties *per se* (1,2), antidiabetic character (3), and stimulatory action upon both the expression of glucotransporter genes (4) and the transport and metabolism of glucose in extrapancreatic tissue (2,5,6), exerts a dual effect in normal rat and human adipocytes (6-9), stimulating not only the mechanism of lipogenesis but also that of lipolysis. These effects of GLP-1 in fat, like those in liver (10) and muscle (11,12), are exerted through a GLP-1-specific receptor (13-15), structurally or functionally distinct (10,11,16) from that in the pancreas (17). In these three extrapancreatic tissues, GLP-1, like insulin, increases the activity of several kinases (PI3K, PKB, p44/42 MAPKs, p70s6k), some of them being determinant for the peptide action (6,18,19).

GLP-1 also inhibits gastric emptying and controls food intake by enhancing satiety (3). Morever, changes in GLP-1 secretion were reported in morbidly obese patients (20-22).

Exendin(1-39)amide (Ex-4), a non-mammalian peptide, shares 53% of its amino-acid sequence with GLP-1; it is also insulinotropic (23) and exerts GLP-1-like effects upon parameters related with the glucose metabolism in rat liver and skeletal muscle (24). Its truncated form, exendin(9-39)amide (Ex-9), has proven to be an antagonist of the GLP-1 receptor in various cell systems (25) and also of its effects in rat pancreas (26), liver cells and muscle tissue (24); however, in human myocytes, both exendins increase glucose transport and metabolism (12,27) and have proven to be an agonist of the GLP-1 receptor in adipocyte (28) and myocyte (16) cell lines.

In recent studies, we have compared the effect of GLP-1 to that of other hormones such as insulin and glucagon on lipolysis and lipogenesis in adipocytes isolated from normal subjects (9). The major aim of the present study, already reported in part in abstract form (Arnés, *et al*, Diabetologia 47: abs, 55, 2004), was to determine possible differences in the expression of the glucotransporter GLUT4, and in the effect of GLP-1 upon glucose transport and lipid metabolism in adipocytes from obese patients, as compared to normal subjects.

Materials and methods

Reagents. Human GLP-1(7-36)amide (GLP-1, Bachem AG, Bubendorf, Switzerland); pork insulin (Novo Biolabs, Bagsvaerd, Denmark); pork glucagon (Lilly Co., Indianapolis, IN, USA); exendin(1-39)amide (Ex-4) and exendin(9-39)amide (Ex-9) as gifts from Dr John Eng (VAMC, NY, USA); bovine serum albumin (BSA), glycerol and cytochalasin B (Sigma Chemical Co., St. Louis, MO, USA); aprotinin (Trasylol®, Bayer Leverkusen, Germany); [2-^{14}C]sodium acetate (Amersham Pharmacia Biotech, Buckinghamshire, UK); 2-deoxy-D-[1,2-^{3}H(N)]glucose (2-DOG, Moravek Biochemicals, Brea, CA, USA); dioctyl phthalate (Acros Organics, NJ, USA); Ultima Gold scintillation liquid (Packard, Gröninger, The Netherlands); adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD) (Boehringer Mannheim, S.A., Barcelona, Spain); and C-terminal GLUT4 rabbit polyclonal antibody (Wak-Chemie Medical GmbH, Bad Soden, Germany) were obtained from the cited sources. All other commonly used chemicals were from Sigma or Merck (Merck Pharma Quimica, S.A., Barcelona, Spain).

Biological material. Residual samples of subcutaneous abdominal fat tissue were collected, after informed consent was obtained, from 25 morbidly obese patients (22 F/3 M) undergoing bariatric surgery. They were 42.2±2.1 years old, with a mean body mass index (BMI) of 50.3±1.3 kg/m^2. Their fasting plasma D-glucose and HDL concentration averaged 5.69±0.25 mM (n=23) and 41.0±2.7 mg/dl (n=19), respectively. The patients examined for each variable under consideration were not selected in any specific manner.

Most of the data collected in normal control subjects, and used for the purpose of comparison with some of the measurements made in the present study, were already reported elsewhere (9). For glucotransporter measurements, tissue samples from a group of normal subjects (6 F/2 M; 39±3 years; BMI: 24.9±0.5 kg/m^2; fasting plasma D-glucose: 5.02±0.13 mM) were collected during inguinal hernia surgery. In all cases, the comparison between control subjects and obese patients was restricted to results obtained under similar experimental conditions. The tissue samples were used either to extract plasma membranes and/or RNA, as previously described in detail (4), or to isolate adipocytes.

The study was approved by the Ethics Committee of the Fundación Jiménez Díaz, Madrid, in accordance with the guidelines proposed in The Declaration of Helsinki.

Cells. Adipocytes were isolated at 37˚C, by enzymatic digestion with collagenase P, from subcutaneous abdominal fat tissue, according to Rodbell (29). Then, cells were initially resuspended in KRB supplemented with 10.9 mM HEPES, 500 KIU/ml Trasylol, 3% BSA and without or with D-glucose, pH 7.4, at a density of 10^6 cells/ml.

GLUT4, protein and mRNA. GLUT4 protein was measured by Western blotting, in 25-50 μg membrane protein samples using a C-terminal GLUT4 rabbit polyclonal antibody, as previously described (4). mRNA was determined by Northern blotting, in RNA extract from 100-200 mg tissue, using the protocol and reagents described (4).

Glucose transport. Cells (10^5) were incubated for 15 min at 37˚C in 400 μl KRB, 10.9 mM HEPES, 500 KIU/ml Trasylol and 2% BSA, pH 7.4, without (basal) or with GLP-1, Ex-4, Ex-9, glucagon or insulin. This was followed by 3-min incubation in the additional presence of 0.2 μCi (6.5 pmol) of 2-deoxy-D-[1,2-^{3}H(N)]glucose (final concentration, 16.3 nM 2-DOG). Adipocytes, after being separated at 10,900 g in 100 μl dioctyl phthalate, were added to 3 ml scintillation liquid for ß-counting. The total glucose content was corrected by the unspecific glucose uptake value obtained in cell samples from each experiment treated in parallel with 0.175 mM cytochalasin B (8).

Lipid metabolism. Lipolysis was determined as glycerol release, following Wieland's enzymatic procedure (30), with some modifications (31). In brief, isolated adipocytes (10^5 cells) were incubated for 60 min at 37˚C in 300 μl KRB supplemented with 10.9 mM HEPES, 500 KIU/ml Trasylol, 3.3 mM D-glucose and 3% BSA, either in the absence (basal) or presence of GLP-1, Ex-4, glucagon or insulin, alone or in combination; thereafter, 0.45 M $HClO_4$ was added to the media, the mixture was maintained for 10 min at 4˚C and then centrifuged at 2,000 x g. The supernatant was neutralized with 20% KCO_3H, separated at 3,000 g, and its glycerol content was spectrophotometrically measured as NADH produced in the presence of ATP, NAD and the appropriate enzymes, from the absorption at 340 nm; known amounts of glycerol were used as standard of reference. Lipogenesis was studied as the incorporation of [2-^{14}C]Na acetate, as precursor, into lipids. Adipocytes (10^5 cells) were incubated at 37˚C for 15 min in 400 μl of the same medium as described above, either without (basal) or with GLP-1, Ex-4, Ex-9 or insulin. This was followed by a 60-min incubation at 37˚C in the additional presence of 0.4 mM [2-^{14}C]Na acetate (0.156 μCi/μmol). Adipocytes, after being separated at 10,900 g in 100 μl dioctyl phthalate, were added to 3 ml scintillation liquid for ß-counting and the data were corrected for background values, as found in vials containing all reagents but no cells and subjected in parallel to the same procedure.

Statistical study. All results, including those already mentioned, are presented as mean values (±SEM) together with the number of individual determinations (n) or degree of freedom (d.f.). The statistical significance ($p<0.05$) of the increments was assessed by one-way analysis of variance, followed by the least significant differences (LSD) test for post-hoc multiple comparisons using the statistical package for the social science (SPSS) software or, when appropriated, by the Student's t-test.

Results

GLUT4 and D-glucose transport. In the adipocytes of eight obese patients, the GLUT4/ß-actin mRNA ratio averaged 0.395±0.052, slightly lower but not significantly different ($p>0.5$) from that found in normal subjects (0.460±0.086; n=4). Likewise, the GLUT4 protein content (Fig. 1) represented, in adipocytes of obese patients, 81.9±14.1% (n=11) of the mean corresponding value found in normal subjects (100.0±15.9%; n=6).

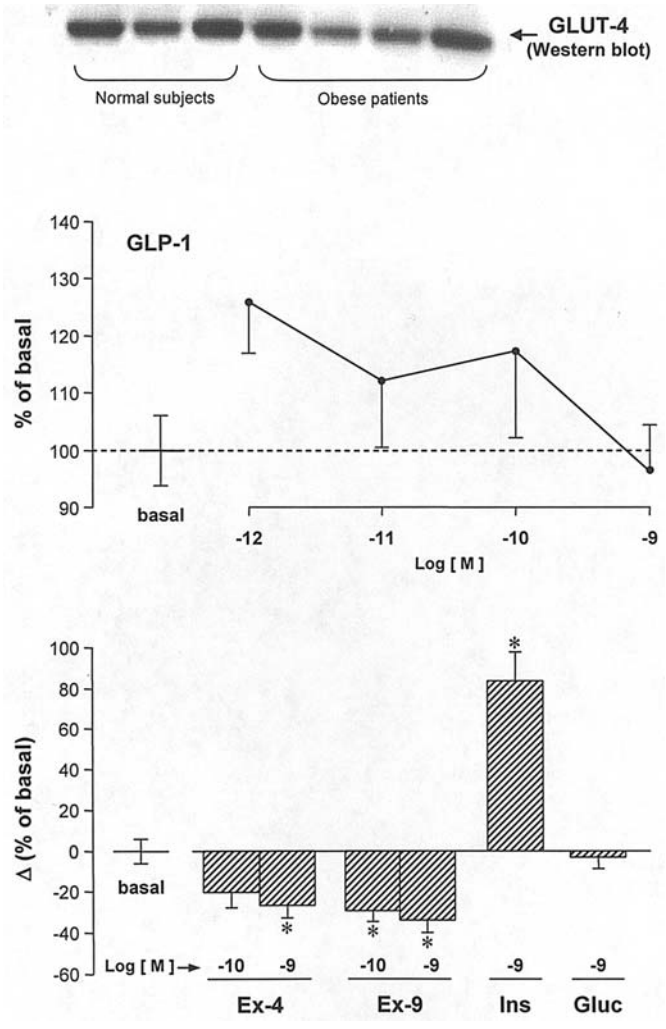

Figure 1. Basal GLUT4 in adipocytes from morbidly obese patients and normal subjects (representative immunoblot) (upper panel), and the effect of GLP-1 (middle panel) and Ex-4, Ex-9, insulin (Ins) and glucagon (Gluc) (lower panel) upon 2-deoxy-D-glucose uptake by isolated adipocytes from five morbidly obese patients. Data (mean ± SEM) are expressed in percent, total or increment, relative to paired basal value. *p<0.04.

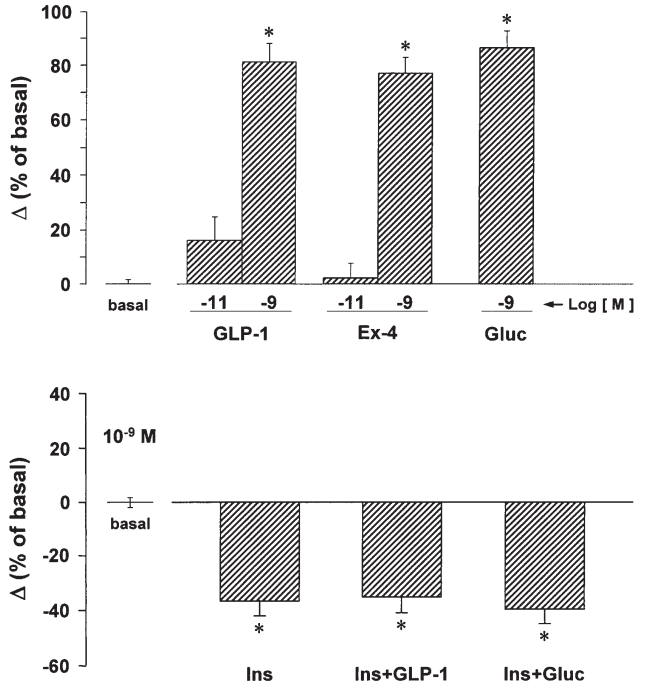

Figure 2. Effect of GLP-1, Ex-4 and glucagon (Gluc) (upper panel) and10^{-9} M insulin (Ins), alone and in combination with 10^{-9} M GLP-1 or glucagon (lower panel), upon the glycerol released by isolated adipocytes from 6-14 morbidly obese patients. Data (mean ± SEM) are expressed in percent increment, relative to paired basal value. *p<0.001.

As shown in Fig. 1, in adipocytes of the obese group, the uptake of 2-deoxy-D-glucose, expressed relative to paired mean basal value (14.95±1.83 fmol/10^5 cells, n=5 subjects), was increased (p<0.001) to 183.5±14.5% by 10^{-9} M insulin but not affected (p>0.80) by glucagon (also 10^{-9} M). GLP-1 did not significantly modify 2-deoxy-D-glucose uptake at any concentration tested. Moreover, the GLP-1 structurally related peptide, Ex-4 (10^{-10}-10^{-9} M), inhibited 2-deoxy-D-glucose uptake, the decrease reaching statistical significance at 10^{-9} M (p<0.05); such was also the case in response to Ex-9 (p<0.05), at both concentrations tested. At equal concentrations (10^{-10} and 10^{-9} M), the uptake of 2-deoxy-D-glucose in the presence of Ex-4 and Ex-9 averaged, respectively, 72.1±4.4% (n=10; p<0.001) and 69.6±3.8% (n=10; p<0.001) of the mean corresponding values found in the presence of GLP-1 (100.0±8.7%; n=10). The inhibitory action of Ex-4 and Ex-9 failed, however, to differ significantly (p>0.2) from one another, the results obtained with the latter agent averaging 96.7±5.3% (n=10) of the mean corresponding values obtained with Ex-4 (100.0±6.1%; n=10).

Lipolysis. The basal rate of lipolysis in the adipocytes of obese patients averaged 21.1±2.3 nmol of glycerol per 10^5 cells (n=14 subjects). Relative to the paired basal value, the rate of lipolysis was increased (p<0.001) to a comparable extent by 10^{-9} GLP-1, Ex-4 and glucagon (Fig. 2). Insulin (10^{-9} M) inhibited basal lipolysis (p<0.001) and abolished the lipolytic effect of 10^{-9} M GLP-1 or glucagon.

The basal lipolysis in obese patients was higher (p<0.05) than that previously found (9) in normal subjects (14.9± 0.9 nmol/10^5 cells, n=11). Relative to the paired basal value, the rate of glycerol release recorded in the presence of either GLP-1 or glucagon (10^{-9} M each) was also higher (p<0.05) in the obese patients than in normal subjects, in which the results averaged, relative to basal values, 143±14% (n=7) in the presence of GLP-1 and 145±12% (n=13) in the presence of glucagon. In normal subjects, the previously reported rate of lipolysis averaged 84±5% (n=15) in the sole presence of insulin (10^{-9} M), and 71±10% (n=12) and 74±10% (n=10) in the presence of insulin (10^{-9} M) combined, respectively, with either GLP-1 (10^{-9} M) or glucagon (10^{-9} M). None of these three percentages found in normal subjects was significantly different (p>0.1) from the corresponding mean value recorded in cells from obese patients (Fig. 2).

Lipogenesis. In the adipocytes of obese patients, the basal rate of lipogenesis (4.9±0.3 nmol/10^5 cells, n=6 subjects) was higher (p<0.001) than that previously found (9) in cells from normal subjects (2.9±0.2 nmol/10^5 cells, n=7).

GLP-1 (10^{-13}-10^{-9} M), Ex-4 and Ex-9 (both 10^{-12}-10^{-9} M) tended to increase lipogenesis; this effect though only achieved statistical significance on a few occasions, i.e. in the presence

Table I. Lipogenesis in adipocytes from obese patients.[a]

	Nil	10^{-13} M	10^{-12} M	10^{-11} M	10^{-10} M	10^{-9} M
Basal	100.0±4.0					
GLP-1		113.7±6.7	111.4±7.7	111.1±7.7	113.4±8.8	104.6±3.9
Ex-4		-	123.8±7.0[b]	-	110.3±7.1	102.8±5.7
Ex-9		-	104.7±4.6	-	113.1±8.1	121.8±7.1[b]
Insulin		-	-	-	-	151.2±6.6[b]

[a]Data (mean ± SEM) are expressed in percent relative to paired basal value (4.9±0.3 nmol/10^5 cells, n=6 subjects). [b]p<0.03 vs basal value.

of either 10^{-12} M Ex-4 (p<0.02) or 10^{-9} M Ex-9 (p<0.03). Nevertheless, when all readings obtained with GLP-1, or with its two related peptides, were pooled, a significant increase in lipogenesis was reached, with mean values of 110.8±3.0% (n=30; p<0.01) in the presence of GLP-1, 112.3±4.2% (n=18; p<0.01) in the presence of Ex-4 and 113.2±4.0% (n=18; p<0.01) in the presence of Ex-9. The latter three percentages were not significantly different from one another (p>0.6) and much lower (p<0.001) than that induced by 10^{-9} M insulin (151.2±6.6%; n=6; p<0.001 versus unity).

These results on lipogenesis are qualitatively comparable to those previously recorded in adipocytes from normal subjects (9) in which, and over the same range of concentrations (10^{-13}-10^{-9} M), the mean value recorded in the presence of GLP-1 (123.4±2.5%; n=35), and expressed relative to paired basal value, was somewhat higher (p<0.005) than in obese patients (see above). On the contrary, the lipogenetic response to insulin (10^{-9} M) was higher (p<0.05) in obese patients than in normal subjects (126±8%; n=7).

As documented in Table I, the trend was towards a decrease in the lipogenetic action of both GLP-1 and Ex-4 as their concentration increased, with a mirror image in the case of Ex-9. The 10^{-9} M/10^{-12} M ratio in lipogenesis was significantly lower (p<0.005) in the case of GLP-1 and Ex-4 (88.4±4.5%; d.f.=20) than in the case of Ex-9 (116.3± 8.5%; d.f.=10).

Discussion

In the present study, both GLUT4 protein and mRNA in adipocytes from obese patients showed a trend toward lower values as compared to normal subjects, although the difference did not reach statistical significance. In other published studies, where statistically significant lower values were reported in overweight subjects (32-34), the patients suffered obesity associated with insulin resistance or type 2 diabetes. Our data also reveal that, in adipocytes from obese subjects, insulin but not GLP-1 (10^{-12}-10^{-9} M) stimulated 2-deoxy-D-glucose uptake, whilst both Ex-4 and Ex-9, at high concentrations (10^{-10}-10^{-9} M), exerted inhibitory action. To our knowledge, the effect of GLP-1, Ex-4 and Ex-9 on 2-deoxy-D-glucose uptake in adipocytes from normal subjects has not yet been investigated. The present results suggest that exendins and GLP-1 use distinct signaling pathways in human adipocytes. In fact, it has been previously observed that, in human myocytes, both exendins, like GLP-1, stimulate glucose metabolism; however, while the three peptides and insulin provoke the immediate hydrolysis of glycosylphosphatidylinositol, suggesting the generation of an inositolphosphoglycan considered as a second messenger in the insulin action, only Ex-9 is able to induce an immediate increase in the cellular cAMP content (12). Also, it has been observed that, in fat cells from either normal or streptozotocin-induced type 2 diabetic rats, GLP-1 and both exendins significantly stimulate D-glucose transport in a dose-related manner, up to 10^{-9} M (6).

The basal rate of lipolysis, expressed per cell, was higher in adipocytes from obese patients than in those from normal subjects (9), and such was also the case for the enhancing effect of GLP-1 and glucagon. The inhibitory action of insulin on either basal or glucagon- and GLP-1-stimulated lipolysis was comparable, however, in normal and obese subjects. The increased basal and hormone-stimulated rates of lipolysis in the adipocytes of obese subjects could be linked, in part at least, to their higher content in triglycerides. Incidentally, no lipolytic effect of either GLP-1 or glucagon was observed in an *in vivo* study conducted in normal subjects (35).

The basal rate of lipogenesis was also higher in the adipocytes of obese versus normal subjects. In this case, however, the response to GLP-1 was lower in the obese patients, with a mirror image in the case of insulin. Moreover, in the obese group, and at variance with the situation found in normal subjects (9), the concentration-response relationship for the lipogenic effect of GLP-1 failed to display a progressive rise at increasing concentrations of the peptide. A comparable situation was found with Ex-4 in cells from obese patients. But the results obtained with GLP-1 and Ex-4 differed from those observed in the case of Ex-9, which exerts increasing lipogenic action as the concentration of the peptide raises. In this regard, possible species differences should be considered; it has been documented that, in rat adipocytes, GLP-1 and both exendins are indeed lipogenic (6).

In conclusion, the present results document both analogies and dissimilarities between the effects of GLP-1 and its structurally related peptides, Ex-4 and Ex-9, as well as insulin, upon two fundamental biochemical variables of the adipocyte metabolism, lipolysis and lipogenesis, when comparing the behavior of cells obtained from obese patients versus normal subjects. It may be premature to speculate on the relevance of the present findings; yet, they should not be ignored, considering the possible use of GLP-1 and its related peptides as tools in the treatment of glucose intolerance and type 2 diabetes *mellitus*, which are common in obese patients. Likewise, the reduced lipogenic action and increased lipolytic

effect of GLP-1 in fat cells from obese subjects should be considered in the perspective of its therapeutic use as a proven anorectic agent in obesity (36).

Acknowledgements

We are grateful to E. Martín-Crespo for technical assistance. This study was supported by grants from the Ministerio de Educación y Cultura (BFI 2003-07399) and the Institute of Health Carlos III (G03/212 and C03/08), Spain. V.S., M.V.T., N.G., A.A. and L.A. are research fellows from the Fundación Conchita Rábago de Jiménez Díaz.

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