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Insulin Receptor Binding and Metabolic Effects of Insulin in Human Subcutaneous Adipose Tissue in Untreated Non-insulin Dependent Diabetes Mellitus

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ABSTRACT

Insulin action at the target tissue level in non-insulin dependent diabetes mellitus was investigated using human adipose tissue. Specific adipocyte receptor binding of insulin and the effects of the hormone on glucose oxidation and lipolysis were determined in subcutaneous adipose tissue. The study included 25 patients with untreated non-insulin dependent diabetes mellitus and 38 healthy control subjects matched for age, sex and body weight. Insulin stimulated adipose tissue glucose oxidation in a dose-dependent way in the control subjects. On the other hand, a marked inhibition of this insulin effect was observed in the diabetics. A weak stimulation was observed only at high unphysiological hormone concentrations ($\geq$ 0.7 nmol/l) and the maximal insulin response was 6 times lower than that in the control subjects. However, neither specific insulin receptor binding nor the antilipolytic effect of insulin were inhibited in diabetes. Similar results with insulin binding and the metabolic effects of insulin were obtained in non-obese normoinsulinemic diabetics as compared to moderately obese hyperinsulinemic diabetics. It is concluded that adipose tissue insulin resistance in non-insulin dependent diabetes mellitus only involves glucose metabolism and not antilipolysis. Furthermore, it may solely be due to postreceptor defects in insulin action and seems not to be influenced by obesity or oversecretion of insulin.

INTRODUCTION

In patients with non-insulin dependent diabetes mellitus the hypoglycemic response to insulin is often attenuated. Insulin resistance in target tissues is an important cause of hyperglycemia in this form of diabetes. When the disease is accompanied by obesity the action of insulin may be further inhibited. Insulin resistance in non-insulin dependent diabetes mellitus is usually attributed to a combination of impaired hormone binding to specific cell surface receptors and inhibition of the signals from the receptor to intracellular metabolic processes [14,15,18,22,26]. It is suggested that alteration of the receptor is an early event that leads to a mild form of insulin resistance, which is subsequently aggravated by impaired intracellular hormone signal [20,22]. The investigation of the insulin action in non-insulin dependent diabetes mellitus in man has been centered mainly on hormone binding to circulating blood cells, where insulin has little, if any, biological effect and on the over-all effect of insulin on glucose metabolism

in vivo. It is at present unclear how insulin action in this disease is altered in the primary target tissues (fat, muscle and liver). It is also not known if insulin action in target tissues differs between obese and non-obese diabetics. The aim of the present study was accordingly to establish whether insulin receptor binding and/or the metabolic effects of the hormone were altered in adipocytes in non-insulin dependent diabetes mellitus. Subcutaneous adipose tissue was obtained from 25 untreated patients with this form of diabetes; 12 of the patients were non-obese and the others were moderately obese. Comparison was made to the finding in 38 healthy control subjects with normal glucose tolerance, who were matched with the diabetics for age, sex and body weight.

MATERIAL AND METHODS

Patients: The series for the study consisted of 25 patients with recently diagnosed and untreated non-insulin dependent diabetes mellitus. Twelve were non-obese and 13 were moderately obese. Moderate obesity was defined as a relative body weight of 115-150% of the average body weight, which was obtained from tables in Documenta Geigy (13). Glucosuria was present in all diabetics but ketoacidosis in none. They were otherwise healthy and there were no signs of physical inactivity secondary to the illness. After the investigation the diabetics received diet therapy, either alone or in combination with sulphonylurea. Thirty-eight healthy volunteers with normal values for either intravenous glucose tolerance (25 g glucose) or oral glucose tolerance (100 g glucose) served as control subjects; 18 were classed as moderately obese and 20 as non-obese. Some of these subjects have been included in previous reports as control subjects. The obese subjects had not followed any form of slimming program during the six months preceeding the study. Clinical data of diabetic patients and control subjects are present in Table 1. They were matched with respect to age, sex and body-weight. The patients and control subjects consumed a diet consisting of 40% carbohydrate, 40% fat, 20% protein and 7-8.5 MJ, according a 24-h recall. All subjects and patients were informed in detail about the study and their consent was obtained. The study was approved by the Hospital's Ethical Committee.

The investigation was performed at 8 a.m. after an overnight fast at the Out-Patient Department. Venous blood samples were taken for the determination of glucose (11) and serum immunoreactive insulin (30). Gluteal specimens of subcutaneous adipose tissue were obtained surgically. Local anesthesia was induced with prilocaine chloride, which was given in such a way that it did not affect the metabolism of adipose tissue (1).

Adipocyte insulin receptor binding: Isolated fat cells were prepared by Rodbell's method (29). They were incubated in 0.5 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing dialyzed bovine serum albumin (40 g/l), glucose (5 mmol/l) mono ^{125}I-(Tyr A_{14})-insulin (0.05 nmol/l), and unlabelled insulin (0-50 nmol/l). The final cell concentration was 8% (vol/vol). After incubation for 60 min at 24°C in triplicate, 10 ml of ice-cold saline was added and the cells were centrifuged through 1.2 ml of

Table 1. Clinical characteristics of the subjects included in the study

		Number of subjects	Sex, M/F	Age, years	Body weight, % of average	Blood glucose mmol/l	Serum insulin, mU/ml	Cell volume, picolitre
ALL	Diabetics	25	14/11	52 ± 2	113 ± 4	10.7 ± 0.6	15 ± 2	976 ± 57
	Controls	38	20/18	48 ± 2	111 ± 3	4.6 ± 0.1	10 ± 1	1016 ± 47
	p					< 0.001	< 0.05	
NON-OBESE	Diabetics	12	7/5	50 ± 2	97 ± 3	11.7 ± 0.7	8 ± 3	874 ± 72
	Controls	20	10/10	46 ± 3	97 ± 2	4.5 ± 0.1	7 ± 1	864 ± 41
	p					< 0.001		
OBESE	Diabetics	13	7/6	53 ± 3	128 ± 3	9.8 ± 0.9	21 ± 8	1070 ± 82
	Controls	18	10/8	50 ± 3	127 ± 2	4.8 ± 0.1	21 ± 2	1151 ± 67
	p					< 0.001	< 0.02	

silicone oil; they were then removed from the oil surface for radioactivity determination. Non-specific binding was measured as the amount of ^{125}I-insulin remaining in the cell layer in the presence of unlabelled insulin (20 μmol/l). All the values presented were corrected for non-specific binding, which amounted to 2-3%. The method has been described in detail (5,7). It was previously shown that (a) insulin binding reaches a maximum after 30 min and remains constant for at least 90 min, (b) insulin degradation in the incubation medium is insignificant and (c) the coefficient of variation within one subject for insulin binding is 8% (5,7).

Adipose tissue metabolism. Explants of adipose tissue (about 100 mg) were preincubated for 30 min and then incubated in triplicate for 2 h at 37°C in 1 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) to which dialyzed bovine serum albumin (40 g/l), glucose (5 mmol/l), (U-^{14}C)-glucose (2 x 10^9 cpm/l) and insulin (0-7 nmol/l) were added. A mixture of (CO_2:O_2) (5:95) was used as gas phase. After incubation glycerol release and $^{14}CO_2$ production were determined as described in detail previously (5,7). These measures were used as indices of lipolysis and glucose oxidation, respectively. The coefficient of variance within one subject for the metabolic studies was 8%. Insulin degradation in the incubation medium, as determined by the tricholoroacetic acid precipitation method (5), was 5%. We have discussed in detail previously why the metabolic effects of insulin are preferably detemined on segments of fat rather than on isolated fat cells (7).

Adipocyte cellularity: The fat cell volume and surface area, and the number of fat cells incubated were determined as described in detail previously (5,7).

Chemicals: Crystalline, glucagon-free porcine insulin was a generous gift from Vitrum AB (Sweden). Mono ^{125}I-(Tyr A_{14})-insulin (specific activity about 200 Ci/g), was from Novo (Denmark). U-^{14}C-glucose (specific activity 270 mCi/mmol) was from the Radiochemical Centre (England). Bovine serum albumin (fraction V) was from Armour Pharmaceutical Company (England).

Statistical methods: The reported values are the mean ± SE. Student's unpaired t-test was used for statistical comparison.

RESULTS

Fat cell size was enhanced in the obese diabetic and obese non-diabetic groups: the values for diabetics and control subjects were however, similar (Table 1). The fasting serum insulin level was twice as high ($p<0.02$) for the obese diabetics than for the obese control subjects and 50% higher ($p<0.05$) in the whole diabetic group as compared to the whole control group (Table 1).

Basal metabolism of adipose tissue is shown in Fig. 1. Lipolysis tended to be slower and glucose oxidation was less pronounced in the whole diabetic group as compared to the whole group.

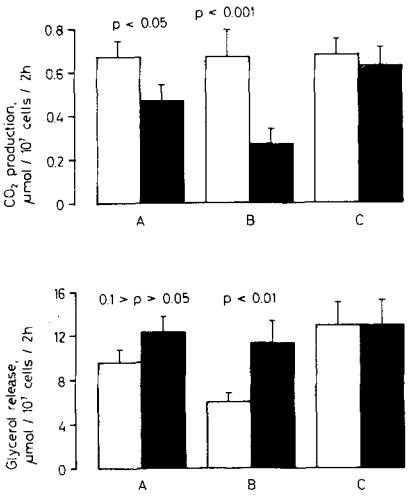

Fig. 1 Basal glucose oxidation and basal lipolysis in adipose tissue of patients with untreated non-insulin-dependent diabetes mellitus (closed symbols) and healthy control subjects with normal glucose tolerance (open symbols). The incorporation of radioactive glucose into CO_2 (glucose oxidation) is shown in the upper graphs and the rate of glycerol release (lipolysis) is depicted in the lower graphs. A: The results with all subjects. B: The results with non-obese subjects. C: The results with moderately obese subjects. Values are mean ± SE. Student's unpaired t-test was used for statistical comparison.

When the material was divided according to body-weight it was observed that the non-obese subjects accounted for the entire difference between diabetes and the control state. Thus, in the non-obese diabetes group basal glucose oxidation was reduced by 2/3 and the basal rate of lipolysis was doubled in comparison with the non-obese control group. In obese subjects, on the other hand, basal lipolysis and basal glucose oxidation were almost identical in diabetes and the control state.

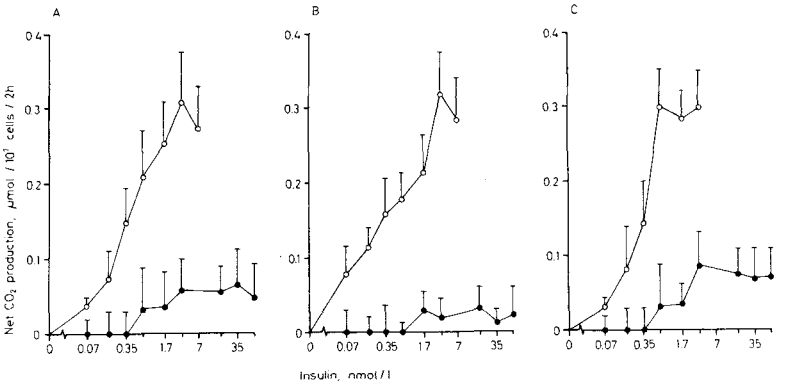

Fig. 2 Insulin-induced stimulation of glucose oxidation in human adipose tissue in untreated non-insulin dependent diabetes mellitus (open circles) and the control state (filled circles). Insulin-induced CO_2-production minus basal CO_2-production was calculated and plotted versus the insulin concentration. See legend to Fig. 1 for further details.

The effect of insulin on glucose oxidation is shown in Fig. 2. In the control subjects there was a marked dose-dependent insulin effect. The results with non-obese and obese subjects were quite similar. In the diabetics there was only a small insulin effect occurring solely at high unphysiological hormone concentrations ($\geq$ 700 pmol/l). Since the dose-response curves in diabetics were scattered it was not possible to calculate ED_{50}. *In the whole diabetes group the amplitude of the dose-response curve was* 6 times lower than in the control group. The results with non-obese and obese diabetics were similar.

Fig. 3 shows the antilipolytic effect of insulin. It is evident that this action of the hormone was not inhibited in untreated non-insulin dependent diabetes mellitus. In the whole material and in obese subjects the amplitudes of the dose-response curves were in the same order of magnitude in diabetes and the control state. Furthermore, insulin sensitivity (i.e. the left-right position of the dose-response curve) was almost identical in these groups: ED_{50} was 35 pmol/l. Insulin sensitivity in non-obese diabetics *was also the same as in non-obese control subjects* (ED_{50} = 35 *pmol/l*). *However, the* antilipolytic effect of insulin was higher in the non-obese diabetics as compared to the non-obese control group at all hormone concentrations tested. The latter may indicate that insulin responsiveness (i.e. the maximum hormone effect) was actually enhanced in non-obese diabetics. This was further investigated in Table 2 which shows insulin responsiveness as calculated from each subject's dose-response curve. The absolute values for insulin responsiveness were almost twice as high in the non-obese diabetes group in comparison to the non-obese control group. The relative values for the maximum antilipolytic effect were, however, almost identical in both groups. Insulin *maximally inhibited basal lipolysis by 35%. The latter is probably due to that* both basal lipolysis (Fig. 1) and insulin-induced antilipolysis (Fig. 3) were enhanced in non-obese diabetics.

Table 2. Responsiveness of the antilipolytic effect of insulin in non-obese subjects with untreated non-insulin dependent diabetes mellitus and in non-obese control subjects.

	Responsiveness μmol/10^7 cells/2 h	Percent of basal
Control	2.00 $\pm$ 0.41	34 $\pm$ 5
Diabetes	3.49 $\pm$ 0.47	36 $\pm$ 7
p	< 0.01	

In each subject glycerol release at the maximum effective insulin concentration was substracted from the basal glycerol release. The obtained value represents responsiveness (the maximum antilipolytic effect) in absolute terms. This value was also divided by the basal value to obtain the relative responsiveness.

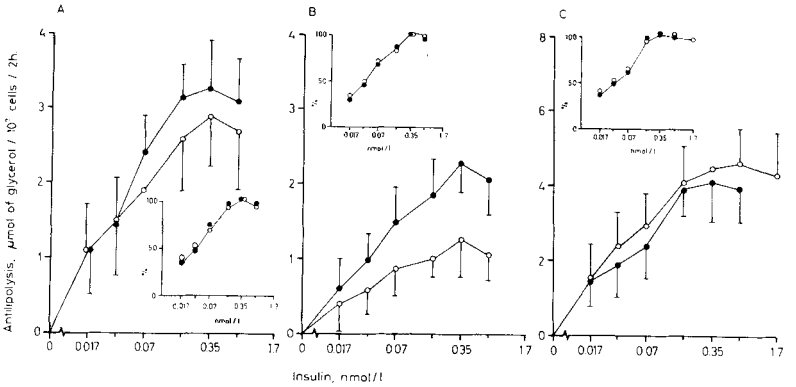

Fig. 3: Antilipolytic effect of insulin in human adipose tissue in untreated non-insulin dependent diabetes mellitus and the control state. Basal glycerol release minus insulin-induced glycerol release was calculated and plotted versus the insulin concentration. The corresponding insulin sensitivity plot is given in the inset. In the latter graph the antilipolytic effect of insulin is expressed as percentage of the response to insulin at the maximum effective concentration (%) and is plotted versus the insulin concentration (nmol/l). See legends to Figs. 1 and 2 for further details.

It is observed in the control subjects that insulin inhibited lipolysis at lower hormone concentration than at which there was an effect of insulin on glucose oxidation. Similar differences in the sensitivities of the effects of insulin on lipolysis and glucose oxidation have previously been demonstrated in human adipose tissue (7).

Specific insulin binding to fat cells is shown in Figure 4. There was no evidence of decreased binding in untreated diabetes. The results were similar in non-obese and moderately obese diabetes patients.

The results of the comparison between diabetes and control groups were the same whether expressed in terms of fat cell number or in terms of fat cell surface area.

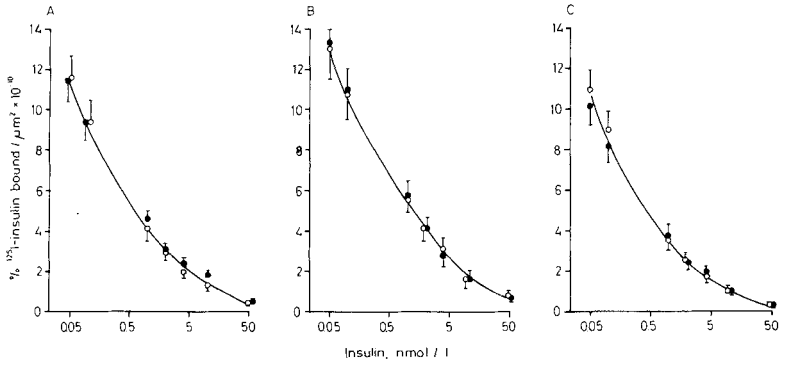

Fig. 4: Insulin binding to isolated human adipocytes in untreated non-insulin-dependent diabetes mellitus and the control state. Specific % ^{125}I-insulin binding was determined and plotted versus the total insulin concentration. See legends to Figs. 1 and 2 for further details.

DISCUSSION

It is well established that non-insulin dependent diabetics are resistant to the hypoglycemic effect of insulin [15,22,26]. In the present untreated patients with this disease there was a marked alteration of the ability of insulin to stimulate glucose oxidation in adipose tissue. A small insulin effect was observed solely at high unphysiological hormone concentrations. In contrast, adipose tissue of control subjects responded to insulin added at physiological concentrations and the maximal insulin effect was 6 times greater in these subjects than in the diabetics. Thus, the present data demonstrate a marked insulin resistance in adipose tisue in untreated non-insulin dependent diabetes mellitus, which suggests that fat tissue is involved in the over-all impairment of insulin action on glucose utilization in this disease.

Inhibited action of insulin on glucose metabolism in human adipose tissue in untreated non-insulin dependent diabetes has previously been observed in hyperobese (average body weight > 150%) Pima Indians [19] and in a small group of patients with normal fasting insulin levels [7]. However, the influence of obesity and fasting hyperinsulinemia on insulin action was not considered in these studies. Non-insulin dependent diabetics are often obese and hyperinsulinemic. Both these factors may induce insulin resistance independently of diabetes [22]. In this study a similar degree of adipose tissue insulin resistance was observed in non-obese diabetics with normal fasting insulin levels as in obese diabetics with fasting hyperinsulinemia. These results indicate that neither obesity nor enhanced insulin secretion are of importance for adipose tissue insulin resistance in non-insulin dependent diabetes mellitus.

The present results with insulin action on lipolysis in diabetics differed markedly from those with insulin-induced glucose oxidation. The antilipolytic effect of insulin was not inhibited in adipose tissue of untreated non-insulin dependent diabetics. In the whole material insulin action on lipolysis was almost identical in diabetes and the control state both regarding insulin sensitivity and insulin responsiveness (maximum hormone effect). Furthermore, neither non-obese nor obese diabetics were resistant to the antilipolytic effect of insulin. These data strongly support our original hypothesis [2,7] that antilipolysis is not involved in diabetes associated insulin resistance. Further support for this theory is the recent finding of normal antilipolytic effect of insulin in adipose tissue of non-insulin dependent diabetics on long term treatment with diet plus sulphonylurea [21].

The first step in the action of insulin is its binding to specific cell surface receptors. From recent review articles it would appear to be a common belief that reduced receptor number is an important factor underlying insulin resistance in insulin sensitive tissues in non-insulin-dependent diabetes mellitus [14,15,18,22,26]. However, in the present study adipocyte insulin receptor binding in a large group of non-obese and obese patients with untreated non-insulin dependent diabetes mellitus was not lower than in healthy subjects with normal glucose tolerance, who were matched for age, sex and body weight. These results are at variance with numerous studies which have demonstrated a de-

creased number of insulin receptors on circulating lymphocytes, monocytes and erythrocytes in non-insulin dependent diabetes mellitus [3,12,16,23,24,27,28]. However, evidence of decreased insulin binding to circulating blood cells is probably irrelevant since these cells are not natural target cells for insulin action. The results differ also from those published by Kolterman and co-workers who observed decreased adipocyte insulin binding in untreated non-insulin dependent diabetics [20]. In that investigation, however, the diabetic patients were significantly older than the control subjects. Age matching of the groups is probably important in any comparison in respect of adipocyte insulin binding, since several reports have demonstrated that insulin binding to human fat cells decrease with age [8,21,25]. It is also possible that regional variations in adipocyte insulin binding play a role. We investigated gluteal fat cells and Kolterman and co-workers investigated abdominal fat cells. We have recently observed that insulin binding varies between different adipose sites in man [4,5]. On the other hand, normal insulin binding to adipocytes has recently been observed in some rare groups of untreated non-insulin dependent diabetics, namely ones with normal insulin secretion [7] and young hyper-obese Pima Indians [19]. Furthermore, normal insulin binding to fat cells was also observed in non-insulin dependent diabetics receiving long-term diet and sulphonylurea therapy [9,21]. Thus, when previous and present findings are considered together the adipocyte insulin receptor appears not to be impaired in any form of non-insulin dependent diabetes mellitus. This would seem to call into question the role of the insulin receptor in the development of insulin resistance in this disease.

It should be noted that in the present binding experiments fat cells were incubated at $24^{o}C$. At this temperature some of the insulin bound is internalized. In theory the latter fraction may differ between diabetics and non-diabetics. However, in human fat cells we have recently observed [5,6] an excellent correlation [$r>0.9$] between insulin binding at $24^{o}C$ and $37^{o}C$ (where a large portion of insulin is internalized) and between insulin binding at $24^{o}C$ and $16^{o}C$ (where no insulin is internalized). This indicates that differences in insulin internalization between diabetics and non-diabetics have little bearing upon the present results with insulin receptor binding. Furthermore, the observation of normal sensitivity of the antilipolytic effect of insulin argues further for that the insulin receptor was normal in our diabetics, since insulin sensitivity is thought to reflect insulin action at the receptor level [17].

Apparently, the presently observed inhibition of insulin-induced glucose metabolism is solely due to a post-binding alteration of insulin action. Furthermore, this resistance to insulin appears not to involve the antilipolytic effect of the hormone.

The molecular mechanisms behind defects in insulin action on glucose metabolism at the postreceptor level in fat cells of non-insulin diabetics are unclear. However, decreased ability of insulin to stimulate adipocyte glucose transport has recently been observed in this form of diabetes [10,19]. Such a defect may in part explain the inhibition of insulin-induced adipose tissue glucose metabolism found in this study. However, additional defects in the intracellular action of insulin may also cause the presently

observed insulin resistance, particularly in the non-obese subjects where we also found a marked reduction of basal glucose oxidation. In the latter respect it is of interest to compare adipose tissue metabolism in our two diabetes groups. Although insulin action did not differ between the two groups basal lipolysis and basal glucose oxidation were markedly altered in normoinsulinemic non-obese diabetics but unchanged in moderately obese hyperinsulinemic diabetics. It is thus possible that adipose tissue metabolism may be regulated differently in these two forms of diabetes.

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