Studies on the Pathogenesis of Lipoatrophic Diabetes: A Case of Congenital Systemic Absence of Adipose Tissue Associated with Insulin-Resistant Diabetes Mellitus and Hepatosplenomegaly

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Summary. A case of coexisting lipoatrophy, hyperlipemia, insulin-resistant diabetes mellitus and hepatosplenomegaly (fatty liver and early cirrhosis, as shown by biopsy) is described. Investigations attempting to explain the pathogenesis of these disturbances are presented. From the urine of the patient both the insulin antagonizing (Louis' factor) and lipid mobilizing substance (Chalmers' factor) were extracted. Injection of these extracts obtained from the patient's urine induced 1) insulin resistance, 2) hyperlipemia and 3) fatty infiltration of the liver in mice. The pathogenetic hypothesis that humoral factors cause a constant increase in lipolysis and therefore prevent triglyceride storage in the adipocytes is discussed.

Etude sur la pathogénie du diabète lipoatrophique: un cas d'absence systémique congénitale de tissu adipeux associée à un diabète sucré insulino-résistant et à une hépatosplénomégalie

Résumé. Les auteurs décrivent un cas de lipoatrophie, d'hyperlipémie, de diabète sucré insulino-résistant et d'hépatosplénomégalie (foie gras et début de cirrhose hépatique, comme le montre la biopsie). Les investigations pour tenter d'expliquer la pathogénie de ces troubles sont rapportées. La substance antagonisant l'insuline (facteur Louis) ainsi que la substance mobilisant les lipides (facteur Chalmers) ont été extraites de l'urine de la patiente. En injectant ces extraits obtenus des urines de la patiente, on a provoqué expérimentalement chez la souris 1) une insulino-résistance, 2) une hyperlipémie et

3) une infiltration graisseuse du foie. L'hypothèse de facteurs humoraux provoquant l'augmentation constante de la lipolyse et par conséquent empêchant le stockage des triglycérides dans les adipocytes est discutée en tant que mécanisme pathogénique dans le diabète lipoatrophique.

Untersuchungen über die Pathogenese des lipoatrophischen Diabetes: ein Fall von kongenitalem, systematischem Schwund des Fettgewebes mit insulinresistentem Diabetes mellitus und Hepatosplenomegalie

Zusammenfassung. Es wird über einen Fall von Lipoatrophie, verbunden mit Hyperlipämie insulinresistentem Diabetes mellitus und Hepatosplenomegalie (biopsisch: Fettleber mit beginnender Cirrhose) berichtet. Untersuchungen zur Aufklärung der Pathogenese dieser Störungen werden vorgelegt. Aus dem Harn der Patientin wurden sowohl die Insulin-antagonistische (Louis Faktor) wie auch die lipids mobilisierende Substanz (Chalmers Faktor) extrahiert. Injektionen dieser Extrakte aus dem Harn der Patientin führten bei Mäusen 1) zu Insulinresistenz, 2) zu Hyperlipämie und 3) zur Fettinfiltration der Leber. Die pathogenetische Hypothese, wonach humorale Faktoren eine konstante Erhöhung der Lipolyse verursachen und damit die Ablagerung von Triglyceriden in den Adipozyten verhindern, wird diskutiert.

Key words: Hyperlipaemia, Lipid Mobilizing Substance, Lipoatrophic diabetes, Louis factor, Insulin resistance, Urinary polypeptides, chalmers faktor.

The purpose of this paper is to present a case of lipoatrophic diabetes mellitus and to discuss the results of its pathophysiological investigation.

Clinical data

In a female, J.M., born in 1953 (case record No. 405/12961/67), a progressive loss of adipose tissue has been observed since the age of 18 months. Initially it was restricted to the subcutaneous tissue of the calves, palms, cheeks and buttocks. By the age of 2 years the whole subcutaneous adipose tissue was already markedly atrophied. Polydipsia was observed from the age of 4 years. In 1967, when the patient was 14 years old, diabetes mellitus and hepatomegaly were recognized.

At this time treatment with 100 u. of insulin daily was started but this did not influence significantly the average daily glycaemia and glucosuria. No further increase in body weight was noticed. The patient's family history revealed that her maternal great-uncle suffered from maturity onset diabetes mellitus, but five siblings are healthy.

Clinical examination. Height — 152 cm, weight — 41 kg, BP — 130/80 mm Hg. Skin dry, atrophic and flaccid. Numerous xanthomata on both elbows and on the posterior, upper areas of the thighs. Subcutaneous adipose tissue markedly atrophied. The absence of adipose tissue set in relief the muscles and subcutaneous veins. In the pubic area the hair was scanty with a distribution of the female type. The liver was greatly

Fig. 1a

Fig. 1b

Fig. 1. a) The silhouette of the patient. b) The face with lipoatrophy. c) The outlines of the veins and muscles on the surface of the calves are distinctly visible; they are not covered by adipose tissue

enlarged (to the transverse umbilical line) and hard. The spleen protruded 3 cm from the costal margin.

Routine laboratory investigations are presented in Table 1.

Liver biopsy disclosed extensive vacuolar degeneration of cell nuclei, fatty degeneration of the cytoplasm and dilatation and fibrosis of the portal spaces, which suggested early cirrhosis. Peripheral blood vessels: no changes were observed by funduscopy. Capillaroscopic, oscillometric, photoplethysmographic, polarographic and reangiographic studies of the arterioles and capillaries revealed *functional abnormalities*. Electromyography; slight changes suggesting a discrete involvement of the peripheral motor neurons. This finding confirmed the clinical diagnosis of diabetic neuropathy. The histochemical investigations of the skeletal muscle biopsy specimens gave normal results. The muscle fibres were of normal size (up to 40 μ in diameter) without any evident structural changes and the arrangement of fibres in bundles was normal. The serum level of creatine phosphokinase was 0.05 u. [9].

Clinical metabolic investigations. The standard diet administered during the studies contained 400 g of carbohydrates, 80 g of protein and 60 g of fat (Table 2).

Carbohydrate metabolism. The 24-hour blood glucose (11) measurements during treatment with crystalline insulin — 48 u. in the morning, and 40 u. in the afternoon — Were: — 7 h — 232, 10 h — 236, 12 h — 280, 15 h — 300, 18 h — 240, 22 h — 296, 0 h — 264, 3 h — 256 mg%. The blood glucose after i.v. administration of 1.00 g of sodium tolbutamide was as follows: fasting — 304, after 10 min — 324, 20 min — 198, 30 min — 290, 45 min — 318, 60 min — 304 mg%.

Blood glucose and free fatty acid levels (20) after intravenous insulin administration were:

Time	Blood glucose in mg%	FFA in μ Eq/l
Fasting	206	700
30 min after 100 u. of insulin i.v.	234	760
60 min	180	760

After 60 min a repeated dose of 100 u. of insulin was administered i.v.

90 min	148	648
120 min	134	353

Blood glucose level, FFA and insulin-like serum activity (ILA) after oral administration of 100.0 g of glucose were as follows:

Time	Blood glucose in mg%	FFA in μ Eq/l	ILA μ u/ml	normal values
Fasting	330	1330	750	310 $\pm$ 140
30 min	380	1330	610	430 $\pm$ 130
60 min	346	1000		
90 min	426	1000		
120 min	336	660		
180 min	362	800	850	330 $\pm$ 160

ILA was determined in serum diluted 1:15 in our own modification [29, 30] of the method of Ball-Merrill [2].

Lipid metabolism. Blood lipids: total lipids [10, 10a] — 2000 mg%, phospholipids [7] — 248 mg%, cholesterol [22] — 340 mg%, triglycerides [10] — 1309 mg%. The serum lipolytic activity [19, 19a] after administration of heparin (25 mg) was 24 u. (normal value up to 25 u.). Electrophoretic separation of serum lipoproteins (26); alpha lipoproteins — 7.1% (normal values 27–33%); beta lipoproteins — 67.2% (normal values 50–60%), "tail" (chylomicrons) 25.7% (normal values 13–17%).

Protein metabolism. Serum: paper electrophoresis (21) — total proteins — 6.6 g%, albumins — 48.2%, globulins alpha₁ — 2.6% globulins alpha₂ — 7.0%, globulins beta — 17.6%, globulins gamma — 24.6%. Chromatographic analysis of the urine (28) revealed the presence of glycine, alanine, glutamine, leucine and histidine. These aminoacids were present in the urine in amounts considerably exceeding normal ones.

Therapy. Effects of different types of treatment are shown in Table 2.

Table 1. Results of routine laboratory examinations

<i>Blood</i>	
ESR	— 42/65 mm
Blood cell count	— normal
Bilirubin	— 1.9 mg%
Urea	— 16 mg%
Uric acid	— 1.5 mg%
Prothrombin index	— 85%
<i>Serum</i>	
Sodium	— 139.1 mEq/l
Potassium	— 4.1 mEq/l
Calcium	— 4.2 mEq/l
Phosphorus	— 2.1 mEq/l
Iron	— 74 γ %
<i>Enzymes</i>	
Aminotransferase-aspartic	— 54 u.
Aminotransferase-alanine	— 86 u.
Aldolase (10)	— 38.5 u (normal < 3.8 u)
Phosphatase alk.	— 5.6 Bodansky u.
Phosphatase acid	— 1.2 u.
<i>Urine</i>	
Daily volume	— 1.5–3.5 l
24 h glucosuria	— 100–250 g
Ketonuria	— $\bigcirc$
Albuminuria	— $\bigcirc$
Urobilinogen	— +
Addis test	— normal
GFR	— 90 ml/min
Tubular reabs.	— 97.6%
Cultures	— no bacteria
17-KS/24 h (3, 3a)	— 4.8 mg
17-OHCS/24 h (27)	— 3.4 mg
<i>Functional tests</i>	
BSP retention, 45 min	— 35%
BMR	— +35%
131 I thyroid uptake, 6 h	— 11%
131 I thyroid uptake, 24 h	— 18% (decreased)

Animal experiments

Isolation of the factor antagonistic to insulin from the urine according to the procedure of Louis et al. [14, 16, 18]. The urinary extract was bioassayed on intact mice. First the minimal dose of insulin causing hypoglycaemic shock in mice fasting over 12 h was determined. In the control group it ranged from 0.06 to 0.15 u. of insulin per animal. The test involved administration of twice the minimal dose of insulin causing hypoglycaemic shock to mice injected intraperitoneally, 24 h earlier, with 1 ml of the urinary extract suspension. The extract obtained from the urine was suspended in Krebs buffer (pH = 7.4). The proportions were as follows: extract obtained from 1 l of urine was suspended in 10 ml of buffer solvent. The injection of 1 ml of such a preparation prevented a decrease of glycaemia and hypoglycaemic symptoms after insulin administration in all the treated mice.

In contrast, in the control group of mice injected with 1 ml of 0.9% NaCl, hypoglycaemic symptoms after insulin injection occurred in all animals (Table 3).

Isolation of the lipid mobilizing substance (LMS). LMS was isolated from the patient's urine 1) after 48 h of total fast and 2) during feeding with a standard diet — according to the method described by Chalmers [6]. In each type of experiment the extracts obtained from 3 l of urine were suspended in 15 ml of Krebs buffer and used for biological tests on mice. The mice were injected with 0.9% saline 1); with the extracts from urine of 2) fasting and 3) fed healthy subjects; and from the patient's urine 4) after 48 h of fast and 5) during feeding with a standard diet.

despite the increase in the insulin dosage and while eating a high caloric diet could indicate a defect in the storage of the calories in the form of fat. The increase in the caloric value of the diet resulted in increasing glucosuria, in spite of augmentation of the insulin dosage. This disorder became clinically noticeable when the patient was 18 months old. As in other cases the lipoatrophy was not observed immediately after birth [25]. The patient's fasting blood ILA levels were slightly elevated. 30 min after an oral glucose load, however, the ILA value was lower than the fasting one,

Table 2. Observations during different types of treatment

Treatment ^a	24 h glycaemia mg%	24 h glucosuria g	Choles- terol mg%	FFA μEq/l	ketones in blood (21) mg%	Body weight kg
No insulin for 30 days	7 h — 240 10 h — 326 12 h — 286 15 h — 304 18 h — 260 22 h — 296 0 h — 380 3 h — 222	140—260	340	1330	5.1—8.1	41.0
Crystalline insulin twice daily. 200 u. — 10 days	7 h — 268 15 h — 176 22 h — 264	120—240	320	900	4.2	41.6
Crystalline insulin 240 u. twice daily + Dexamethasone 6 mg — 10 days	7 h — 325 15 h — 240 22 h — 228	110—250	335	1360	6.2	42.4
Crystalline insulin 240 u. twice daily thyroidea sicca 0.2 mg twice daily — 30 days	7 h — 242 15 h — 216 22 h — 232	80—180	276	1300	5.1	42.9
Tolbutamide 1.5 g daily — 20 days	7 h — 258 15 h — 302 22 h — 286	64—210	262	1280	4.8	41.2
Phenformin 100 mg daily — 20 days	7 h — 246 15 h — 262 22 h — 302	80—160	276	1120	5.8	42.0
Crystalline insulin 280 u. twice daily, diet: 500 g of CHO, 100 g of protein and 100 g of fat — 14 days	7 h — 256 15 h — 219 22 h — 272	160—390	320	1180	5.4	41.8

^a During all types of studies a standard diet 400 g of CHO, 80 g of protein, 60 g of fat, was fed.

The comparisons of the blood FFA levels and the total lipid contents in the liver of mice injected with 0.9% saline, control urinary extracts and the LMS extracts obtained from the patient's urine showed interesting differences in the activity of tested extracts. The results of these experiments are presented in Table 4.

Sections of liver from mice receiving LMS from the patient's urine were stained with Sudan III and showed more numerous lipid deposits compared with the controls.

Discussion

In this patient the relative unresponsiveness of the blood glucose to marked changes in the daily insulin dose was striking. Keeping a constant body weight

suggesting an abnormal response of the pancreatic islets.

The influence of such conditions as hyperfunction of thyroid and adrenal cortex was clinically excluded. Hamvi [8] observed normal secretion of growth hormone in a *patient* with lipoatrophic diabetes.

The results of the animal experiments with the urinary extracts from the patient's urine confirm the finding of Louis et al. [15], who in 1963, obtained the insulin antagonizing extract from the urine of 4 lipoatrophic diabetics, as well as from 1 patient with maturity onset, insulin resistant, diabetes mellitus. Injection of this extract in healthy volunteers and also in dogs, caused transitory decrease in glucose tolerance and relative resistance to insulin. The polypeptides found in urinary extracts as prepared by Louis did not show any chemical or physiological properties of

ACTH or of growth hormone [15]. In later studies Louis *et al.* [17] obtained insulin antagonizing polypeptides from the urine of the majority of diabetics with diabetic nephropathy and albuminuria. They failed to obtain such polypeptides from the urine of diabetics without albuminuria. In other studies it was suggested that the presence of Louis polypeptides in the human organism is related to pituitary function [15]. Louis *et al.* succeeded in isolating these specific polypeptides from bovine and also human pituitary glands

revealed by experiments in mice, suggests its pathogenic role and confirms Louis' hypothesis.

Lipoatrophic diabetes includes disturbances which are hard to explain by the biologic influences of the Louis' factor alone. Hence we attempted to isolate the fat mobilizing substance (LMS) of Chalmers [4, 5, 6, 31], though Aarseth [1] showed the absence of this factor in 1 case of lipoatrophic diabetes.

Chalmers elaborated his procedure primarily in order to obtain LMS from the urine of healthy fasting

Table 3. Activity of the urinary factor antagonistic to insulin bioassayed on mice

Preparation injected	Nr. of animal	Insulin dose in units per animal				
		0.15	0.18	0.22	0.26	0.30
1 ml of extract suspension	1—6	○	○	○	○	○
	7—12	○	○	○	○	○
	13—18	○	○	○	○	○
per 1 animal	19—24	○	○	○	+	+
Control: 1 ml of 0.9% saline per 1 animal	25—30	+	+	+	+	+
	31—36	+	+	+	+	+
	37—42	+	+	+	+	+
	43—48	×	+	×	+	+

○ — no hypoglycaemia

+

×

Table 4. Activity of the lipid mobilizing substance (LMS) estimated by changes in blood FFA levels and liver lipid contents in mice

Groups of animals and types of urinary extracts injected ^a	No. of animal	Blood FFA in $\mu\text{Eq/l}$		Liver lipid contents in % of liver weight	
		Injections			
		before	after	before	after
Control group of mice 0.9% saline	1—18	420 ± 18	416 ± 22	3.1 ± 0.10	3.3 ± 0.01
		$p > 0.05$		$p > 0.05$	
Control group of mice LMS — urinary extract, urine of healthy subjects after 48 h of fast ^b	19—36	398 ± 19	458 ± 20	3.0 ± 0.07	3.5 ± 0.13
		$p < 0.09$		$p < 0.08$	
Control group of mice LMS — urinary extract, urine of healthy fed subjects	37—54	410 ± 12	390 ± 18	2.9 ± 0.01	3.1 ± 0.09
		$p > 0.05$		$p > 0.05$	
Tested group of mice LMS — urinary extract of patient's urine after 48 h of fast	55—72	424 ± 23	890 ± 36	3.3 ± 0.17	5.0 ± 0.19
		$p < 0.001$		$p < 0.001$	
Tested group of mice LMS — urinary extract of patient's urine under standard diet ^b	73—90	430 ± 12	730 ± 28	3.5 ± 0.08	4.7 ± 0.14
		$p < 0.05$		$p < 0.05$	

^a injected i.p. with 0.2 ml 3 times and 0.5 ml twice, every other day, of saline or urinary extracts as described in the text.

^b $p < 0.01$

[15]. The latter resembled the polypeptides found in the urine of patients with lipoatrophic diabetes mellitus.

Using Louis' procedure [18] the insulin antagonizing extract was obtained from the urine of the case under discussion. Biological activity of this extract, as

subjects [45]. Later he extended it to investigate the presence of LMS in the urine of patients with various diseases [4]. Following Chalmers' procedure the urinary extract increasing the blood FFA levels and liver TG in mice was extracted from the urine of the case under study.

These biological effects indicate the strong lipid mobilizing properties of the extract. It is of interest that the biological activity of LMS from the patient's urine was strikingly high compared with extracts obtained from the urine of healthy subjects in fasting conditions and that this activity did not disappear after feeding, as it did in healthy subjects. The latter observation confirms the earlier findings of Chalmers concerning the possibility of isolating LMS from the urine of lipoatrophic diabetics.

In the case under study, 2 kinds of urinary extracts were obtained: one of them diminishing the responsiveness of mice to exogenous insulin (Louis' type of extract), the other increasing the FFA concentration in blood and TG liver content as bioassayed on mice (Chalmers' type of extract).

The possibility of inducing the essential components of the lipoatrophic diabetes 1) hyperlipemia, 2) fatty infiltration of the liver, 3) insulin-resistance — in normal mice injected with the polypeptide-containing extracts of the patient's urine suggests that the essential role in the pathogenesis of this syndrome may be attributed to humoral factors. The pathogenetic polypeptides are probably present in the blood and excreted in the urine. They are probably responsible for the marked stimulation of lipolysis within the adipose tissue of the human as well as of the experimental animals.

The biochemical mechanism of these changes, the source and the chemical characteristics of pathogenic substances are far from being clear. Most interesting was the finding of both the Louis' and Chalmers' factors in the investigated case. The separate appearance of these factors has already been described. Their common appearance has been found probably for the first time. It is not possible to determine which of them is of primary importance and what their mutual relationship is. It is possible that cases of lipoatrophic diabetes are pathogenetically heterogeneous.

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