Insulin Resistance in Limb and Trunk Partial Lipodystrophy (type 2 Köbberling-Dunnigan syndrome)

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We studied insulin action in two patients with limb and trunk partial lipodystrophy with hirsutism and acanthosis nigricans. Glucose was normal in one of the patients and slightly above normal in the other during an oral glucose tolerance test (OGTT). An intravenous glucose tolerance test (IVGTT) was normal in both patients. Basal and glucose-stimulated insulin levels were elevated in both the OGTT and IVGTT in both patients. The response of plasma glucose to exogenously administered insulin was decreased. A euglycemic-hyperinsulinemic clamp performed in patient no. 2 indicated insulin resistance, which was not corrected by reducing the increased basal level of serum free fatty acids (FFAs). Binding of insulin to neck adipocytes was normal in both subjects, but glucose transport and oxidation in these cells was impaired. Insulin binding to abdominal adipocytes was increased in one patient whose adipocytes displayed higher glucose transport at low insulin concentrations. Glucose oxidation was decreased in abdominal adipocytes of both patients. We conclude that insulin resistance in Köbberling-Dunnigan type 2 partial lipodystrophy is not related to an alteration of the insulin molecule or to changes in insulin binding, but is more likely associated with a postreceptor defect, since glucose oxidation was impaired in adipocytes of the neck and abdomen.

THREE CLINICAL SYNDROMES share as a common distinguishing feature a partial absence of subcutaneous fat (lipodystrophy). In progressive partial lipodystrophy (Barraquer-Simon syndrome), fat is absent in the face and, in most cases, in the trunk, with normal or excessive fat deposition in the pelvic girdle and lower limbs. The other two types, referred to as the Köbberling-Dunnigan syndrome, comprise two clinical phenotypes occurring either as familial disease or as sporadic cases. In type 1, loss of subcutaneous fat is confined to the limbs, sparing the face and trunk. In type 2, the trunk is affected but the face of the individual looks cushingoid, with excessive fat on the face, neck, and supraclavicular fossae.[1]

Previous studies have indicated that the insulin resistance of these syndromes may involve both receptor and postreceptor abnormalities. However, the studies were performed either in circulating cells (monocytes and erythrocytes)[2-4] or in cultured fibroblasts,[5-7] which are not classic target tissues of insulin.

This report describes the results of in vivo evaluation of glucose metabolism and in vitro studies of adipocytes taken from the neck and lower abdomen in two patients with sporadic cases of type 2 Köbberling-Dunnigan syndrome.

CASE REPORTS

Case 1

Patient no. 1, a 31-year-old white woman, noted increased fat in the face, neck, and supraclavicular fossae near the onset of puberty and periods of oligomenorrhea since menarche. No other members of the family were affected. Pertinent physical findings included an increase of subcutaneous fat tissue in the face, neck, and upper torso and a loss of adipose tissue below these regions. She had facial hirsutism and acanthosis nigricans, particularly in the neck and axillary regions and around the areolae of the breasts (Fig 1). The remainder of the physical examination was normal, with no hepatomegaly or neurologic abnormalities. No abdominal ultrasound examination was performed. Routine laboratory studies including blood cell counts, urinanalysis, and determination of blood urea and plasma creatinine and glucose were normal. The serum triglyceride level was 2.25 mmol/L, total cholesterol 5.17 mmol/L, low-density lipoprotein cholesterol 3.56 mmol/L, and high-density lipoprotein cholesterol 0.36 mmol/L. The testosterone level was 0.76 nmol/L, dehydroepiandrosterone (DHEA) 52.1 nmol/L, androstenedione 10.1 nmol/L, and estradiol 124 pmol/L.

Case 2

Patient no. 2, a 22-year-old black woman, noted an increase in subcutaneous fat in the face, neck, and upper trunk from puberty and oligomenorrhea since menarche at the age of 14. No other members of the family presented the same features. Physical examination showed a muscular woman with increased subcutaneous fat in the face, neck, mandibula, and supraclavicular fossae and loss of subcutaneous adipose tissue below these regions. Acanthosis nigricans was present in the axillary folds and in the flexures of the elbows and groins, and there was severe hirsutism in the face, chest, and midline of the abdomen. The external genitalia showed moderate labial hypertrophy and clitoromegaly. On abdominal ultrasonography, the left ovary was enlarged, whereas the right ovary was of normal size. Cysts were not seen. Hepatomegaly was absent, and no neurologic abnormalities were found. Routine laboratory studies including blood cell counts, urinalysis, and determination of urea, creatinine, and glycemia were within the normal range. The triglyceride level was 4.09 mmol/L, total cholesterol 4.44 mmol/L, low-density lipoprotein cholesterol 2.35 mmol/L, and high-density lipoprotein cholesterol 0.62 mmol/L. The testosterone level was 3.8 nmol/L, DHEA, 33.2 nmol/L, androstenedione 15.3 nmol/L, and estradiol 171 pmol/L.

Methods

Informed consent was obtained from both patients, and approval was granted by the Ethics Committee of Hospital das Clínicas, University of São Paulo Medical School, to perform the following studies: oral (OGTT) and intravenous (IVGTT) glucose tolerance tests, intravenous insulin tolerance tests (ITTs), hyperinsulinemic clamp, evaluation of insulin biological activity, and insulin binding, glucose transport, and oxidation assays in adipose cells from biopsies obtained from the neck and lower abdomen.

The control group included four female subjects aged 23 to 37 years with body mass index less than 26 kg/m^2.

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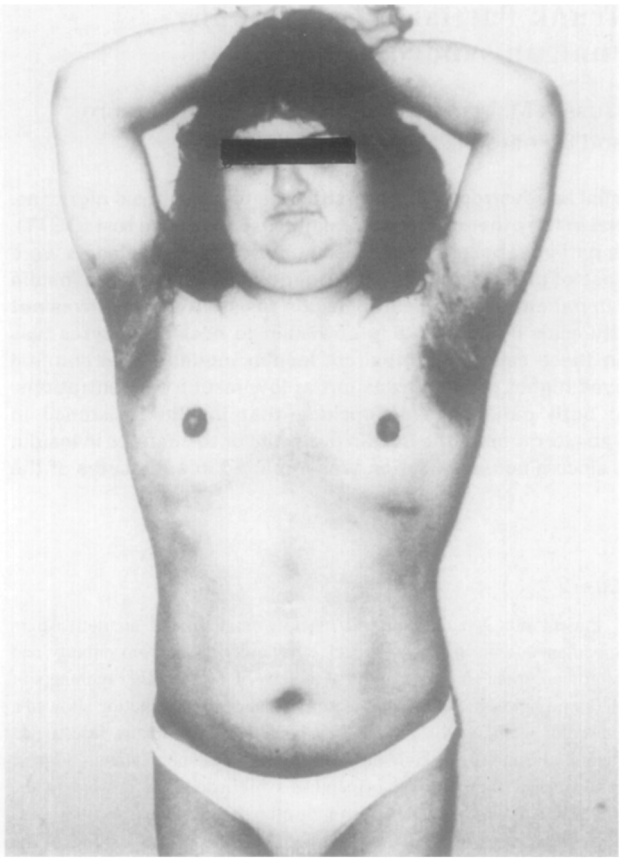

Fig 1. Distribution of subcutaneous fat in patient no. 1. Note the lack of subcutaneous fat in the abdomen, the cushingoid face, and acanthosis nigricans in the axillae.

OGTT. Seventy-five grams of glucose was administered, and blood samples were obtained (180 minutes) for determination of plasma glucose and serum insulin.

IVGTT. Twenty-five grams of glucose in a 50% solution was administered as a bolus, and frequent blood samples were taken (60 minutes) for measurement of glucose and insulin levels.

ITT. Insulin (0.025 mU/kg body weight) was injected intravenously, and frequent sampling was performed (30 minutes) for glucose determination.[8]

Euglycemic-hyperinsulinemic clamp. In patient no. 2, a euglycemic-hyperinsulinemic clamp was performed according to the method of DeFronzo et al.[9] Plasma insulin was acutely increased and maintained for 2 hours at approximately 100 μU/mL by a primed continuous infusion. Plasma glucose concentration was held constant at 5 mmol/L by a variable glucose infusion. The clamp was repeated after overnight suppression of free fatty acids (FFAs) with acipimox.[10]

The glucose level was measured by the glucose-oxidase method of Wasko and Rice,[11] and the insulin level by the Desbuquois and Aurbach[12] radioimmunoassay technique. Cholesterol was assayed with an enzymatic method,[13] and triglycerides with the peroxidase-coupled method of McGowan et al.[14] Plasma androgen and estradiol levels were measured by radioimmunoassay according to the method of Abraham,[15] and plasma FFA levels by the method of Chromy et al[16] as modified by Demacker et al.[17]

Insulin biological activity. Insulin was extracted from the patient's serum, and its biological activity was determined according to the method of Freychet et al[18] by measurement of U-^{14}C-glucose transport in rat adipose cells using increasing doses of the patient's own insulin.

Preparation of isolated adipocytes. Samples of adipose tissue from the neck and lower abdomen were obtained in the operating room after lidocaine anesthesia. Adipose tissue (10 g) removed from each area was immediately placed in cold saline and brought to the laboratory for metabolic studies. Adipocytes were isolated by a modification of the collagenase digestion method of Rodbell,[19] as described in a previous publication.[20] We used methods for binding studies and glucose transport and oxidation that were previously published.[20]

Materials. Crystalline porcine insulin for in vitro studies and for iodine labeling was generously supplied by Novo-Nordisk (Copenhagen, Denmark). Collagenase (4197 CLS) was purchased from Worthington Biochemical (Freehold, NJ). Bovine serum albumin (fraction V, A-4503), Na-^{125}I (NEZ-033H), and D-(U-^{14}C) glucose (NEC-042X) were supplied by New England Nuclear (Boston, MA).

RESULTS

Patient no. 2 had elevated serum triglycerides, which were only slightly above normal in patient no. 1. The androgens, testosterone, DHEA, and androstenedione, measured in the midfollicular phase were increased in patient no. 2. The other patient had elevated DHEA and androstenedione, but of a lesser magnitude. The higher hyperandrogenemia in patient no. 2 was associated with a virilized condition not found in patient no. 1.

Results for the OGTT and IVGTT are displayed in Fig 2. Insulin levels before (basal) and during testing were elevated. These findings suggest a state of systemic insulin resistance. The response of glucose levels in the ITT (Fig 3) also indicates a reduced effect of exogenous insulin.

The amount of glucose metabolized during the euglycemic-hyperinsulinemic clamp in subject no. 2, assuming that endogenous glucose production was suppressed, was 100.5 mg/m^2/min, with normal values in our laboratory being 253 ± 12 mg/m^2/min (mean ± SEM). Fasting plasma FFA levels before starting the clamp were 487 μEq/L (normal value, 320 ± 30). After overnight suppression of plasma FFA with acipimox, a nicotinic acid analog (750 mg orally), as suggested by Fulcher et al,[10] a new clamp was performed at a basal FFA level of 274 μEq/L. Despite the appropriately reduced FFA level, the amount of glucose metabolized remained low at 99 mg/m^2/min.

Insulin Receptor Binding

Figure 4 displays the binding of ^{125}I-insulin to isolated adipocytes from patients and controls. Adipose cell size (diameter), specific insulin binding at tracer insulin concentrations, insulin receptor sites per cell, and high-affinity (Ke) and low-affinity (Kf) constants are shown in Table 1. Adipocyte cell diameter was greater in the atrophic region (lower abdomen). The binding of insulin to neck adipocytes at tracer insulin concentrations was normal in both patients. However, the results for binding to lower-abdominal adipocytes were enhanced in patient no. 2 and reduced in patient no. 1, due to parallel changes in receptor concentration without changes in Ke.

Glucose Transport

Glucose transport was impaired in adipocytes taken from the neck in both patients (Fig 5). Transport was at the lower limit of normal and above in patients no. 1 and 2, respectively, at insulin levels up to 20 μU/mL in adipocytes taken from the abdomen; however, it was below normal at higher insulin concentrations.

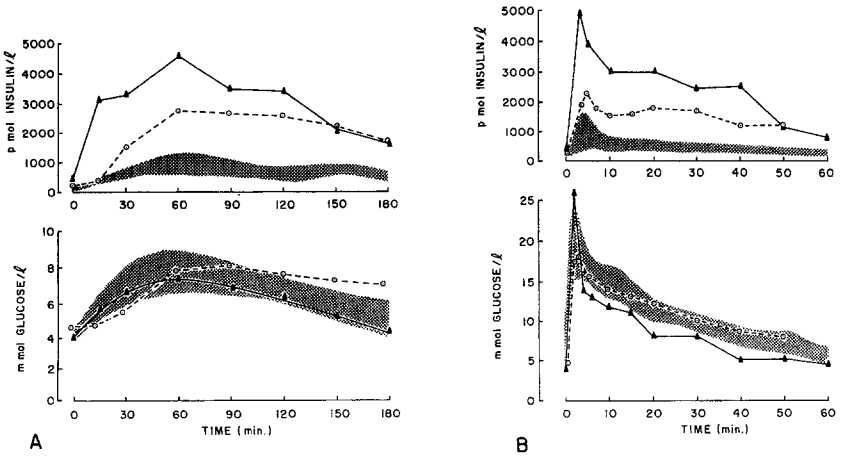

Fig 2. Plasma glucose and serum insulin levels during an OGTT (A) and IVGTT (B) in patients no. 1 (△) and 2 (○). Shaded area represents the range of normal values from this laboratory.

Glucose Oxidation

Glucose oxidation was reduced in neck adipocytes from both patients. In abdominal adipocytes, CO_2 production was in the low-normal range at low insulin concentrations in patient no. 1, whereas it was severely impaired in patient no. 2 (Fig 5).

DISCUSSION

The diagnosis of partial lipodystrophy (type II Köbberling-Dunnigan syndrome) was established by symmetrical lipoatrophy of the trunk and limbs, with a cushingoid rounded, full face with excess fat on the face, neck, and supraclavicular areas.[1] As described previously, this lipodystrophy was associated with severe insulin resistance and acanthosis nigricans.[21,22]

Insulin resistance was clearly shown by enhanced insulin secretion during the OGTT and IVGTT in the presence of normal or slightly elevated glucose levels and resistance to the test dose of exogenous insulin. Patient no. 2 had a significant reduction in the glucose disposal rate during a euglycemic-hyperinsulinemic clamp before and after FFA suppression.

The metabolic abnormalities associated with this syndrome in four published cases[1,23,24] were variable. They ranged from severe hyperlipemia and insulin-resistant diabetes in three patients to impaired glucose tolerance and mild hypertriglyceridemia in the remaining one. All of these cases occurred as a familial disease suggesting an X-linked dominant mode of inheritance.[1]

We now present two sporadic cases of this syndrome. We did not identify other family members affected with this disease. Some findings cited in the original description such as hyperinsulinemia and acanthosis nigricans are consistent with severe insulin resistance, also associated with ovarian dysfunction and hirsutism or virilism in one of the familial cases and present in our two patients.

Insulin biological activity was normal in both patients when tested directly using adipocytes of rodents, thus ruling out insulin resistance due to an altered insulin molecule. Clinically, acanthosis nigricans is an indication of biologically active endogenous insulin with a significant correlation with the degree of hyperinsulinemia.[21,22]

Fat cell size was surprising in our patients. We expected that cells in the atrophic area would be smaller and those in

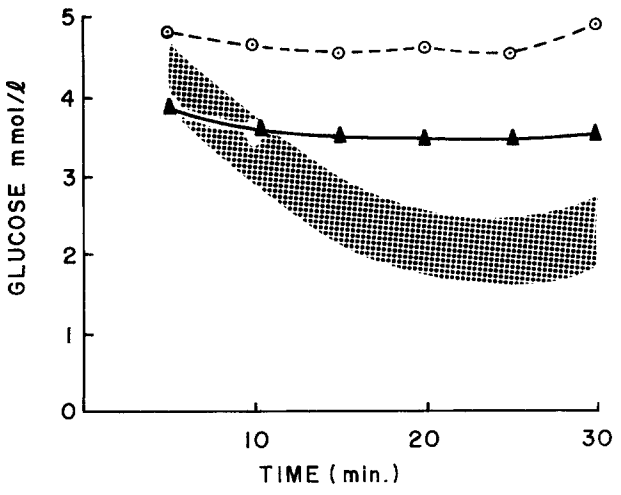

Fig 3. Plasma glucose levels in subjects no. 1 (▲) and 2 (○) during an ITT (0.025 U/kg body weight). Shaded area represents the range of normal values from this laboratory.

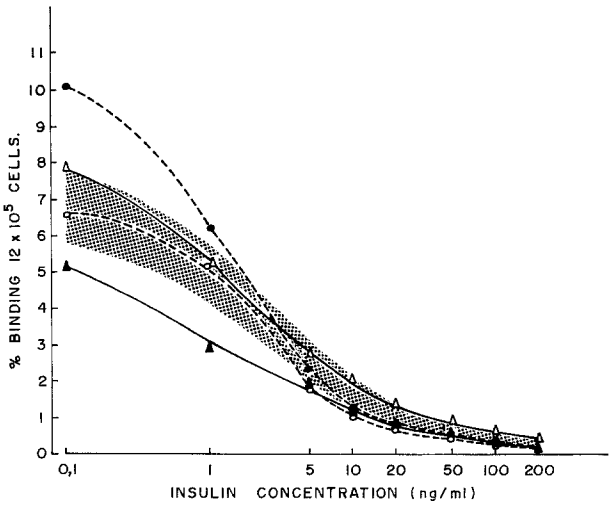

Fig 4. Specific ^{125}I-insulin binding to adipocytes from patients no. 1 (neck, △; lower abdomen, ▲) and 2 (neck, ○; lower abdomen, ●). Shaded area represents the range of normal values from this laboratory.

Table 1. Adipose Cell Diameter, Insulin Binding, Insulin Receptor Sites per Cell, Ke, and Kf

	Adipose Cell Diameter (μm)		Insulin Binding (%)		Receptor Sites per Cell		Ke (nmol/L)		Kf (nmol/L)	
	N	LA	N	LA	N	LA	N	LA	N	LA
Case 1	82	155	7.9	5.1	478,158	130,075	1.35×10^8	1.45×10^8	0.135×10^8	0.135×10^8
Case 2	85	140	6.6	10.2	163,593	245,390	0.59×10^8	1.43×10^8	0.134×10^8	0.134×10^8
Control	75		5.7		322,118		0.58×10^8		0.139×10^8	
Range	40-110		4.0-8.1		217,080-410,040		$0.45\text{-}0.75 \times 10^8$		$0.135\text{-}0.141 \times 10^8$	

Abbreviations: N, neck; LA, lower abdomen.

hypertrophic areas larger. However, in our patients, adipose cell size measured in a calibrated microscope in isolated cells was greater in the atrophic low-abdomen region. The enlarged adipose cells were fragile, and during the preparation we observed crushing of cells from the neck. Thus, small cells remained for the study. In a study of a patient with partial cephalothoracic lipodystrophy by Bernstein et al,[25] adipose cells surgically removed from the atrophic area were smaller than those from the hypertrophic area, but atrophic cell volume was 65% of that of hypertrophic cells, suggesting loss of fat cells.

Binding at tracer insulin concentration in lower-abdomen adipose cells was enhanced in patient no. 2 and reduced in patient no. 1. These results conflict with those observed by Rosenbloom et al,[6] Copeland et al,[26] and Howard et al,[7] who found normal insulin binding of cultured fibroblasts from patients with congenital lipoatrophic diabetes. Dörfler et al[5] found a 50% lower insulin binding to cultured fibroblasts in acquired lipoatrophic diabetes. Wachslicht-Rodbard et al,[4] evaluating insulin-receptor interactions in circulating cells (red blood cells [RBCs] and monocytes) from 11 patients with lipoatrophic diabetes with complete or partial lack of adipose tissue (three of them with face and neck fat spared) and severe insulin resistance, observed heterogeneity of insulin binding to monocytes (three patients had decreased binding due to decreased receptor number, two had normal tracer binding with decreased receptor affinity, and four had normal or increased insulin binding). Insulin binding to RBCs demonstrated similar heterogeneity, with a good correlation between RBC and monocyte data. Their data suggest that insulin resistance in lipoatrophic diabetes is heterogenous and may involve both receptor and postreceptor abnormalities. Robbins et al[27] described decreased insulin receptors on erythrocytes in patients with partial lipodystrophy who had increased fat in subcutaneous depots on the face, neck, and torso. The cells used for all of the previous studies of the insulin receptor in lipoatrophic diabetes are not the classic target tissues for insulin. A novel feature of our study is the examination of adipocytes from different regions of the body.

Binding of insulin to neck adipocytes at tracer insulin concentrations was normal in our patients. However, insulin action was impaired, since glucose transport and oxidation were diminished, indicating a postreceptor defect. In abdominal adipocytes, despite normal insulin binding and glucose transport, a defect in glucose oxidation was observed.

In a similar study performed by Davidson and Young[28] in isolated adipocytes from subcutaneous fat removed from the left flank of a patient with probable Köbberling-Dunnigan syndrome type 1, glucose oxidation and incorporation into glycogen and lipids were greatly diminished in basal conditions and after incubation with an elevated concentration of insulin. The results were considered to be dependent on the small size of the cell, since the percent stimulation by insulin of glucose conversion to CO_2 and glycogen was comparable to that found in their controls.

The different insulin-binding pattern measured in vitro in the abdominal adipocytes, also observed by Wachslicht-Rodbard et al[4] in circulating cells, may reflect differences in the fundamental pathogenesis of the disease or suggest that the receptor abnormality could be a secondary manifestation to multiple factors independent of the basic pathogenesis of the disease.[3]

Plasma FFAs are high in total and partial lipodystrophy,[28,29] suggesting that the dystrophic adipocyte is capable of synthesizing and breaking down lipids. The increased FFA levels associated with an enhanced rate of FFA oxidation could impair insulin-stimulated glucose uptake in peripheral tissues.[30]

Thus, it could be speculated that the systemic insulin resistance could be secondary to an abnormality in adipose tissue via the enhanced lipolysis and increased plasma FFAs. However, the clamp studies in our subject no. 2 suggest that, at

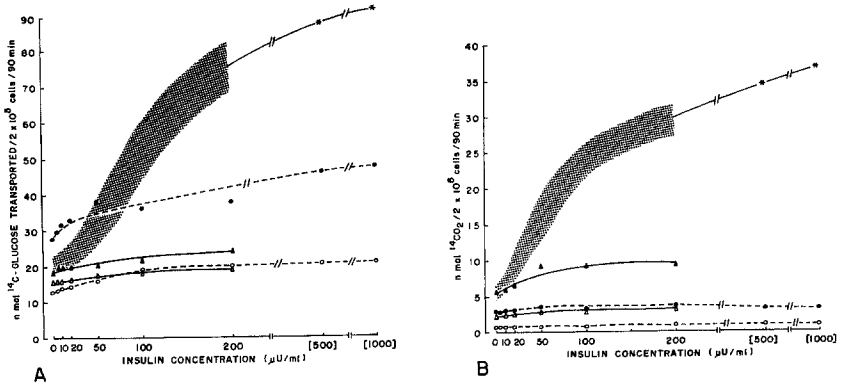

Fig 5. Curves for the effect of increasing doses of insulin on D-(U-^{14}C)glucose transport (A) and CO_2 production (B) by adipocytes of patient no. 1 (neck, △; lower abdomen, ▲) and 2 (neck, ○; lower abdomen, ●). Shaded area represents the range of normal values from this laboratory.

least in the syndrome in discussion, the increase in FFAs has no significant role in the insulin resistance of this syndrome.

We conclude that insulin resistance in type 2 lipodystrophy is not related to changes in insulin binding, but is probably a consequence of a postreceptor defect in adipocytes from the neck and abdomen. The postreceptor defect may be related to decreased glucose transport or to an altered enzymatic pathway distal to the cellular transport of glucose.

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