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Enzyme Activities in Quadriceps Femoris Muscle of Obese Diabetic Male Patients

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Summary. In biopsy samples of the lateral part of the quadriceps femoris muscle of 6 obese diabetic male patients and of 11 obese males with a normal glucose tolerance, the activities of 7 enzymes of energy metabolism were estimated: hexokinase, cytoplasmic glycerol-3-phosphate: NAD dehydrogenase, triosephosphate dehydrogenase, lactate dehydrogenase, citrate synthase, malate dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase. The obese diabetic male patients exhibited decreased activities of enzymes of carbohydrate breakdown and cytoplasmic NAD regeneration. Enzymes connected functionally with aerobic metabolism were less affected. The unchanged activity of 3-hydroxyacyl-CoA dehydrogenase points to an increased role of fatty acid catabolism in the muscle.

Key words: Human obesity, carbohydrate tolerance, skeletal muscle, enzyme patterns, muscle enzymes, energy metabolism.

Previous studies [15] concerning the enzyme activity patterns of energy metabolism of skeletal muscle in obese males with a normal blood glucose curve disclosed changes in some enzyme activities suggestive of a reduced capacity of glucose utilization. The extent of these changes was correlated with increasing weight and other indicators of body composition. This observation stimulated the present study, where we tried to find out whether changes of enzyme activity become more intense or acquire a different character in conditions with a manifest disorder of glucose tolerance.

Patients and Methods

The investigation was performed on 17 obese males; their data are summarized in Table 1. The first control group consisted of 11 obese males with normal carbohydrate tolerance, the other group of 6 obese male patients with markedly decreased carbohydrate tolerance. As a criterion of carbohydrate tolerance we used the sum of basal blood glucose values and three stimulated blood glucose values i.e. 30, 60 and 120 min after 50 g oral glucose load. According to Danowski et al. [5] the sum of these four values (G sum) up to 500 mg/100 ml corresponds to normal carbohydrate tolerance and that exceeding 801 mg/100 ml, indicates a marked decrease of carbohydrate tolerance or manifest diabetes mellitus. The sum of immunoreactive insulin values (IRI sum) was calculated in the same manner as G sum. None of the obese patients had been treated by medication nor diet. A thorough medical examination was per-

Table 1. Anthropometric and metabolic characteristics of the non-diabetic and diabetic obese male patients

	Non-diabetics	P	Diabetics
n	11		6
Age years	30 ± 3	n.s.	39 ± 5
Body weight (kg)	120 ± 4.5	n.s.	106 ± 11.0
Body fat (%)	35 ± 1.4	n.s.	34 ± 1.5
G sum (mg/100 ml)	432 ±12.0	<0.001	996 ± 63.0
IRI sum (μU/ml)	262 ±21.0	<0.001	137 ± 15.0
NEFA (mmol/l)	840 ±54.0	<0.05	1134 ±117.0

Results are expressed as means ± SEM. Significance was assessed with Student's t-test.
G sum – sum of blood glucose values 0, 30, 60 and 120 min after administration of 50 g glucose by mouth
IRI sum – sum of plasma values of immunoreactive insulin 0, 30, 60 and 120 min after administration of 50 g glucose by mouth

Table 2. Enzyme activities (U/g wet weight) and significant enzyme activity ratios of the quadriceps femoris muscle of the non-diabetic and diabetic obese male patients

n	Non-diabetics 11	P	Diabetics 6	% Difference
TPDH	242 ±10	<0.001	114 ±25	−53
LDH	196 ±19	<0.01	104 ±22	−53
GPDH	13.5± 1.3	<0.02	7.5± 1.6	−44
HK	2.2± 0.12	<0.001	1.4± 0.10	−34
MDH	147 ±21	<0.01	66 ±14	−55
CS	8.0± 0.3	<0.02	5.6± 0.8	−30
HOADH	5.1± 1.1	n.s.	5.8± 1.7	+13
TPDH/HOADH	62 ± 9	<0.01	25 ± 7	
LDH/HOADH	52 ±10	<0.05	26 ± 7	
GPDH/HOADH	3.6± 0.6	<0.05	1.7± 0.5	
HK/HOADH	0.6± 0.1	<0.05	0.3± 0.05	
MDH/HOADH	38.0± 8.6	<0.05	15.7± 4.8	

Results are expressed as means ± SEM. Significance was assessed with Student's t-test.
TPDH – triosephosphate dehydrogenase; LDH – lactate dehydrogenase; GPDH – glycerol-3-phosphate dehydrogenase; HK – hexokinase; MDH – malate dehydrogenase; CS – citrate synthase; HOADH – hydroxyacyl-CoA-dehydrogenase

formed during the first week after their admission on a standard diet consisting of 110 g protein, 400 g carbohydrates and 85 g fat (2600 kcal/day). During this week the muscle biopsy samples were obtained, usually on the 4th day. All patients were informed in detail about all planned procedures and their respective aim, and agreed to participate in the trial. Enzyme activities were assessed in samples of muscle tissue (15–30 mg) obtained with a biopsy needle [14] from the lateral part of the quadriceps femoris muscle. The samples were cleaned, when necessary, from adhering fat. Parallel controls inspected under a light microscope showed no apparent contamination with adipose tissue. The samples were weighed immediately and transferred into small glass-teflon homogenisers containing 0.2 ml of phosphate buffer solution (50 mmol/l K-Na phosphate, 5 mmol/l EDTA, 0.1% Triton X 100, pH 7.25). The volume was made up to 20 times (v/w) that of the sample, homogenized in a glass-teflon homogenizer and centrifuged at 15,000 g for 10 min at 0–4°C in a cooled centrifuge. Homogenization of the sediment and centrifugation was then repeated in the same amount of buffer; the supernatants were pooled and used for estimation of enzyme activities.

The activities of the following enzymes were estimated: hexokinase (HK) (EC 2.7.1.1), cytoplasmic glycerol-3-phosphate:NAD dehydrogenase (GPDH) (EC 1.1.1.8), triose phosphate dehydrogenase (TPDH) (EC 1.2.1.12), lactate dehydrogenase (LDH) (EC 1.1.1.27), citrate synthase (CS) (EC 4.1.3.7), malate dehydrogenase (MDH) (EC 1.1.1.37) and hydroxyacyl-CoA-dehydrogenase (HOADH) (EC 1.1.1.35). Enzyme activities were estimated according to the methods of Bücher et al. [3], Stern et al. [13] and Wakil [16], using an Eppendorf recording photometer at 25°C. Enzyme activities were expressed as international units per gram wet weight of tissue specimen. Dimensionless enzyme activity ratios were used for comparative purposes only. By showing the differences or changes of activity of an enzyme against the background of that of another enzyme, they allow conclusions about the prevalent change of one or another enzyme or enzyme group and thus demonstrate changes of enzyme activity patterns. At the same time they are independent of the reference unit as, e.g., g wet or dry weight, g protein etc.

The procedures of muscle sampling and estimation of enzyme activities have been reported in detail previously [1]. Muscle biopsy, assessment of body composition [8], of the blood glucose level [9], immunoreactive insulin in blood [7] and NEFA [6] in blood were performed under standard conditions after a 12-hour overnight fast. Student's t-test was used for statistical evaluation of differences. The results are given as mean ± SEM.

Results

The mean activities of the enzymes estimated and significant enzyme activity ratios are presented in Table 2. The activities of the 7 enzymes of the main pathways of energy-supplying metabolism were expressed as international units per g wet weight of muscle, because no difference in protein content was found. The protein content of the muscle of the diabetic obese patients was in 3 cases 154 ± 11 mg protein/per g wet weight and in 6 cases of the non-diabetic obese patients 151 ± 15 mg protein per g wet weight. Six of the seven enzymes measured showed significantly lower activities in the obese diabetic group as compared with the group of non-diabetic obese patients. The activity of MDH was lower by 55 per cent, of TPDH and LDH by 53 per cent, of GPDH by 44 per cent, of HK by 34 per cent and CS by 30 per cent each. No significant difference was found of the activity of HOADH.

The obese diabetic patients also showed significant differences in enzyme activity ratios of TPDH, LDH, GPDH, HK and MDH to HOADH, with a relative predominance of HOADH.

Discussion

The results of this study showed that obese diabetic patients displayed significantly lower activities of all

enzymes estimated except of HOADH. It seems that the decline of enzyme activities was not uniform, it was particularly marked in enzymes taking part in carbohydrate breakdown (TPDH, LDH) and cytoplasmic NAD regeneration from $NADH_2$ (GPDH, MDH, LDH). It seemed to be less marked in the cases of HK and CS activities, i.e. enzymes connected with aerobic metabolism (HK activity in muscle seemed to be tied with that of CS – see 2, 12).

A relatively high activity of citrate synthase in the diabetic group deserves attention in connection with the findings of elevated citrate levels in the tissues of diabetic animals [4].

The unchanged activity of HOADH suggests an increased role of fatty acid catabolism in obese diabetics. This is further borne out by enzyme activity ratios showing a relative prevalence of HOADH.

Similar results were reported by Standl et al. [11], using a different method, in insulin-dependent diabetics. To interpret the altered profile of the enzyme patterns of energy metabolism of obese diabetics, the different supply of energy substrates should also be considered. In this respect the significantly elevated blood NEFA levels in this group are of interest. A high supply of free fatty acids can modify the energy metabolism of skeletal muscle, inducing preferential fatty acid utilization followed by adaptive changes in enzyme activities [10].

Thus, we can conclude that the obese diabetic male patients exhibited decreased enzyme activities of carbohydrate breakdown and cytoplasmic NAD regeneration. Those of aerobic metabolism seem to be less affected. The unchanged activity of HOADH points to an increased role of fatty acid catabolism.

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