Diabetologia (1998) 41: 1343–1354

Diabetologia

Regional difference in insulin inhibition of non-esterified fatty acid release from human adipocytes: relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway

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Summary Increased mobilization of non-esterified fatty acids (NEFA) from visceral as opposed to peripheral fat depots can lead to metabolic disturbances because of the direct portal link between visceral fat and the liver. Compared with peripheral fat, visceral fat shows a decreased response to insulin. The mechanisms behind these site variations were investigated by comparing insulin action on NEFA metabolism with insulin receptor signal transduction through the insulin receptor substrate-1 (IRS-1) pathway in omental (visceral) and subcutaneous human fat obtained during elective surgery. Insulin inhibited lipolysis and stimulated NEFA re-esterification. This was counteracted by wortmannin, an inhibitor of phosphaditylinositol (PI) 3-kinase. The effects of insulin on antilipolysis and NEFA re-esterification were greatly reduced in omental fat cells. Insulin receptor binding capacity, mRNA and protein expression did not differ between the cell types. Insulin was four times more effective in stimulating tyrosine phosporylation of the insulin receptor in subcutaneous fat cells ($p < 0.001$). Similarly, insulin was two to three times more effective in stimulating tyrosine phosphorylation of IRS-1 in subcutaneous fat cells ($p < 0.01$). This finding could be explained by finding that IRS-1 protein expression was reduced by $50 \pm 8\%$ in omental fat cells ($p < 0.01$). In omental fat cells, maximum insulin-stimulated association of the p85 kDa subunit of PI 3-kinase to phosphotyrosine proteins and phosphotyrosine associated PI 3-kinase activity were both reduced by 50% ($p < 0.05$ or better). Thus, the ability of insulin to induce antilipolysis and stimulate NEFA re-esterification is reduced in visceral adipocytes. This reduction can be explained by reduced insulin receptor autophosphorylation and signal transduction through an IRS-1 associated PI 3-kinase pathway in visceral adipocytes. [Diabetologia (1998) 41: 1343–1354]

Keywords Tyrosine kinase, insulin receptor substrate-1, phosphaditylinositol 3-kinase, catecholamines, re-esterification, lipolysis.

The intracellular mechanism responsible for insulin receptor-mediated stimulation of glucose transport is partly understood [1–4]. In contrast, much less is known about the signalling pathways that couple the insulin receptor to lipid metabolism. In fat cells, insulin inhibits the mobilization of non-esterified fatty acids (NEFA) in two synergistic ways [5]. Firstly, insulin decreases the rate of lipolysis (i.e. activates antilipolysis). Secondly, insulin increases the rate of re-synthesis of triglycerides from the NEFA that are formed by lipolysis, i.e. the re-esterfication effect.

Although the intracellular signalling cascade which regulates antilipolysis has not been studied to the same degree as glucose transport or mitogenesis, similar early steps in the insulin receptor signalling cascade are likely to be involved. The insulin receptor is activated by binding of insulin, which causes the receptor to undergo autophosphorylation on tyrosine residues [1–4]. Autophosphorylation activates the tyrosine kinase of the receptor and allows the receptor

Received: 27 February 1998 and in revised form: 12 May 1998

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Abbreviations: IRS, Insulin receptor substrate; PI 3-kinase, phosphatidylinositol 3-kinase; NEFA, non-esterified fatty acids; p85, 85 kDa (kilodalton) subunit; EC_{50}, half-maximum effective concentration; ANOVA, analysis of variance.

to tyrosine phosphorylate intracellular substrates, in particular insulin receptor substrate-1 (IRS-1) [1–4]. One of several proteins which dock to phosphorylated IRS-1 (and other tyrosine phosphorylated proteins) is the 85 kilodalton subunit (p85) of phosphatidylinositol (PI) 3-kinase, which through this association increases the activity of PI 3-kinase [6]. Studies using the PI 3-kinase inhibitor wortmannin show that in fat cells, PI 3-kinase participates in the antilipolytic effect of insulin and also in the stimulation of glucose transport [7–9]. The mechanism that underlies insulin-stimulated re-esterfication of NEFA is less well understood.

Regional variations in the metabolic effects of insulin on human fat cells have been observed [10]. Insulin has a much stronger antilipolytic effect in subcutaneous than visceral fat cells. Since the visceral fat depot is in direct contact with the liver through the portal circulation, an inability of insulin to inhibit lipolysis in visceral fat cells may be of great clinical importance for the metabolic complications to upper body obesity [11–13]. In upper body obesity the visceral fat mass is increased and impaired insulin action in visceral fat (as opposed to peripheral adipose tissue) will promote mobilization of "portal" NEFA and disturb liver metabolism. This effect in turn can cause glucose intolerance, dyslipidaemia, hyperinsulinaemia and insulin resistance.

Early studies suggest that the affinity of the insulin receptor for insulin is lower in omental (visceral) fat than in subcutaneous (peripheral) fat cells from both lean and obese subjects [14, 15]. This difference could, however, explain only a small part of the regional variations in the antilipolytic response to insulin. The major part was attributed to unidentified "post receptor" differences. Thus, whether regional variations in insulin receptor signalling occur in subcutaneous or omental fat depots is not known. In addition, whether site differences in insulin action on lipolysis and NEFA re-esterification occur in adipocytes from different sites remains to be established. We examined regional variations in adipocyte insulin receptor expression and tyrosine autophosphorylation, IRS-1 tyrosine phosphorylation and phosphorylation as well as enzymatic activity of PI 3-kinase. In addition, we have evaluated site differences in insulin action on lipolysis and NEFA re-esterification.

Subjects and methods

Subjects. The study group consisted of 27 men and 28 women who underwent either elective cholecystectomy because of gallstone disease or gastric banding because of obesity. Laparoscopic surgery was performed in most of the cases. None of the patients were on regular medication, had any known metabolic disorder (besides obesity), or had recently changed his or her food and exercise habits. There was no selection for age (which ranged from 23 to 68 years) or body weight (body mass index ranged from 19 to 37 kg/m^2). All of the subjects gave their written and informed consent and the study was approved by the ethic's committee at Huddinge Hospital. General anesthesia was induced by a short-acting barbiturate and maintained by phentanyl and nitrous oxygen. The patients had fasted overnight and received i. v. saline before the biopsy. The subcutaneous adipose tissue was obtained from an abdominal incision at the beginning of surgery and the omental fat specimens were taken from the major omentum, 5–10 min later. Because of the laparoscopic procedures, only small amounts (1–3 g) of tissue were obtained from each depot. Therefore all of experiments listed below were not performed on the same set of fat cells. Each experiment was always performed on omental and subcutaneous tissue from the same individual, which provided a direct comparison of the two adipose depots in each assay.

Isolation of fat cells and determination of cell size and number. Fat cells were isolated and cell size and number determined as described previously [16]. Adipose tissue was transported immediately to the laboratory and isolated fat cells were prepared by collagenase treatment. Direct microscopic determination of the fat cell diameter was done using 200 cells and mean fat cell volume and weight were calculated. The total lipid content in each incubation was determined gravimetrically after organic extraction. The number of fat cells was calculated by dividing the total lipid weight by the mean cell weight. Fat cell volume, determined in 43 subjects was similar in subcutaneous and omental fat cells; 540 ± 70 pl compared with 472 ± 61 pl (mean ± SEM) for the whole study group.

Lipolysis experiments. The following experiments were performed on 19 subjects. The lipolysis assay was performed and data evaluated as described previously [16]. Briefly, 0.2 ml of diluted suspensions of isolated fat cells were incubated in duplicate for 2 h with or without increasing concentrations of noradrenaline or human insulin or both. All incubations were done at 37 °C in Krebs-Ringer phosphate buffer (pH 7.4) supplemented with glucose (1 g/l), bovine serum albumin (20 g/l) and ascorbic acid (0.1 g/l) with air as the gas phase. The ligands were added simultaneously at the start of the incubation. The same batches of collagenase and albumin and the same stock solutions of agonists were used throughout the study. In our dilute incubation system there is minimal influence of adenosine contamination. Therefore, adenosine deaminase was not added to the incubation. The glycerol concentration after the 2-h incubation was determined in a cell-free aliquot by a bioluminescence method [17].

Both agonists induced a concentration-dependent stimulation or inhibition of glycerol release that reached a plateau at the highest agonist concentrations. Concentration-response curves for glycerol release were used to determine the agonist concentration that generates a half-maximal response. These half-maximum effective concentration (EC_{50}) values (expressed as log mol/l) were determined by linear regression analysis of log-logit transformation of the ascending or descending part of the individual concentration-response curves. Lipolysis rates were related to fat cell number. Maximum lipolytic or antilipolytic effects were used as a measure of agonist responsiveness.

Simultaneous determination of glycerol and NEFA release. The simultaneous production of NEFA and glycerol was studied in 18 subjects. The fat cell incubation procedures used were as described above for the lipolysis experiments with one modification; 0.25 % (wt/vol) non-esterified acid-free bovine serum albumin was used as acceptor of NEFA. Since in isolated adipocytes, the rate of agitation of the incubator might influence

re-esterification [18], the shaking rate in the incubation bath was kept constant in all experiments (20 cycles/min). At the end of the incubation, one aliquot of the medium was removed for glycerol determination as described above and another aliquot was used for the measurement of NEFA as described in detail [19]. The assay involved pretreatment with sodium dodecyl sulphate to liberate the NEFA from the albumin before the activation of fatty acids by acylCoA synthetase. This was followed by oxidation of the resulting thioesters by acyl-CoA oxidase. The H_2O_2 formed (reflecting the amount of NEFA) was subsequently measured in a horseradish peroxidase-catalysed luminol reaction, using a luminometer (LKB Wallac, Turku, Finland). The molar ratio of NEFA to glycerol was calculated and used as an index of NEFA-re-esterification. The validity of this method has been reported previously [20].

Determination of insulin receptor mRNA. Insulin receptor mRNA was investigated in 20 subjects and quantitated with solution hybridization as described previously [21]. Extracts of total nucleic acids were prepared from the cell samples, and nucleic acid content was determined by spectrophotometry. A PstI fragment of 266 base pairs from the human insulin receptor cDNA clone phINSR-13–1 spanning exons 12–14 was cloned into pGEM4 and used for in vitro transcription. For synthesis of the labelled antisense probe, phINSR266 was cleaved with BamHI (New England Biolabs, Beverely, Mass., USA) and transcribed with T7 RNA polymerase (Promega Biotec Madison, WI, USA) in the presence of [^{35}S]UTP (Amersham, Aylesbury, UK). To produce an unlabelled sense standard RNA, phINSR266 was cleaved with *Hind* III (New England Biolabs) and transcribed with SP6 RNA polymerase to yield a 328-base transcript.

The conditions for hybridization and RNase treatment have been described [21]. Insulin receptor mRNA levels were compared with standard curves of parallel incubations with known amounts of unlabelled sense RNA. The background radioactivity, measured in RNase-treated samples containing the same amount of probe – but neither unlabelled sense transcript nor nucleic acid extract – was subtracted from all values. The insulin receptor RNA determinations were based on at least three dilutions of the extracts within the range of linear increase in acid perceptible radioactivity (0.1–10 μg RNA). Only radioactivity values more than twice the background were accepted for quantification of the specific RNA. The mRNA was related to either the total nucleic acid content or the content of mRNA for gamma-actin in the nucleic acid extract. Gamma-actin was measured with the same solution hybridization technique as described above. The probe was kindly donated by Mats Gåfvels, Novum, Huddinge Hospital, Huddinge, Sweden.

Phosphorylation of the insulin receptor. The following experiments were performed in samples obtained from 21 individuals. Two ml of human adipocytes (10% packed cell volume) were incubated for 10 min at 37 °C with the indicated concentration of insulin. The incubation was terminated by rapid centrifugation of the cells and removal of the infrantant below the fat cake. Seven hundred μl of extraction buffer consisting of 1% Triton X-100, 50 mmol/l Tris, pH 7.4, 1 mmol/l per-vandate (50:1 molar mixture of orthovanadate:H_2O_2), 1 mmol/l PMSF, 25 mmol/l benzamidine and 0.15 mol NaCl were added to the fat sample. After vortexing, the fat was removed by centrifugation. An aliquot of 150 μl of the extract was used for measurement of total insulin receptor. An antibody to the carboxyl terminus of the insulin receptor beta subunit (α-CT) was prepared as described [22], added and after an overnight incubation at 4 °C, the immunoprecipitate was collected on Pansorbin (Calbiochemi), washed as described [22] and suspended in 0.1% Triton X-100, 50 mmol/l Tris, pH 7.4, 0.1% albumin, and 1 mmol/l per-vanadate. This suspension was divided into three samples of 90 μl each and 100,000 cpm of ^{125}I[A-14]-insulin was added to the suspension. The third sample also received 1 μmol/l native insulin to estimate non-specific insulin binding. After an overnight incubation at 4 °C, the suspension was collected by centrifugation, washed and the amount of specifically bound insulin determined as described [22].

The amount of tyrosine phosphorylated insulin receptor in fat cell extracts was similarly determined, however phosphotyrosine antibody 4G10 termed α-PY (UBI, Lake Placid, N.Y., USA) was used in place of α-CT. Also 250 μl of extract was processed for this determination rather than 150 μl. The amount of tyrosine phosphorylated receptor in the extract was expressed as a percentage of the total receptor population calculated as follows: per cent phosphorylated = specific insulin binding in the α-PY precipitate/specific insulin binding in the α-CT precipitate X 100.

Protein expression of the insulin receptor. The total protein amount of insulin receptor was determined by Western blot analysis of omental and subcutaneous fat cells from 9 subjects. Aliquots of cell extract (40 μg) were made soluble in Laemmli buffer with 100 mmol/l dithiothreitol and heated (95 °C) for 6 min. The samples were subjected to SDS-PAGE (6% resolving gel), transferred to PVDF membranes, and blocked in Tris-buffered saline and 0.1% Tween 20. The membranes were incubated with anti-Insulin receptor antibody. Membranes were washed and incubated with secondary antibodies. Insulin receptors were made visible by enhanced chemiluminescence and quantified by densitometry (Bio Rad, Richmond, Calif., USA). Samples from subcutaneous and omental fat depots from each subject were always run on the same gel.

IRS-1 tyrosine phosphorylation and protein expression. Cell extracts (750 μg) from subcutaneous and omental fat obtained from 5 subjects were immunoprecipitated with anti-IRS-1 antibody (gift from Dr. Morris White, Joslin Diabetes Center, Boston, Mass., USA). Thereafter the immune complex was washed as indicated [23]. The samples were re-suspended in Laemmli buffer with 100 mmol/l dithiothreitol and heated (95 °C) for 6 min. The proteins were separated by SDS-PAGE (6% resolving gel), transferred to PVDF membranes, and blocked in Tris-buffered saline and 1.0% Tween 20. The membranes were incubated with either anti-IRS-1 (total protein expression) or anti-phosphotyrosine (IRS-1 phosphorylation) antibodies (PY-20; Signal Transduction Laboratories, Lexington, Kan., USA). Membranes were washed and incubated with secondary antibodies. IRS-1 was made visible by chemiluminescence and quantified by densitometry. Samples from subcutaneous and omental fat depots from each subject were run on the same gel.

Phosphotyrosine associated PI 3-Kinase activity. Aliquots of cell extract (750 μg) from 10 subjects were immunoprecipitated with a polyclonal anti-phosphotyrosine antibody (Signal Transduction Laboratories) coupled to protein A-sepharose. The immune complex was washed as described [23]. The pellet was re-suspended in 50 μl of Tris-NaCl buffer, and 12 mmol/l $MgCl_2$ and phosphatidylinositol (10 μg) were added. PI 3-Kinase activity was assessed essentially as described previously [24]. The lipid product was resolved by thin-layer chromatography and incorporation of label from [γ-^{32}P]-ATP into phosphatidylinositol was quantitated using a PhosphorImager (Bio-Rad). Samples from subcutaneous and omental fat depots from each subject were run on the same TLC plate.

Western blotting for p85. Aliquots of cell extract (250 µl) from 14 subjects were incubated with α-PY antibody and the immunoprecipitate collected as described above. After washing, the immunoprecipitates were subjected to SDS PAGE as described previously using a 7.5% resolving gel [22]. The resolved proteins were transferred to an Immobilion-P membrane, blocked as described and probed with α-p85 (UBI, Lake Placid N.Y., USA). Total p85 was measured by subjecting 5 µl of the fat cell extract to Western blot analysis. The p85 band was scanned by laser densitometer and the amount of p85 in the α-PY immunoprecipitate was expressed as a per cent of the total amount of p85 in the extract or as optical density units. Omental and subcutaneous cell extracts from one subject were analysed in parallel.

Drugs and chemicals. Bovine serum albumin (fraction V, lot 63F-0748), non-esterified acid-free bovine serum albumin (lot A-6003), *Clostridium histolyticum* collagenase type I, glycerol kinase from *Escherichia coli* (&4509), acyl-CoA synthetase (EC 6.2.1.3) from *Pseudomonas specials*, acyl-CoA oxidase from Candida lipolytic, horseradish peroxidase (EC 1.11.1.7., type VI; 250–300 U/mg), sodium dodecyl sulphate, ascorbate oxidase, Triton X-100, and ATP were obtained from Sigma Immunochemicals (St. Louis, Mo., USA). Insulin (human) and noradrenaline came from Pharmacia (Stockholm, Sweden). ATP monitoring reagent containing firefly luciferase came from LKB Wallac. Protein A-sepharose was purchased from Sigma. Enhanced chemiluminescence reagents were purchased from Amersham (Arlington Heights, Ill., USA). Phosphatylinositol was from Avanti Polar Lipids (Alabaster, Ala., USA). The aluminum backed Silica Gel 60 thin-layer chromatography plates were purchased from EM Separations (Gibbstown, N.J., USA). All other chemicals were of the highest grade of purity commercially available.

Statistical analysis. Values are presented as mean ± SE. EC_{50} values were logarithmically transformed to normalize the data. Analysis of variance (ANOVA) corrected for repeated measurements and two-tailed *t*-test were used for statistical comparisons. All statistical calculations were performed with a statistical software package (STATVIEW II; Abacus Concepts, Inc., Berkeley, Calif., USA).

Results

Interactions between insulin, noradrenaline, and lipolysis. Previous studies with rat fat cells suggested that the degree of antilipolysis induced by insulin depends on the rate of lipolysis before the addition of insulin [25]. Thus, the antilipolytic effect of insulin is more pronounced in rodent adipose tissue when lipolysis is raised. To determine whether human adipocytes behave in a similar fashion, omental and subcutaneous fat cells were incubated with different concentrations of insulin in combination with different concentrations of the lipolytic catecholamine, noradrenaline (Fig. 1). Our results show that the antilipolytic effect of insulin is dependent on the rate of lipolysis in both cell types from human adipose tissue, although this influence was most pronounced in subcutaneous cells. In the absence of noradrenaline or in the presence of low concentrations of noradrenaline, the antilipolytic response to insulin was small and became progressively more prominent at high lipolysis rates produced by greater amounts of noradrenaline. Maximum antilipolysis was achieved with 100 pmol/l insulin.

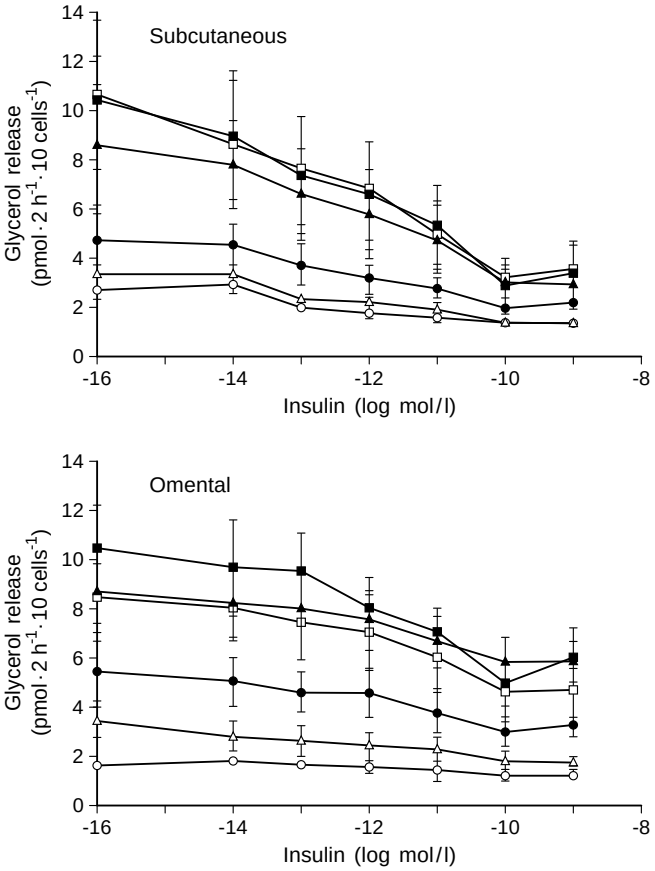


Fig. 1. Interactions between insulin, noradrenaline (Na) and lipolysis in subcutaneous (upper graph) and omental (lower graph) fat cells. Cells were incubated with insulin and noradrenaline in different concentration combinations; –○– Na 0 mol/l, –△– Na 1 nmol/l, –●– Na 10 nmol/l, –□– Na 0.1 µmol/l, ▲ Na1 µmol/l, ■ Na 10 µmol/l. Glycerol release was determined. Values are mean ± SEM. $n = 8$ for both cell types

Since regional differences in basal and noradrenaline-induced rates of lipolysis between human omental and subcutaneous fat cells have been described [10, 11], we matched the rate of lipolysis for each cell type in each individual experiment before measuring the antilipolytic effect of insulin. Fat cells were incubated in the presence of increasing noradrenaline concentrations ranging from 1 nmol/l to 10 µmol/l with insulin absent or present in the concentration range of 10 fmol/l to 100 pmol/l. The comparisons between omental and subcutaneous fat cells from within one subject were made with the insulin concentration-response curve carried out with the most similar rate of noradrenaline-stimulated lipolysis.

In both cell types there was a concentration dependent inhibition of lipolysis by insulin (Fig. 2). The antilipolytic response was, however, greater for subcu-

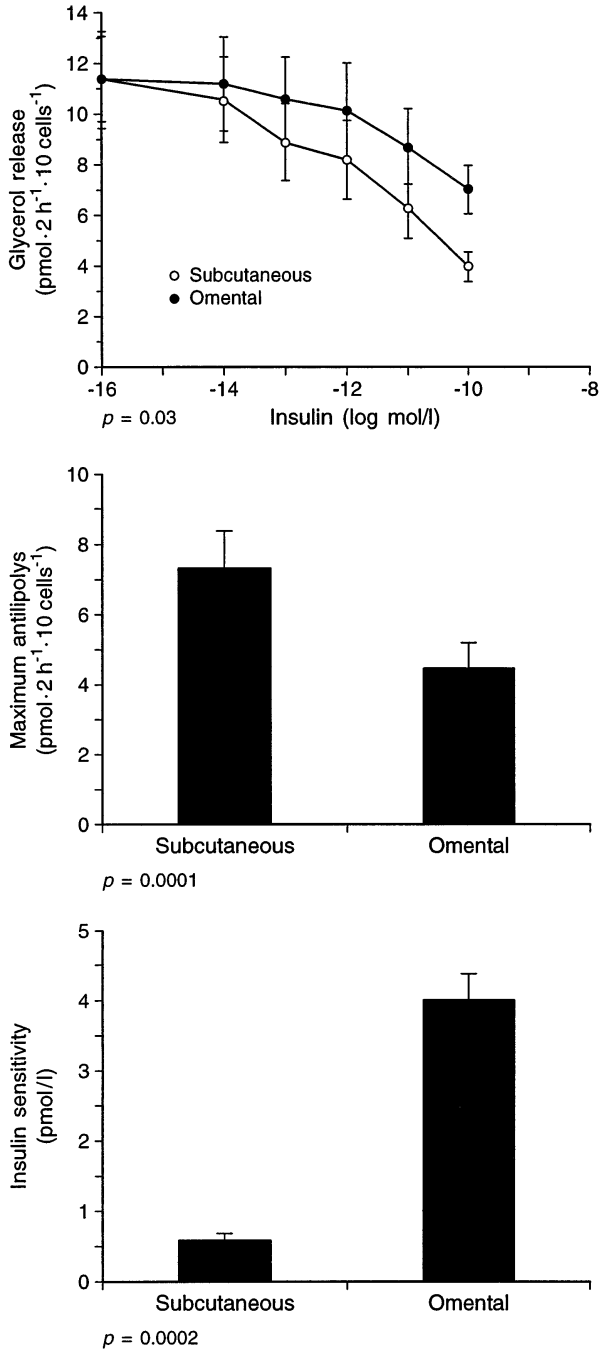


Fig. 2. Antilipolytic effect of insulin in subcutaneous and omental fat cells from the same subject. Fat cells were incubated with noradrenaline in combination with insulin. The figure shows comparison between the insulin concentration-response curves from omental and subcutaneous cells that had the most similar rate of noradrenaline-stimulated lipolysis (i.e., with no insulin present in the incubation medium). Upper graph shows the complete mean concentration-response curves. Middle graph shows the maximum antilipolytic effect (noradrenaline alone minus noradrenaline in presence of the maximum effective insulin concentration). Lower graph shows the concentration of insulin causing half-maximum inhibition of lipolysis. Values are mean ± SEM. $n = 19$. Statistical differences were determined by the two-way ANOVA (upper graph) or the *t*-test (middle and lower graphs)

Table 1. Lipolysis in human fat cells

	Subcutaneous	Omental	*P* value
Basal	7.1 ± 1.2	2.8 ± 0.6	< 0.001
Noradrenaline-Induced			
Insulin Experiments	11.4 ± 1.7	11.4 ± 1.9	NS
Maximum	17.7 ± 2.3	15.5 ± 2.7	NS
Noradrenaline concentration in the insulin experiments (log mmol/l)	− 7.0 ± 0.6	− 7.1 ± 0.3	NS

Fat cells were incubated in the absence or presence of various concentrations of noradrenaline. Glycerol release was determined with no noradrenaline present (basal), at the concentration of noradrenaline which was used in the insulin experiments and at the maximum effective noradrenaline concentration. Results are mean ± SEM for $n = 11$ subjects. Values were compared using paired *t*-test. NS = not significant

taneous cells. The maximum antilipolytic effect differed almost twofold ($p < 0.001$) and insulin sensitivity differed almost 10-fold, i.e. the EC_{50} was approximately 1 pmol/l for omental cells and 0.1 pmol/l for subcutaneous cells ($p < 0.01$). The mean noradrenaline concentration was almost identical for the experiments with omental and subcutaneous cells and was submaximal for the stimulation of lipolysis in both cell types (Table 1).

Re-esterification of non-esterified fatty acids. Fat cells were incubated with either a submaximal or maximal effective concentration of noradrenaline (10 nmol/l and 10 μmol/l, respectively), and the ratio between NEFA and glycerol release (apparent re-esterification) was determined (Fig. 3). Insulin induced an almost identical concentration-dependent increase in re-esterfication, i.e. a lowering of the NEFA to glycerol ratio, in subcutaneous cells at both concentrations of noradrenaline ($p < 0.01$ by one-way ANOVA). In the absence of insulin, the ratio was approximately 2.5, indicating that about 17% of the NEFA formed by lipolysis underwent re-esterification since the ratio should be 3.0 when there is no re-esterification during lipolysis [20]. At the maximum effective insulin concentration of 1 pmol/l, this ratio decreased to 1.5, indicating that half of the NEFA formed during lipolysis was re-esterified. These findings indicate that insulin maximally stimulated re-esterification about threefold above basal in subcutaneous cells. It induced only a marginal increase, however, in re-esterification in omental cells at both the low and high noradrenaline concentrations ($p > 0.1$ by one-way ANOVA). At both noradrenaline concentrations, insulin action was significantly more pronounced in subcutaneous cells than in omental cells. The results were similar when comparing the insulin concentration-response curves (two-way ANOVA) or the maximal insulin effects (paired *t*-test). The initial NEFA to glycerol ratio did not differ between cell types.

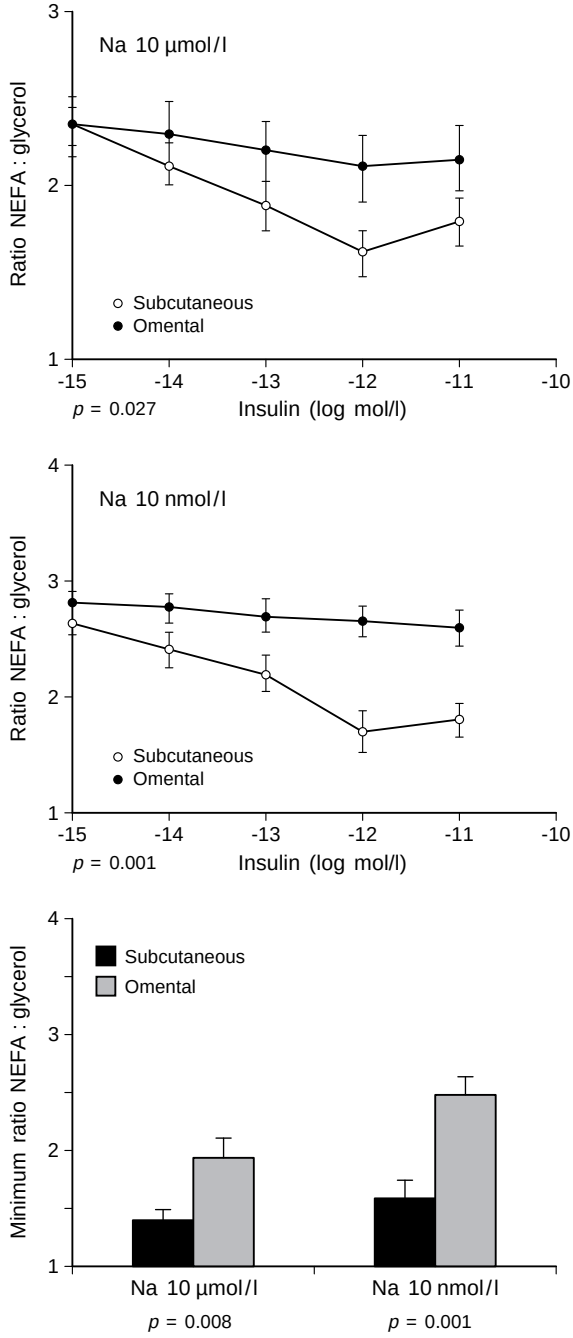


Fig. 3. Apparent re-esterification of NEFA in subcutaneous (subc) and omental (om) fat cells from the same subject. Cells were incubated with either 10 µmol/l or 10 nmol/l of noradrenaline (Na) and insulin was added in concentrations indicated in the figure. Release of glycerol and NEFA were determined in the same test tube and the ratio of NEFA:glycerol was calculated. Concentration-response curves are depicted in upper and middle graphs, which were compared using two-way ANOVA. Lower graph shows results with the maximum effective insulin concentration, which were compared using the *t*-test. Values are mean ± SEM. $n = 18$

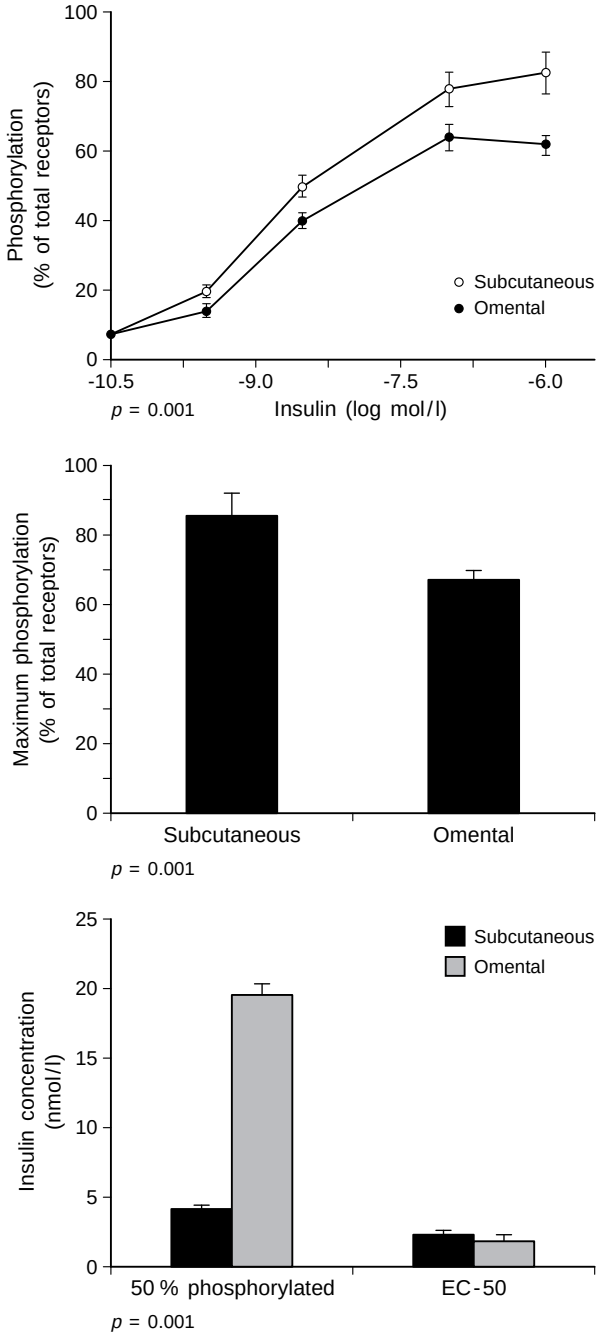


Fig. 4. Tyrosine autophosphorylation of the insulin receptor in subcutaneous (subc) and omental (om) fat cells of the same subject. Fat cells were incubated with insulin (0, 0.3, 3, 100 and 1000 nmol/l) and insulin receptor tyrosine phosphorylation was determined as described in "Subjects and methods". Upper graph shows complete concentration-response curves. Middle graph depicts receptor phosphorylation at the maximum effective insulin concentration. The concentration of insulin causing 50 % of the total receptor population to be phosphorylated and causing half-maximum phosphorylation effect (EC-50) are shown in the lower graph. Values are mean ± SEM. Statistical difference was determined using two-way ANOVA (upper graph) or the *t*-test (middle and lower graphs). $n = 21$

Table 2. Expression and activity of insulin receptor and PI 3-kinase in human fat cells

	Subcutaneous	Omental	*P* value
Insulin receptor binding, amol/10^7 cells (n = 21)			
Tracer Insulin	90 ± 11	75 ± 12	NS
High Insulin	235 ± 38	220 ± 38	NS
Insulin receptor mRNA (n = 26)			
pg/μg TNA	0.08 ± 0.01	0.11 ± 0.02	NS
pg/ng actin	17 ± 2	18 ± 2	NS
PI 3-kinase optical density, activity units (n = 14)	490 ± 95	408 ± 75	NS
Maximal Insulin-Stimulated Activation of PI 3-kinase			
Absolute value	4.8 ± 0.5	3.1 ± 0.5	0.013
% of total PI 3-kinase	1.5 ± 0.3	0.8 ± 0.1	0.008
EC_{50} nmol/l	2.0 ± 0.5	8.0 ± 0.5	0.002

Values are mean ± SEM. n = number of subjects. For maximal insulin-stimulated activation of PI 3-kinase, fat cells were incubated with or without insulin (0, 0.3, 100 and 1000 nmol/l) and PI 3-kinase activation at maximum effective insulin concentration was determined. Values were compared using paired t-test

Insulin-induced tyrosine phosphorylation of the insulin receptor. In both cell types insulin stimulated autophosphorylation of the receptor in a concentration-dependent fashion (Fig. 4). It was though more effective in subcutaneous than in omental cells at each concentration. At its maximum effective concentration, about 85 % of the insulin receptors underwent autophosphorylation in subcutaneous cells, as compared with 65 % phosphorylated in omental cells ($p < 0.001$). The concentration of insulin required to phosphorylate 50 % of the total receptor population was 5 nmol/l in subcutaneous and 20 nmol/l in omental cells ($p < 0.01$), which is a fourfold difference. Although the insulin sensitivity of autophopshorylation, as determined by the concentration of insulin that produced half-maximal activation, did not differ between the two cell types, i. e. EC_{50} for both cell types was approximately 3 nmol/l. Insulin receptor expression in subcutaneous and omental fat is presented in Table 2. Neither insulin receptor binding capacity nor steady-state insulin receptor mRNA differed between the two groups. Furthermore, insulin receptor protein expression was similar between both fat depots (relative expression for omental/subcutaneous fat was 0.98 ± 0.20; NS).

Insulin-induced tyrosine phosphorylation of IRS-1. Insulin-stimulated (1,000 nmol/l) tyrosine phosphorylation of IRS-1 was greater in subcutaneous compared with omental fat cells (Fig. 5). A concentration relationship for insulin-stimulated IRS-1 tyrosine phosphorylation was shown in subcutaneous and omental adipocyte preparations (Fig. 6). Consistant with our finding for the insulin receptor, insulin was 2–3 times more effective in inducing tyrosine phosphorylation of IRS-1 at all concentrations tested in subcutaneous cells than in omental cells ($p < 0.01$ by ANOVA). The concentration of insulin that produced half-maximal activation for both cell types was approximately 3 nmol/l. To make direct compari-

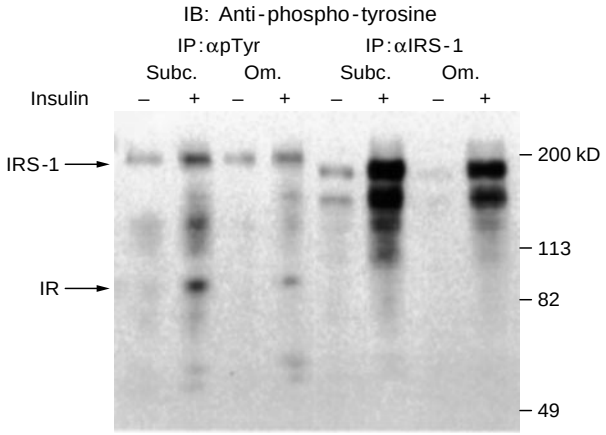


Fig. 5. Basal and insulin-stimulated (1000 nmol/l) tyrosine phosphorylation in subcutaneous (subc) and omental (om) fat cells. Aliquots of fat cell lysate were immunoprecipitated with anti-phospho-tyrosine (αpTyr) or insulin-receptor substrate-1 (αIRS-1) antibodies. Proteins were subjected to SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with anti-phospho-tyrosine antibodies (RC20, Signal Transduction Laboratories). On *left* position of insulin receptor substrate 1 (IRS-1) and insulin receptor β-subunit (IR) is indicated. On *right* position of the molecular weight markers is indicated

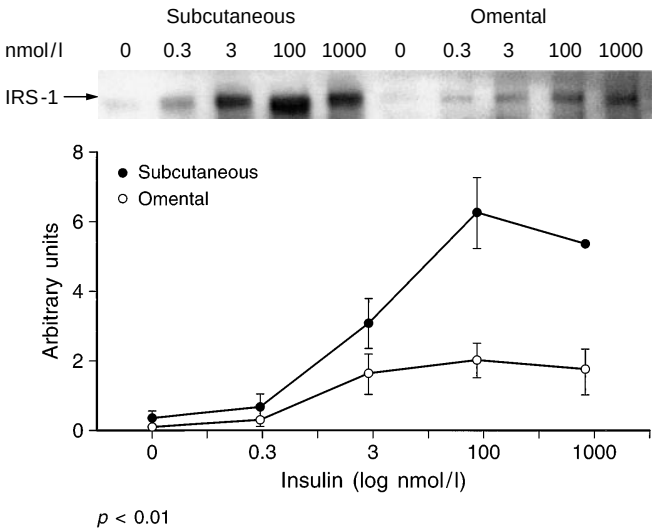


Fig. 6. Tyrosine phosphorylation of the insulin receptor substrate-1 in subcutaneous (subc) and omental (om) fat cells. Fat cells were incubated with insulin (0, 0.3, 3, 100 and 1000 nmol/l) and tyrosine phsophorylation of IRS-1 was determined as described in "Subjects and methods". Upper panel shows representative autoradiograph of insulin-stimulated IRS-1 tyrosine phosphorylation in subc or om fat cells. The graph (lower panel) depicts the complete mean concentration-response curves for tyrosine phosphorylation of IRS-1 in subc or om fat cells (n = 4). Statistical difference was determined using two-way ANOVA (graph)

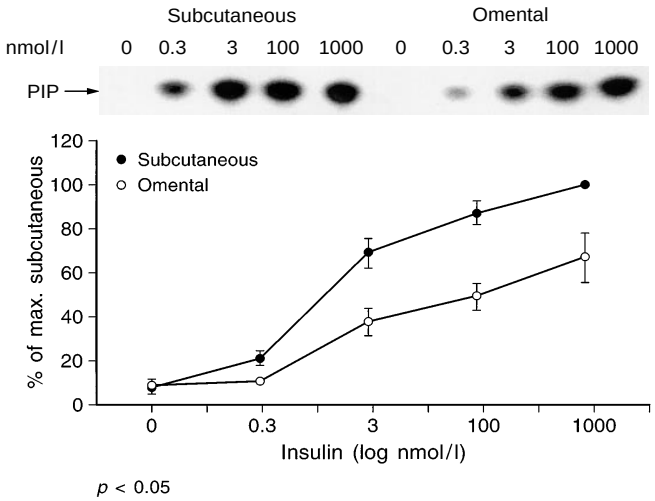


Fig. 7. Phosphotyrosine associated phosphatidylinositol 3-kinase (PI 3-kinase) activity in subcutaneous (subc) and omental (om) fat cells. Fat cells were incubated with insulin (0, 0.3, 3, 100 and 1000 nmol/l) and phosphotyrosine associated PI 3-kinase activity was determined as described in "Subjects and methods". Upper panel shows a representative autoradiograph of phosphotyrosine associated PI 3-kinase activity in subc or om fat cells from the same individual. The graph depicts the complete mean concentration-response curves for phosphotyrosine associated PI 3-kinase activity in subc or om fat cells ($n = 10$). Statistical difference was determined using two-way ANOVA (graph)

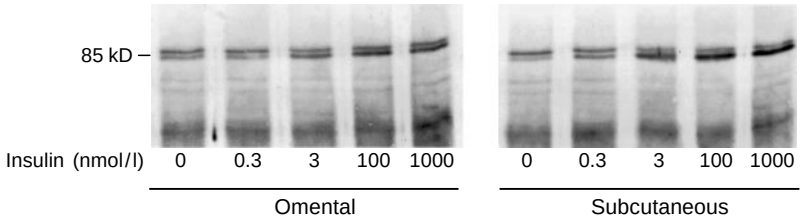
sions of the relative abundance of IRS-1 expression between the depots, IRS-1 protein content was assessed in subcutaneous and omental fat from the same subject. Expression of IRS-1 was 50 ± 8 % lower in omental fat ($p < 0.01$).

Phosphotyrosine associated PI 3-kinase activity. A concentration relation for insulin-stimulated phosphotyrosine associated PI 3-kinase activity in subcutaneous and omental adiopocytes was established (Fig. 7). At each concentration, insulin was more effective in increasing tyrosine associated PI 3-kinase activity in subcutaneous adipocytes than in omental adipocytes ($p < 0.05$ by ANOVA). Mean insulin-stimulated tyrosine associated PI 3-kinase activity was one-third to one-half lower in omental compared with subcutaneous adipocytes. The concentration of insulin that produced half-maximal activation for both cell types was approximately 3 nmol/l.

Association of the p85α subunit of PI 3-kinase in anti-phosphotyrosine immunoprecipitates. Insulin induced a concentration-dependent increase in the association of p85 protein with the α-PY precipitate in both cell types (Fig. 8), but the effect was greater at all concentrations ($p < 0.01$ by ANOVA) in subcutaneous cells (Fig. 9). The maximum effect was twofold higher ($p < 0.01$) and the insulin sensitivity was about fourfold higher ($p < 0.01$) in subcutaneous compared with omental cells, i.e. EC_{50} for insulin sensitivity was 2.0 nmol/l for subcutaneous cells compared with 8.0 nmol/l for omental cells (Table 2). The results were similar when the data were expressed either as absolute values for the p85 protein or as a percentage of total p85 protein (Table 2). The total protein amount of p85 did not differ significantly between the omental and subcutaneous fat cells.

The effect of the PI 3-kinase inhibitor wortmannin on insulin action in subcutaneous fat cells. Wortmannin was added to the incubation media in a final concentration of 100 nmol/l which has been shown to induce a complete and selective inhibition of PI 3-kinase activity in rat fat cells [7]. At higher concentrations (> 500 nmol/l), wortmannin can interfere with PI 4-kinase activity, and can be a non-specific inhibitor of various kinases [7]. Glycerol release, NEFA release or NEFA/glycerol ratio under basal (no insulin) conditions was not altered by wortmannin. Conversely, the ability of insulin to inhibit noradrenaline-induced glycerol release was completely counteracted. Furthermore, wortmannin partly, but significantly, counteracted the ability of insulin to inhibit noradrenaline-induced NEFA release and significantly counteracted the insulin-induced decrease in the NEFA to

Fig. 8. Association of the 85 kilodalton (kDa) subunit of PI 3-kinase to tyrosine phosphorylated products in cells from the same subject. Fat cells were incubated with insulin (0, 0.3, 3, 100 and 1000 nmol/l) and association of the p85 a subunit of PI 3-kinase to tyrosine phosphorylated proteins was determined as described in "Subjects and methods". Cell extracts were immunoprecipitated with a αPtyr antibody, the immonoprecipitates were then subjected to electrophoresis and immunoblotted with a p85α antibody. The results of one typical Western blot with subcutaneous and omental fat cells from the same subject are depicted. The mean ± SEM for all experiments is shown in Figure 9. In this experiment a second band was detected that migrated at a slightly higher molecular weight position than the p85 band. This upper band was not observed in all Western blots and, when present, was not influenced by insulin treatment



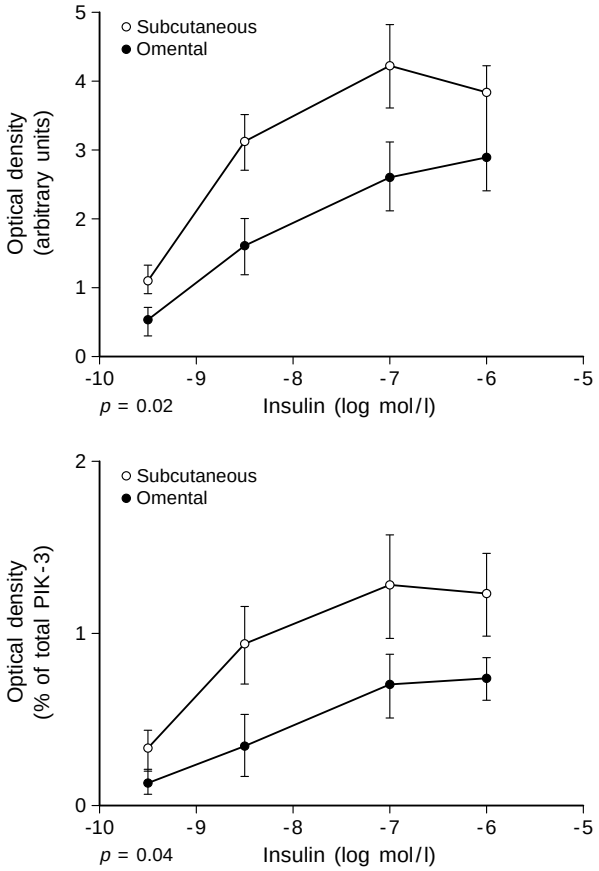


Fig. 9. Association of the p85α kDa subunit of PI 3-kinase to tyrosine phosphorylated products in subcutaneous (subc) and omental (om) fat. Fat cells were incubated with insulin (0, 0.3, 3, 100, 1000 nmol/l) and association of the p85a subunit of PI 3-kinase to tyrosine phosphorylated proteins was determined as described in "Subjects and methods". Absolute values (upper graph) and values expressed as a function of total amount of the 85 kDa subunit of PI 3-kinase (lower graph) are depicted. Stastical differences were determined using a two-way ANOVA. Values are mean ± SEM. $n = 14$

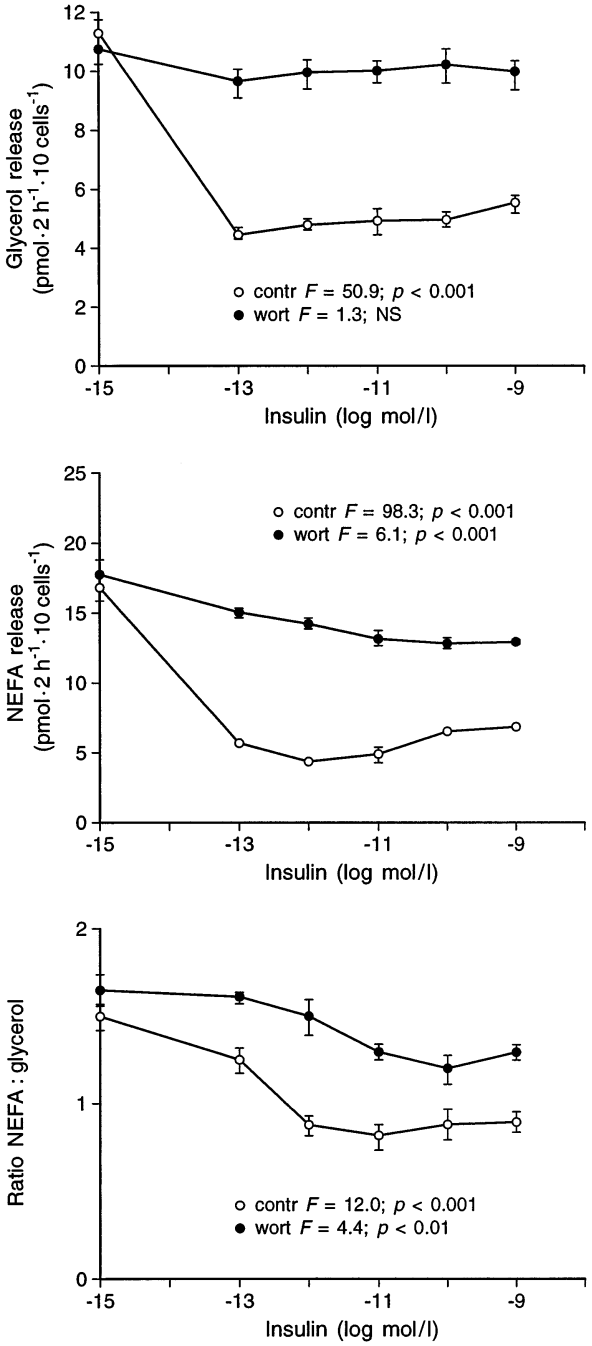


Fig. 10. Effect of wortmannin on insulin action in subcutaneous fat cells. Cells were incubated in the absence (contr) or presence of wortmannin (wort). Release of glycerol and NEFA were determined and the ratio of NEFA: glycerol was calculated. Noradrenaline (10 μmol/l) was always present in the incubation medium. The results were statistically evaluated for a concentration-dependent effect of insulin by one-way ANOVA. NS = not significant. Values are mean ± SEM. $n = 6$. The contr and wort curves were also compared using two way ANOVA. A difference between control and wortmannin was observed in the experiments with glycerol (upper graph; $p < 0.001$), NEFA (middle graph; $p < 0.01$) and NEFA glycerol ratio (lower graph; $p < 0.01$)

glycerol ratio. Thus, maximum insulin induced inhibition of NEFA release was 75 ± 2 % in the absence and 27 ± 5 % in the presence of wortmannin ($p < 0.001$). The maximum insulin-induced decrease in the NEFA to glycerol ratio was 0.7 ± 0.1 in the absence and 0.3 ± 0.1 in the presence of wortmannin ($p < 0.01$). The initial NEFA to glycerol ratio (with no insulin present) was lower and the maximum insulin inhibition of glycerol release occurred at a lower hormone concentration when results in Figure 10 were compared with those in Figure 3. This difference could result from individual variations in either NEFA to glycerol ratio or insulin sensitivity or both. A subgroup of study participants are presented in Figure 10, whereas all subjects investigated are described in Figure 3. Because of the lack of a significant insulin effect on the NEFA to glycerol ratio in omental fat cells, no studies with wortmannin were done.

Discussion

The mechanism by which insulin regulates NEFA metabolism in human adipocytes is poorly understood. Here we show pronounced regional differences in the ability of insulin to inhibit the release of NEFA from human fat cells. Firstly, insulin-induced antilipolysis was much less pronounced in omental than in subcutaneous fat cells, confirming an earlier observation [14]. Both the maximum response to insulin and the sensitivity to insulin were enhanced in subcutaneous fat. Secondly, we provide the first evidence that insulin stimulation of NEFA re-esterification differs between omental and subcutaneous fat cells. The effect of insulin was pronounced in subcutaneous fat cells but only small in omental fat cells. According to classical drug-receptor theories a combined change in hormone sensitivity and responsiveness indicates alterations at both receptor and post-receptor levels [26, 27]. Previous studies have shown an increased insulin receptor affinity in subcutaneous fat cells compared with omental adipocytes [14, 15]. This phenomenon cannot explain all of our findings. Although the increased sensitivity of the antilipolytic effect of insulin in subcutaneous cells could be due to enhanced receptor affinity [26], the observation of a reduced maximum response to insulin by omental cells for antilipolysis and re-esterification cannot be explained by binding affinity differences [27]. Thus we examined other events in the insulin receptor signalling cascade which are believed to lead to classical biological responses in adipocytes [1–4].

Insulin binding, protein expression, and steady-state level of insulin receptor mRNA was similar between subcutaneous and omental fat cells. Despite this, we observed a striking difference in insulin receptor autophosphorylation between the two fat cell depots. At each insulin concentration, insulin-induced receptor autophosphorylation was more pronounced in subcutaneous fat than in omental fat cells. Maximal insulin stimulation resulted in almost 100 % insulin receptors autophosphorylation in subcutaneous cells, whereas only two-thirds of the receptors underwent autophosphorylation in omental cells. The EC_{50} (3 nmol/l) for insulin stimulation of autophosphorylation, calculated using maximum stimulation as 100 % rather than total receptor number, was similar between omental and subcutaneous fat cells. Importantly, the concentration of insulin needed to autophosphorylate half of the total receptors was four times greater for omental cells than for subcutaneous cells. Thus, different phosphorylation sites could be present between the two cell types. These differences could partly explain the finding that insulin-induced antilipolysis and re-esterification were more pronounced in subcutaneous cells. Additional variations in the autophosphorylation process could exist between the depots. For example, at least six tyrosine moieties undergo phosphorylation during insulin stimulation [1]. Alternatively, differences in insulin receptor phosphorylation between the two depots could be related to differences in the level of serine phosphorylation.

IRS-1 is one of the earliest down-stream components of the insulin receptor signalling pathway following receptor autophosphorylation. We show a dramatic difference in IRS-1 tyrosine phosphorylation between the two cell types. Similar to the results for insulin receptor autophosphorylation, we noted that insulin-induced tyrosine phosphorylation of IRS-1 was greatly reduced in omental compared with subcutaneous cells. The latter finding was accompanied by a 50 % reduction in IRS-1 protein expression in omental cells, and could occur in response to differences in the insulin concentration between the portal (high) and peripheral (low) circulation [11]. For example, in 3T3-L1 adipocytes, high insulin concentrations lead to the degradation of IRS-1 [28].

To further characterize the insulin signalling transduction pathway downstream of IRS-1, we assessed the amount and enzymatic activity of phosphotyrosine associated PI 3-kinase in subcutaneous and omental fat cells. Protein expression of the 85 kDa isoform of PI 3-kinase was similar between subcutaneous and omental fat cells. The ability of insulin to increase the association of the p85 subunit of PI 3-kinase and to stimulate the phosphotyrosine associated enzymatic activity of PI 3-kinase were, however, greatly reduced in omental fat cells. This could be related to reduced insulin receptor phosphorylation in omental compared with subcutaneous fat cells. Alternatively, reduced protein expression and tyrosine phosphorylation of IRS-1 could account for the regional differences in PI 3-kinase activity. Adipocytes are known, however, to express other IRS proteins (IRS2/3) which contain tyrosine phosphorylation sites for PI 3-kinase [29, 30]. Thus, regional differences in the expression of IRS-2 or IRS-3 in human adipocytes might compensate for the changes in IRS-1 expression and activity. For example, it has been suggested IRS-2 compensates for reduced expression and activity of IRS-1 in adipocytes from subjects with non-insulin-dependent diabetes [31].

The ability of insulin to inhibit glycerol and NEFA release were partly counteracted by wortmannin. Furthermore, wortmannin counteracted the ability of insulin to stimulate re-esterification of NEFA. Thus, these results strongly support a role for PI 3-kinase in mediating insulin action on lipolysis and NEFA-esterification. Although insulin did not prevent liberation of NEFA by lipolysis, it inhibited NEFA release by stimulating re-utilization of NEFA for the re-esterification process. These speculations are based on measurements of glycerol and NEFA in the incubation medium. Although there are some methodological problems to consider regarding the

interpretation of these data [5, 20], glycerol is probably a valid indicator of lipolysis, since it is not re-utilized by fat cells to an appreciable extent. Furthermore, NEFA cannot be re-esterified but can be trapped intracellularly. Although the latter intracellular pool is generally considered to be small, changes in its size during lipolysis might influence the calculation of re-esterification.

Consistent with previously reports on rat fat cells [7–9], we show antilipolysis is activated by a PI 3-kinase-mediated pathway human adipocytes. Furthermore, we show for the first time that the re-esterification process is partly mediated via a PI 3-kinase dependent process. The antilipolytic effect of insulin is considered to be the most sensitive of its metabolic actions [32]. Our data support this notion. A large rightwards shift in the position of the insulin dose-response curve for PI 3-kinase is apparent when compared with that of lipolysis. This finding is reminiscent of the difference in the dose-response curves between insulin action on glucose transport and insulin receptor binding, the latter being shifted to the right compared with the former in human fat cells [33]. This discrepancy between insulin receptor binding and downstream metabolic event could be due to "spare" insulin receptors. Only a small fraction of insulin receptors are needed to reach maximum stimulation of a down-stream events such as stimulation of glucose transport or inhibition of lipolysis. Since the response of PI 3-kinase to insulin is comparable to the insulin binding curve and to the insulin-mediated receptor autophosphorylation, it is possible that the insulin signal is amplified downstream from PI 3-kinase, thus, allowing maximal antilipolysis when only a small percentage of receptors are activated. This fraction could be smaller than for activation of glucose transport, since a maximum stimulation of glucose transport in human fat cells is achieved at 35 % of maximum insulin receptor autophosphorylation [34].

The present observations on regional variations of insulin action in fat depots have potential clinical importance. Nevertheless, caution should be exercised in extrapolation of these in vitro results to the in vivo situation. For example, rapid infusion of glucose in vivo results in increased NEFA re-esterification, but no immediate decrease in the rate of lipolysis in vitro [35]. The increased delivery of NEFA to the liver by the visceral fat depots is believed to be an important pathophysiological factor contributing to several of the metabolic complications in obesity [11–13]. Our results provide evidence that NEFA release is more pronounced in omental than in subcutaneous adipose tissue, due to a combination of decreased antilipolytic and re-esterification effect of insulin in the omental region. These finding could have great implications for obesity. Future studies are warranted to ascertain whether differences in the insulin signal transduction pathway occur between obese and lean subjects.

Other possibilities for the regional variations in final events in insulin action on lipolysis include the ability of insulin to activate phosphodiesterase type III, a key enzyme responsible for insulin antilipolytic action [36]. Alternatively, phosphatases such as LAR and PTP1 b, which can dephosphorylate the insulin receptor [37], could also have a role in the differential regulation of metabolic events between the two fat depots. Although these phosphatases can be measured in human fat cells [38], the methods were not available for analysis of small samples at the time of our studies. Furthermore, differences in insulin sensitivities between the two sites could be related to a different membrane lipid composition. For example, alterations in the membrane lipid composition can have a profound influence on insulin sensitivity in skeletal muscle [39].

In summary, insulin receptor autophosphorylation and signal transduction through a IRS-1 and PI 3-kinase dependent pathways are decreased in human omental fat cells. This could, at least in part, explain why the ability of insulin to induce antilipolysis and stimulate re-esterification of NEFA is blunted in omental compared with subcutaneous fat cells.

Acknowledgements. This study was supported by grants from the Swedish Medical Research Council, the Swedish Diabetic Association, Karolinska Institute, Swedish Heart and Lung Foundation and the foundations of Söderberg, Bergvall, Gustav V, and Queen Victoria, Belvén and Novo Nordisk. J.R. Zierath was the recipient of a Junior Individual Grant from the Foundation for Strategic Research.

References

1. Kahn CR (1994) Insulin action, diabetogenesis and the cause of Type II diabetes. Diabetes 43: 1066–1084, 16: 2, 117
2. Lee J, Pilch PF (1994) The insulin receptor: structure, function, and signalling. Am J Physiol 266:C319–C334
3. White MF, Khan RC (1994) The insulin signalling system. Biochem Mol 269: 1
4. Quon MJ, Butte AJ, Taylor I (1994) Insulin signal transduction pathways. Trends Endocrinol Metab 5: 369–376
5. Arner P (1992) Metabolism of fatty acids: an overview. In: Bray G, Ricquier D, Spiegelman BM (eds) Obesity, JB Lippincott Co., Philadelphia, pp 294–308
6. Fry MJ (1994) Structure, regulation and function of phosphoinositide 3-kinases. Biochimica et Biophysica Acta 1226: 237–268
7. Okada T, Kawano Y, Sakakibara T, Hazeki O, Ui M (1994) Essential role of phospatidylinositol 3-kinase in insulin-induced glucose transport and antilipolysis in rat adipocytes. Studies with a selective inhibitor wortmannin. J Biol Chem 269: 3568–3573
8. Lam K, Carpenters CL, Ruderman NB, Friel JC, Kelly KL (1994) The phosphatidylinositol 3-kinase serine kinase phosphorylates IRS-1. Stimulation by insulin and inhibition by wortmannin. J Biol Chem 269: 20648–20652
9. Rahn T, Ridderstråle M, Tornqvist H et al. (1994) Essential role of phosphatidylinositol 3-kinase in insulin-induced activation and phosphorylation of the cGMP-inhibited

cAMP phosphodiesterase in rat adipocytes. Studies using the selective inhibitor wortmannin. FEBS Lett 350: 314–318
10. Leibel RL, Edens NK, Fried SK (1989) Physiological basis for the control of body fat distribution in humans. Annu Rev Nutr 9: 417–443
11. Kissebah AH, Krakower GR (1994) Regional adiposity and morbidity. Physiol Rev 74: 761–811
12. Lemieux S, Després JP (1994) Metabolic complications of visceral obesity: contribution to the aetiology of Type II diabetes and implications for prevention and treatment. Diabetes Metab 20: 375–393
13. Abat N, Garg A (1995) Heterogeneity in adipose tissue metabolism: causes, implications and management of regional adiposity. Prog Lipid Res 34: 53–70
14. Bolinder J, Kager L, Östman J, Arner P (1983) Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. Diabetes 32: 117–123
15. Livingston JN, Lerea KM, Bolinder J, Kager L, Backman L, Arner P (1984) Binding and molecular weight properties of the insulin receptor from omental and subcutaneous adipocytes in human obesity. Diabetologia 27: 447–453
16. Lönnqvist F, Thörne A, Nilsell K, Hoffstedt J, Arner P (1995) A pathogenic role of visceral fat beta$_3$-adrenoceptors in obesity. J Clin Invest 1109–1116
17. Hellmér J, Arner P, Lundin A (1989) Automatic luminometric kinetic assay of glycerol for lipolysis studies. Anal Biochem 177: 132–137
18. Edens NK, Leibel RL, Hirsch J (1990) Mechanism of free fatty acid re-esterification in human adipocytes in vitro. J Lipid Res 31: 1423–1431
19. Näslund B, Bernström K, Lundin A, Arner P (1993) Release of small amounts of free fatty acids from human adipocytes as determined by chemiluminescence. J Lipid Res 34: 633–642
20. Arner P (1995) Techniques for the measurement of white adipose tissue metabolism: a practical guide. Int J Obes 19: 435–442
21. Norgren S, Arner P, Luthman H (1994) Insulin receptor ribonucleic acid levels and alternative splicing in human liver, muscle and adipose tissue: tissue specificity and relation to insulin action. J Clin Endocrinol Metab 78: 757–762
22. Liu R, Zhu J, Jospe N, Furlanetto RW, Bastian W, Livingston JN (1995) Deletion of tysine 1.21 and creates a temperature-sensitive alteration in insulin binding by the insulin receptor. J Biol Chem 270: 476–482
23. Goodyear LA, Giorgino F, Sherman LA, Carey JO, Smith RJ, Dohm GL (1995) Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. J Clin Invest 95: 2195–2204
24. Jackson TR, Stephens LR, Hawkins PT (1992) Receptor specificity of growth factor-stimulated synthesis of 3-phosphorylated inositol lipids in Swiss 3T3 cells. J Biol Chem 267: 16627–16636
25. Thomas SHL, Wisher MH, Brandenburg D, Sönksen PH (1979) Evidence that the anti-lipolytic and lipogenic effects of insulin are mediated by the same receptor. Biochem J 184: 355–360
26. Kenakin T (1984) The classification of drugs and drug receptors in isolated tissue. Pharmacol Rev 36: 165–214
27. Kahn CR (1978) Insulin resistance, insulin sensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27: 1893–1902
28. Rice KM, Turnbow MA, Garner CW (1993) Insulin stimulates the degradation of IRS-1 in 3T3-L1 adipocytes. Biochem Biophys Res Commun 190: 961–967
29. Sun XJ, Wang LM, Zhang Y et al. (1995) Role of IRS-2 in insulin and cytokine signalling. Nature 377: 173–177
30. Lavan BE, Lane WS, Lienhard GE (1977) The 60-kDa phosphotyrosine protein in insulin-treated adipocytes is a new member of the insulin receptor substrate family. J Biol Chem 272: 11439–11443
31. Rondinone CM, Wang LM, Lonnroth P, Wesslau C, Pierce JH, Smith U (1977) Insulin receptor substrate (IRS) 1 is reduced and IRS-2 is the main docking protein for phosphatidylinositol 3-kinase in adipocytes from subjects with non-insulin-dependent diabetes mellitus. Proc Natl Acad Sci USA 94: 4171–4175
32. Zierler KL, Rabinovitz B (1964) Effect of very small concentrations of insulin on forearm metabolism. Persistence of its action of potassium and free fatty acids without its effect on glucose. J Clin Invest 43: 950–962
33. Pedersen O, Gliemann J (1981) Hexose transport in human adipocytes: factors influencing the response to insulin and kinetics of methylglucose and glucose transport. Diabetologia 20: 630–635
34. Thies RS, Molina JM, Ciaraldi TP, Freidenberg GR, Olefsky JM (1990) Insulin-receptor autophosphorylation and endogenous substrate phosphorylation in human adipocytes from control, obese and NIDDM subjects. Diabetes 39: 250–259
35. Leibel RL, Forse RA, Hirsch J (1989) Effects of rapid glucose infusion on in vivo and in vitro free fatty acid re-esterification by adipose tissue of fasted obese subjects. Int J Obesity 13: 661–671
36. Manganiello VC, Vaughan M (1973) An effect of insulin on cyclic adenosine 3′, 5′-monophosphate phosphodiesterase activity in fat cells. J Biol Chem 248: 7164
37. Goldstein BJ (1993) Regulation of insulin receptor signalling by protein-tyrosine dephosphorylation. Receptor 3: 1–15
38. Ahmad F, Considine RV, Goldstein BJ (1995) Increased abundance of the receptor-type protein-tyrosine phosphatase LAR accounts for the elevated insulin receptor dephosphorylating activity in adipose tissue of obese human subjects. J Clin Invest 95: 2806–2812
39. Borkman M, Storlien LH, Pan DA, Jenkins AB, Chrisholm DJ, Campbell LV (1993) The relationship between insulin sensitivity and the fatty acid composition of the phospholipids of skeletal muscle. N Engl J Med 328: 238–244