Abnormalities in the Adrenergic Control and the Rate of Lipolysis in Isolated Human Subcutaneous Adipose Tissue in Diabetes Mellitus

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Summary. Subcutaneous adipose tissue was obtained from 9 patients with untreated diabetes mellitus and from 13 obese nondiabetics. After incubation with isoprenaline or noradrenaline, glycerol release and tissue cyclic AMP (cAMP) were determined. Basal glycerol release was twice as rapid from the diabetic adipose tissue. With isoprenaline, the cAMP concentration and the glycerol production was significantly higher in the diabetic adipose tissue. Noradrenaline did not increase glycerol production or cAMP concentration in the diabetic adipose tissue. Subcutaneous adipose tissue was also removed from the diabetics after antidiabetic treatment. Basal lipolysis was significantly reduced and noradrenaline significantly increased both glycerol release and cAMP production. With isoprenaline, cAMP production and glycerol release were significantly less after antidiabetic treatment than in the untreated state. The data provide evidence for increased α - as well as β -adrenergic receptor sensitivity in human subcutaneous adipose tissue of untreated diabetic patients.

Key words: Adipose tissue, diabetes mellitus, isoprenaline, noradrenaline, glycerol release, cyclic AMP.

The accelerated lipid mobilisation from the fat depots in human diabetes mellitus is well established by measurements of free fatty acids (FFA) and glycerol in plasma (see 13 for references) as well as by studies on subcutaneous adipose tissue in vitro [7, 8, 12, 18, 32]. Insulin deficiency and/or increased action by adipokinetic hormone are generally considered to be the pathophysiological background. In human adipose tissue the catecholamines noradrenaline (NA)

and adrenaline are unique, with respect to their prominent lipolytic actions, in comparison with a number of other hormones [9] and to their ability to produce both β -adrenergic (stimulatory) and α -adrenergic effects (inhibitory) on lipolysis [10, 33]. To study the apparent changes in the insulin-catecholamine interrelationship which occur in the lipid metabolism of human adipose tissue in diabetes mellitus the following experiments were performed. Subcutaneous adipose tissue was obtained from 1) patients with newly diagnosed diabetes mellitus before and after antidiabetic treatment and 2) from a group of non-diabetic control patients admitted to the hospital because of obesity. Adipose tissue was incubated in the absence or in the presence of either isoprenaline (ISNA) or NA. The release of glycerol to the incubation medium and the maximal tissue levels of cyclic AMP (cAMP) were determined. The diabetic patients constituted a heterogeneous group as regards the type of disease, kind of antidiabetic treatment and body-weight. In other words, this study was undertaken to get a general idea of the adrenergic control of lipolysis in diabetes mellitus in vitro.

Material and Methods

Patients

The nine newly diagnosed diabetic patients included 2 with juvenile-onset and 7 with maturity-onset type of diabetes. The duration of symptoms was from 3 to 6 months. All were initially studied as inpatients. Relevant clinical data are presented in Table 1. Three patients (Nos. 5, 6, 7) had non-acidotic ketosis and 4 (Nos. 3, 4, 6, 9) had high levels of serum triglycerides and one (No. 1) had increased level of serum chole-

Table 1. Clinical data of the patients with diabetes mellitus^a

Pat	Age	Sex	Body-weight % of average		Mean fat cell volume mm ³ × 10 ⁻⁶		Fasting serum- triglyceride mmol/l		Fasting serum- cholesterol mmol/l	
			Biopsy I	Biopsy II	Biopsy I	Biopsy II	Biopsy I	Biopsy II	Biopsy I	Biopsy II
1	65	F	124	111	779	1103	2.4	2.0	13.9	5.3
2	45	M	157	169	896	1006	1.4	2.0	6.7	7.1
3	40	M	123	121	591	444	4.1	2.3	6.0	5.8
4	42	M	146	136	395	262	4.6	2.3	7.3	6.7
5	60	M	113	94	592	779	1.0	0.8	4.3	4.3
6	34	M	80	92	587	782	3.8	2.6	8.1	5.4
7	23	M	96	91	369	693	2.2	1.0	5.1	4.5
8	30	M	86	83	428	349	2.8	2.0	4.3	4.3
9	47	M	111	113	905	703	4.1	0.6	8.0	7.5
Mean 43			115	113	616	680	2.9	1.7	7.0	5.7
±SEM ±4			±9	±9	±68	±94	±0.4	±0.02	±1.0	±0.4
P			NS		NS		< 0.02		NS	

Fasting blood- glucose mmol/l		Mean blood- glucose nmol/l		Urine glucose nmol/24 h		Ketonuria (Ketostix®)		Treatment
Biopsy I	Biopsy II	Biopsy I	Biopsy II	Biopsy I	Biopsy II	Biopsy I	Biopsy II	
9.4	5.4	9.9	5.6	38	0	0	0	Chlorpropamide 250 mg/day
15.6	5.9	16.2	6.3	720	0	0	0	Chlorpropamide 375 mg/day
9.8	4.4	10.4	5.4	350	0	0	0	Chlorpropamide 500 mg/day
13.4	5.1	13.8	5.4	271	0	0	0	Chlorpropamide 250 mg/day
10.9	5.2	10.9	6.1	633	0	++	0	Glipizide 15 mg/day
13.5	4.8	19.7	6.4	1084	31	++	0	Medium-acting insulin 40 I U/day
14.8	8.2	21.4	9.2	866	49	+++	0	Medium-acting insulin 28 I U/day
15.6	5.0	13.5	7.0	148	0	0	0	Medium-acting insulin 24 I U/day
9.5	4.7	10.7	5.4	69	0	0	0	Chlorpropamide 375 mg/day
12.5	5.4	14.1	6.3	464	8			
±0.9	±0.4	±1.4	±0.4	±125	±6			
< 0.001		< 0.001		< 0.005				

^a Biopsy I is before and II after treatment. Fasting blood-glucose, serum-triglyceride and serum-cholesterol was determined at 7 a. m. on the day of biopsy. The mean blood-glucose level was calculated from three to four determinations during 24 h preceding the biopsy. The production of urine-glucose was determined on urine collected during 12 h before and after biopsy. Body weight in percentages of average was calculated from tables computed by Documenta Geigy [15]. P is probability of differences of biopsy I and II (paired t-test). NS = not significant

Table 2. Clinical data of 13-non-diabetic, obese control patients^a

	Age	Sex	Body-weight % of average	Mean fat cell volume mm ³ × 10 ⁻⁶	Serum- triglyceride mmol/l	Serum- cholesterol mmol/l	Glucose tolerance k-value %/min
Mean	34	10F/3M	181	861	2.8	5.8	1.36
±SEM	±3		±11	±55	±0.5	±0.2	±0.11
P vs Diabetic group	NS		< 0.001	< 0.02	NS	NS	

^a Cholesterol and triglycerides were determined on fasting venous blood-samples by the hospital's routine procedure. The k-value was determined according to Ikkos & Luft (23). Body-weight in percentage of average was calculated from tables computed by Documenta Geigy (15). P denotes the significance of differences between the means of the control patients and the untreated diabetic patients in Table 1. NS = not significant

terol. They were all on unrestricted hospital diet and did not receive any antidiabetic treatment before the first study. A fat biopsy specimen was taken at 8 a.m. after an overnight fast.

A second study was undertaken when the diabetes was considered to be well controlled for at least two months on the basis of stable body-weight, normal or slightly elevated fasting blood-glucose level and essential freedom from glucosuria. Three patients (Nos. 6, 7, 8) were treated with insulin and the others with sulphonylurea drugs. On average, there was a small and significant ($p < 0.02$) decrease of the level of serum triglycerides but serum cholesterol and the fat cell size were not significantly changed while the patients were on antidiabetic therapy (Table 1). Then the patients were again admitted to the hospital. At 8 a. m., and before they had received antidiabetic therapy, a second biopsy was performed after an overnight fast. All but two of the diabetic patients were otherwise in good health. Patient No. 1 had mild hypertension, which was treated with 50 mg of chlorthalidone per day. Patient No. 6 had an acute myocardial infarction between the two studies. He was treated with 400 mg of phenytoin per day because of ventricular ectopic beats.

The control group comprised 13 adult patients admitted to the hospital because of obesity. They showed no evidence of hepatic, renal or endocrine disorder and had a normal glucose tolerance [23]. One of them had mild hypertension and epilepsy and was treated with 50 mg of chlorthalidone and 400 mg of phenytoin per day. All were on unrestricted diet before the study. None was subjected to attempts at weight reduction in the 3 months prior to the study. Clinical data of the control patients are presented in Table 2. On average their age and serum cholesterol and serum triglyceride levels did not differ from those of the diabetic patients. However, 4 control patients had elevated serum levels of triglycerides and cholesterol, corresponding to Type IV hyperlipoproteinaemia according to the classification of Fredrickson et al. [17]. The mean fat cell volume was significantly larger ($p < 0.02$) in adipose tissue of the controls ($861 \text{ mm}^3 \times 10^{-6}$) than in that of the diabetics ($616 \text{ mm}^3 \times 10^{-6}$). The body-weight was almost 60% higher in the control patients than in the diabetics when expressed as a percentage of the average and the difference was significant ($p < 0.001$). After one night of fasting a fat biopsy specimen was taken at 8 a.m. on the following morning.

All patients were weighed on standard scales accurate to within 0.1 kg. The weight as a percentage of the average weight of a normal population was calculated from the tables computed by Documenta Geigy [15]. The biopsy procedure has been approved by the Ethi-

cal Committee of the Karolinska Institute. Details of the study were explained to each patient and informed consent was obtained.

Methods

Subcutaneous adipose tissue was excised from the upper lateral part of the thigh, which was anaesthetised with prilocaine chloride as described in detail before [3]. Adipose tissue was divided into portions of approximately 50 mg each, preincubated for 30 min in Krebs-Henseleit bicarbonate (KHB) buffer containing 40 mg/ml of dialysed bovine serum albumin (Armour Pharmaceutical Co. Eastbourne, England; Lot No. R 970) and then incubated in 1 ml of medium of the same type for 2 h. After incubation, two aliquots (0.1 ml) of the medium were removed for glycerol determination as described by Wieland [29] and modified by Chernick [14]. The pre-incubation and incubation procedure have been described in detail [31].

The intracellular cAMP concentration was determined in adipose tissue pre-incubated for 30 min in KHB-buffer, pH 7.4, containing 10 mmol/l of theophylline (ACO, Sweden) and then incubated for 10 min in fresh medium of the same type. Albumin was not included in these experiments, since it has been shown to interfere strongly with the assay for cAMP [4, 27]. Incubation and pre-incubation were carried out in glass vessels with 50 ml of medium per g of adipose tissue. It was observed from several experiments that the maximal tissue level of cAMP occurs after about 10 min of incubation [2] and that the omission of albumin from the buffer did not lead to accumulation of tissue FFA in amounts that could interfere with the production or degradation of cAMP in human adipose tissue incubated for 10 min under basal or stimulated conditions [1]. We have observed that cAMP could not usually be measured in human subcutaneous adipose tissue when tissue pieces weighing less than 100 mg wet weight were analysed without prior incubation [1]. In the present study, the amount of adipose tissue obtained from one biopsy allowed the use of tissue samples weighing only 50–75 mg of wet weight for a single incubation. Furthermore, we have recently observed [2] that the cAMP level significantly decreases during incubation in a basal medium. Therefore, the phosphodiesterase inhibitor theophylline was added to enable measurement of the maximal effect of catecholamines on the tissue level of cAMP as well as the basal tissue level of cAMP.

Agents added *in vitro* were noradrenaline bitartrate (Astra, Sweden) and isopropylnoradrenaline-HCl (Winthrop, England). Both agents were dis-

Fig. 1. Effect of isoprenaline (ISNA) on the metabolism of diabetic and non-diabetic adipose tissue: Subcutaneous adipose tissue was excised from 9 newly diagnosed diabetics before antidiabetic treatment (hatched bars) and from 13 obese non-diabetic patients (open bars). Adipose tissue was pre-incubated and incubated under basal conditions and in the presence of 6×10^{-6} mol/l of ISNA. Tissue cyclic AMP (cAMP) was determined at 10 min and glycerol release at 2 h of incubation. Values are Mean $\pm$ SEM and expressed per g of lipid weight. In the individual experiments four to five fat portions were used for each type of incubation. The statistical symbols are for unpaired comparison of the values for the diabetics with those of the non-diabetic patients (*** = $p < 0.005$, ** = $p < 0.02$, * = $p < 0.05$).

Fig. 2. This figure shows the same experiments as in Figure 1 but the values for cyclic AMP and glycerol release are expressed per 10^7 cells. The symbols are the same as in Figure 1 except that NS = not significant

solved in water and added to the incubation medium in portions of 0.1 ml, or directly dissolved in the incubation medium, to give a final concentration of 6×10^{-6} mol/l. This concentration is known to give a maximal effect on the rate of lipolysis [2, 16]. All incubations and pre-incubations were carried out at 37°C in a water-bath cycling at 40/min. Air was used as the gas phase.

Assay of Intracellular cAMP

cAMP was determined by a modification of the protein-binding method of Gilman [19], as described in detail elsewhere [4]. cAMP-binding protein and ^3H -adenosine-3', 5'-monophosphate, cyclic ammonium salt (spec. act. 15 mCi/mmol) were obtained from Boehringer-Mannheim's "kit assay" for cAMP (Cat. No. 16289). Protein-kinase inhibitor was purchased from Sigma (U. S. A.).

Determination of Cell Size

Two fat specimens, weighing about 30 mg each, were used for the determination of the cell diameter according to the procedure developed by Sjöström et al. [28]. 100 cells were measured and the mean cellular triglyceride content was calculated from the average diameter and the SD of the diameter, according to the formula developed by Hirsch and Gallian [22]. The number of fat cells incubated was calculated using the mean cellular triglyceride weight and the total triglyceride content of the fat portions.

Statistical Analysis

The values given comprise the mean and the standard error of the mean (SEM). Significance of the differences was calculated by Student's paired or unpaired t-test.

Results

The basal rate of glycerol release was about twice as rapid from adipose tissue of the untreated diabetics as from that of the control patients (Figs. 1 and 2), although the latter had a mean fat cell volume which was significantly larger ($p < 0.02$) than that of the diabetics (Table 2). The difference in the rate of lipolysis was significant when calculated per lipid weight ($p < 0.02$ in Fig. 1) as well as per cell number ($p < 0.05$ in Fig. 2). The basal tissue level of cAMP was also higher in adipose tissue of the diabetics, but only when calculated per lipid weight ($p < 0.005$ in Fig. 1).

The addition of 6×10^{-6} mol/l of ISNA increased significantly the rate of glycerol release ($p < 0.01$) as well as the cAMP level ($p < 0.005$) in adipose tissue of both patient groups (Figs. 1 and 2). When expressed per lipid weight (Fig. 1), the total release of glycerol in

Table 3. Tissue levels of cyclic AMP (cAMP) and glycerol release by subcutaneous adipose tissue in vitro, obtained from 6 patients with diabetes mellitus before and after treatment^a

Type of experiment	Tissue cAMP, pmol/10 ⁷ cells					Glycerol release, µmol/10 ⁷ cells/2 hours				
	Basal	ISNA	P	NA	P	Basal	ISNA	P	NA	P
Before treatment	1186 ±188	14499 ±3297	<0.005	1305 ±221	NS	11.9 ±2.5	28.0 ±5.0	<0.001	12.1 ±2.8	NS
After treatment	1053 ±200	7502 ±2102	<0.02	1683 ±276	<0.01	3.7 ±0.5	13.0 ±1.3	<0.001	7.5 ±0.9	<0.005
P ₁	NS	<0.05		NS		<0.02	<0.02		NS	

^a Adipose tissue was incubated in the absence or in the presence of 6×10^{-6} mol/l of isoprenaline (ISNA) or noradrenaline (NA). Tissue cAMP was determined at 10 min and the glycerol after 2 h of incubation. P is probability of differences of basal vs ISNA or NA, before or after treatment and before vs after treatment. NS = not significant. n = 3 to 5 in the individual experiments. Values are mean ± SEM

the presence of ISNA ($p < 0.02$) as well as the ISNA-induced glycerol release over basal ($p < 0.05$) was significantly higher in the diabetic patients. When expressed per lipid weight (Fig. 1) or per cell number (Fig. 2), the tissue concentration of cAMP in the presence of ISNA ($p < 0.02$ in Figs. 1 and 2) as well as the ISNA-induced increase of tissue cAMP over the basal level ($p < 0.02$ in Fig. 1 and $p < 0.05$ in Fig. 2) was significantly higher in the diabetic patients.

NA, 6×10^{-6} mol/l, did not increase significantly either the tissue level of cAMP or the rate of lipolysis in the untreated diabetics (Fig. 3). In the control group, however, NA increased significantly the adipose tissue concentration of cAMP by 60% ($p < 0.001$) and the glycerol release by 50% ($p < 0.001$), as shown in Figure 3. In uncharted experiments, 6×10^{-5} mol/l of NA was also without effect on the rate of lipolysis of adipose tissue of untreated diabetics.

Table 3 shows the effect of antidiabetic therapy on cAMP and glycerol release from subcutaneous adipose tissue obtained from the diabetic patients. Whereas the basal rate of glycerol release decreased significantly ($p < 0.02$), there was no change in the basal tissue level of cAMP. After antidiabetic therapy the response to NA was normalised. Thus, a significant ($p < 0.005$) increment in the rate of glycerol release of about 100% was observed and the tissue level of cAMP increased significantly ($p < 0.01$) by about 60%. In the presence of ISNA both the rate of glycerol release and the tissue level of cAMP were 50% lower after treatment. These findings were also significant ($p < 0.02$ for glycerol and $p < 0.05$ for cAMP).

Discussion

Basal Lipolysis

The basal rate of lipolysis was about twice as rapid in adipose tissue of the untreated diabetics as in the

Fig. 3. Effect of noradrenaline (NA) on the metabolism of diabetic and non-diabetic adipose tissue: Subcutaneous adipose tissue was obtained from 9 newly diagnosed diabetics before receiving antidiabetic treatment and from 10 obese, non-diabetic control patients. Adipose tissue was pre-incubated and incubated under basal conditions (open bars) and in the presence of 6×10^{-6} mol/l of NA (hatched bars). Tissue cyclic AMP (cAMP) and glycerol release were determined at 10 min and 2 h, respectively, of incubation. Four to five fat portions were used for each type of incubation in the individual experiments. Values are Mean ± SEM and expressed per 10^7 cells. The statistical symbols are for paired comparison of basal with NA-induced values (* = $p < 0.001$, NS = not significant)

control group (Figs. 1 and 2), which confirms earlier observations made on juvenile [7, 12, 32], maturity-onset diabetics [8] and obese diabetics [18]. Differences in the basal concentration of tissue cAMP are not a likely explanation of the findings relating to basal lipolysis. No differences existed between the diabetic and non-diabetics groups when cAMP was expressed on a cellular basis (Fig. 2). Furthermore, when the diabetic patients were on antidiabetic therapy, the rate of basal lipolysis was significantly reduced by 70%, ($p < 0.02$), while the basal tissue

level of cAMP was unchanged (Table 3). It is thus possible that, in untreated diabetics, basal lipolysis is increased because of a direct effect on the level of protein kinase or triglyceride lipase. However, stromal cAMP might have influenced the measured basal tissue level of the nucleotide.

Catecholamine-Induced Lipolysis

The mean lipolysis induced by ISNA was of similar rate in adipose tissue of untreated diabetics and control patients when expressed per cell number (Fig. 2), but increased in diabetics when expressed per lipid weight (Fig. 1). However, the ISNA-induced rate of lipolysis per number of cells was significantly ($p < 0.02$) reduced by one-half in adipose tissue of the diabetics when they were on antidiabetic treatment (Table 3). Irrespective of the reference system, the β -adrenergic production of cAMP induced by ISNA was increased in adipose tissue of untreated diabetics (Figs. 1 and 2), results which could partly be due to inhibited phosphodiesterase activity, which is known to be decreased in adipose tissue of diabetics [24]. If decreased activity of phosphodiesterase is the only explanation, changes in the ISNA-induced cAMP-level would be followed by concomitant changes in the basal level of cAMP. However, the ISNA-induced tissue level of cAMP was significantly ($p < 0.05$) reduced by one-half in adipose tissue of diabetic patients on antidiabetic therapy when compared with the untreated state, while the basal level of cAMP was unchanged (Table 3). Thus, the findings with ISNA and cAMP seem to indicate increased β -adrenergic receptor sensitivity as well as decreased phosphodiesterase activity in subcutaneous adipose of untreated diabetics.

Addition of NA failed to increase lipolysis in adipose tissue of untreated diabetics in contrast to the effect of NA on adipose tissue of control patients (Fig. 3). Earlier studies in man have also shown blunting of NA-stimulated lipolysis in adipose tissue of juvenile diabetics [20] and diminished lipolytic response to NA plus phentolamine in adipose tissue of obese maturity-onset diabetics [6]. In the present study, the ability of NA to increase the production of cAMP was abolished in adipose tissue of untreated diabetics. The lipolytic response to NA and also the NA-induced production of cAMP were normalised after antidiabetic therapy. For reasons discussed above the findings with NA are not explained by the action of phosphodiesterase. Instead, they indicate increased α -adrenergic receptor sensitivity in subcutaneous adipose tissue of untreated diabetics. Increased lipolytic activity of adrenaline in diabetic rat adipose tissue has recently been observed [30]. The divergent results could be explained by

the fact that rat adipocytes seem to lack α -receptors [11].

We have observed a strong relationship between glycerol release and tissue cAMP concentration in the presence of ISNA [2] and that the NA-induced peak tissue level of cAMP is correlated with the cell volume of human adipocytes [1]. Therefore, the cAMP data with ISNA and NA were hardly influenced in a significant way by the stroma of human adipose tissue. Since NA and ISNA had divergent effects on glycerol release, the impaired catecholamine induced lipolysis from diabetic human adipose tissue is not explained by alterations in the level of protein kinase or triglyceride lipase.

General Discussion

Increased basal lipolysis [21, 25, 26] and increased α -adrenergic responsiveness [25] can be demonstrated in subcutaneous adipose tissue obtained from obese patients after starvation. Both phenomena are normalised by refeeding [5]. Increased β -adrenergic receptor sensitivity in subcutaneous adipose tissue is also observed after starvation [5]. Thus, it is apparent that the metabolism of human adipose tissue is impaired in a very similar way in starvation and in uncontrolled diabetes. It is therefore possible that the findings in untreated diabetes mellitus are secondary to the catabolic state of the disease. However, the patients in this study were unselected and it may be that some of the pathological findings are primarily associated with the diabetic disease.

Acknowledgements. We thank Mrs. Agneta Börjeson, Raija Jönsson, Else-Britt Lundström and Eva Nilsson for their excellent technical assistance. The investigation was supported by grants from the Swedish Medical Research Council (B76-19X-01034-10A), Nordic Insulin Fund, Swedish Diabetic Association and Swedish Association for Medical Research.

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Received: February 18, 1976, and in revised form: August 3, 1976

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