Sugar Consumption in Vitro of the Muscle Tissue of Diabetic Patients

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SUMMARY

The sugar consumption of healthy pieces of rectus muscle incubated in normal plasma and in an insulin medium is almost identical with the sugar uptake of rat diaphragm.

The sugar consumption of healthy pieces of human rectus muscle is identical in both normal plasma and in those of our insulin-resistant diabetic patients. In four out of our five insulin-resistant diabetics, the rectus muscle pieces consumed none or only a minimum quantity of glucose when incubated in either a healthy human plasma, plasma from the diabetic patient supplying the muscle sample, or in a medium containing a known quantity of insulin. DIABETES *14*:716-18, November 1965.

According to our experience, the number of diabetic patients resistant to insulin has greatly increased during the last few years. Several plasma factors, including insulin antagonists, may offer an explanation for this phenomenon.[1] The most important of these antagonists are insulin antibodies. Because of these and other factors that may result in resistance, we considered it important to carry out in vitro experiments on muscles of insulin-resistant patients, to ascertain the quantity of glucose consumption in vitro from their own plasma. These values are compared with those obtained using plasma from normal patients with and without added insulin and in Ringer's solution in the presence and absence of insulin.

MATERIALS AND METHODS

White rats of the same sex, starved for twenty hours and weighing between 120 and 140 gm. were used for the experiments. Animals were killed by decapitation and the diaphragm, obtained by the usual preparation, was immersed in ice-cold saline for twenty to thirty minutes. The same method was employed with the excised parts from the rectus abdominalis muscle of patients from both sexes and different ages who did not suffer from metabolic diseases, but were subjected to surgical intervention because of duodenal ulcer or cholelithiasis. The operation was always performed under inhalation narcosis. The surgeon excised the parts of the muscle at the commencement of the operation.

Muscle biopsy, also from the rectus abdominalis muscle tissues, was performed under local anesthesia on diabetic patients. In order to assess the effect of the local anesthesia, the same novocain infiltration (cocainum novum hydrochloricum) was performed on the muscle part in situ as during excision. Additional control tests were carried out, in which the muscle parts obtained were soaked in a novocain bath prior to the experiment.

The human muscle was divided into approximately 150 mg. slices, cleaned from fatty and bloody tissue and separated along fibers. Rat hemidiaphragms were used.

Heparinized blood (5 to 10 I.U./ml.) was obtained from fasting patients not suffering from metabolic diseases or from diabetic patients and was immediately centrifuged. The plasma obtained was kept at a temperature of +4° C. and used within three to four hours. Diabetic patients on insulin were given regular Crystalline Insulin some time before the blood samples were collected. The glucose content in all plasmas was made up to 300 mg. per 100 ml.

Two milliliters each of the plasma so prepared and/or bicarbonate-buffer Ringer solution was measured into 20 ml. tubes and sealed with rubber stoppers. Thus, the test of every single plasma was performed simultaneously in two to three tubes.

The prepared muscle parts were placed in the tubes and a gas mixture containing 95 per cent O_2 and 5 per cent CO_2 was circulated within the sealed tubes for ten minutes. Then both the control tubes and those containing the muscle parts were placed in a 37° C. water bath and shaken for ninety minutes at a rate of 150/min. and a 10 mm. stroke. After incubation the muscle was dried at 100° C. for two hours until it attained a constant weight which was established with a torsion balance. Sugar concentration was measured by the Somogyi-Nelson[3] method in both the control plasma and the human muscle. The difference between the average glucose concentration of the control plasma and the incu-

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bated plasma with muscle gave the glucose consumption of the muscle. The milligrams of glucose consumed within ninety minutes was calculated per 10 mg. dried muscle.

Results of the test were assessed only when neither the dried weight of the human rectus muscles nor that of the rat half-diaphragms exceeded 20 to 40 mg., provided the difference in weight of the muscle parts incubated in identical plasma was not more than ± 10 per cent. The difference in sugar consumption of the simultaneously incubated muscles did not exceed ± 5 per cent. If the difference was greater, the experiment was discarded.

RESULTS

Glucose uptake by rat diaphragm muscle and human abdominal muscle incubated in a Ringer solution, in the presence and absence of insulin, is plotted in figure 1. Black dots represent glucose uptake by rat diaphragm muscle and open circles indicate values obtained with human abdominal muscle. The figure shows that the sugar consumption of a piece of healthy human muscle is identical with that of the rat diaphragm and that glucose uptake is proportional to the insulin content of the medium.

Figure 2 shows the sugar consumption of healthy rectus muscle pieces and that of muscle of insulin-resistant diabetic patients. On the left of the figure is shown the effect of the same healthy fasting human plasma on the sugar consumption of the rectus muscle piece of healthy individuals and of insulin-resistant diabetics. The difference is striking. Rectus muscles of diabetic patients consumed little sugar in the healthy plasma, and indeed muscle pieces from two patients did not consume any sugar at all.

GLUCOSE UPTAKE OF HEALTHY HUMAN ABDOMINAL MUSCLE (○) AND OF RAT HEMI-DIAPHRAGM (●)

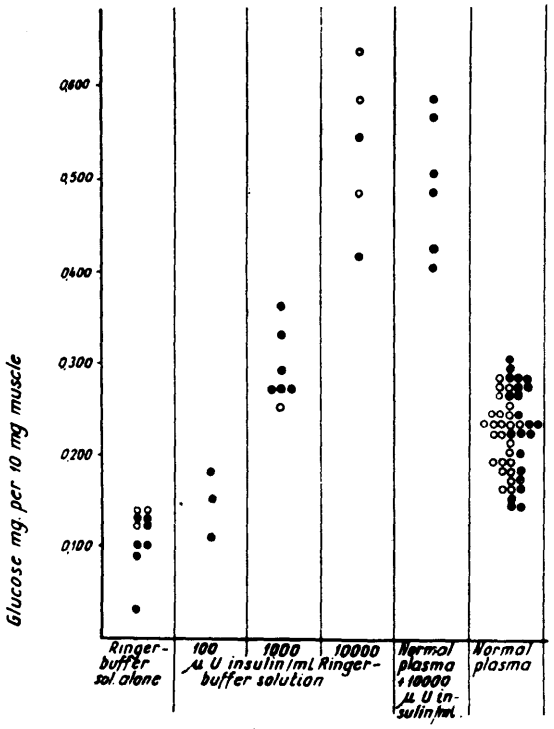

FIGURE 1

THE GLUCOSE UPTAKE OF THE HUMAN ABDOMINAL MUSCLE FROM HEALTHY (H) AND CHRONIC INSULIN-RESISTANT DIABETIC (D) SUBJECTS

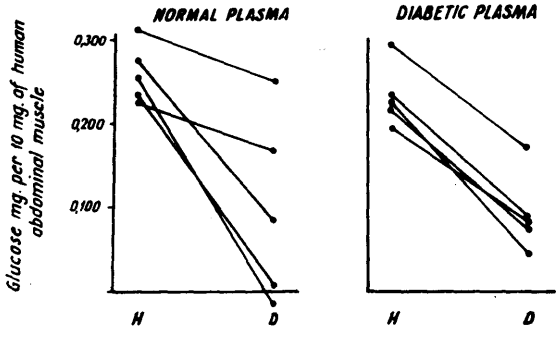

FIGURE 2

On the right of figure 2 the effect of the same insulin-resistant patient's plasma on the sugar consumption of the healthy rectus muscle and that on his own rectus muscle has been plotted. Similar to healthy plasma, the diabetic patient's plasma (see figure 1, column 6) increased the sugar consumption of the healthy rectus muscle; therefore, in the plasma of these patients there is insulin-like activity. At the same time, the sugar consumption of the insulin-resistant diabetics in their own plasma was minimal in four cases.

Table 1 plots a comparison between the sugar consumption of the rectus muscle of healthy individuals with that of insulin-resistant and nonresistant diabetic patients. The table summarizes values obtained on plasma of seven healthy individuals in which the muscle of insulin-resistant patients was tested. It is clear from the table that out of five insulin-resistant patients (H.L., P.I., H.J., J.L., and LL.), the rectus muscle parts of three consumed only a minimal quantity of sugar in healthy plasma. The rectus muscle of four patients consumed unappreciable quantities of glucose in their own plasma and in Ringer solution containing insulin.

Figure 3 gives information on the glucose uptake of insulin-resistant patients' rectus muscle pieces. The black

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TABLE 1

Glucose uptake of the abdominal muscle tissue (mg./10 mg. dry weight/90 min.)

		Clinical data								Experimental data		
Abdominal muscle from:		Sex	Age (yrs.)	Duration of diabetes	Diet CHO gm. per day	Daily insulin requirement I.U.	Fasting blood sugar mg. per 100 ml.	Urine sugar gm. per day	Plasma from healthy subject	Ringer-buffer solution +0.01 I.U. per ml. insulin	Plasma, 1 hr. after 60 gm. of oral glucose	Diabetic patient's plasma
Healthy subjects									0.255*	0.555†	0.495†	
Diabetic patients with chronic insulin resistance	H.L.	F	28	9	250	<360	350-500	150-200	—0.015		—0.093	0.086
	P.I.	F	42	8	200	160	200-300	100-160	0.006	0.086		0.077
	H.J.	M	38	2	200	120	350-400	80-120	0.084	0.047		0.041
	J.L.	F	53	4	200	260	260-360	150-200	0.250	0.108		0.080
	L.L.	M	21	1	220	120	250-400	150-200	0.169	0.266		0.172
Diabetic patients nonresistant to insulin	S.L.	F	65	6	180	BZ 55	120-150	>10	0.188			
	L.J.	F	61	5	200	diet only	100-140	>10	0.242			0.279

*Average value from seven healthy subjects
†Average value from three healthy subjects

columns indicate healthy plasma, the open ones Ringer solution containing insulin, the shaded columns diabetic plasma and the dotted ones plasma which had been obtained from a healthy individual one hour after consumption of 60 mg. glucose.

Each piece of rectus muscle from a healthy individual calculated on 10 mg. dry muscle consumed 0.200 to 0.300 mg. glucose in a healthy fasting plasma. In plasma containing insulin, whether a known quantity of insulin was added to the plasma, or plasma obtained after glucose ingestion was used, the sugar consumption of the muscle considerably increased. The rectus muscle piece of patients H.L., P.L., and H.J. did not consume an appreciable quantity of sugar in healthy plasma, nor in plasma obtained after sugar loading, nor in plasma supplemented with insulin. In patient J.L., sugar consumption in healthy plasma was almost normal but insulin remained ineffective and sugar uptake was also minimal in his own plasma. In patient L.L., sugar consumption was below average.

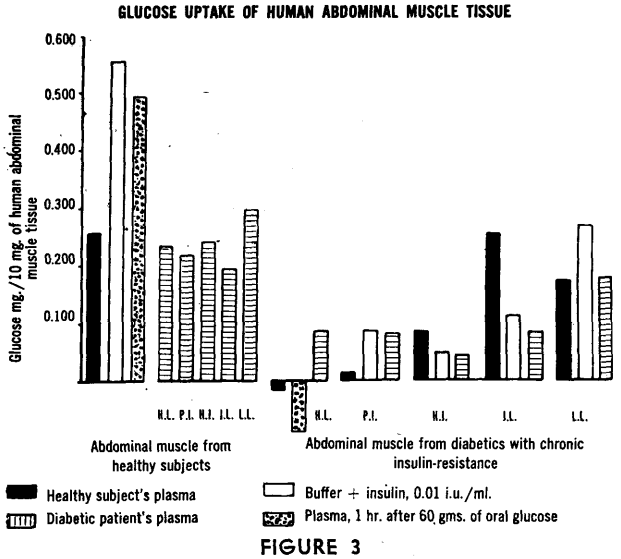

FIGURE 3

DISCUSSION

These experiments reveal that in five out of seven diabetics, muscle tissue was incapable of utilizing glucose even in the presence of a suitable quantity of insulin. In the plasma of our tested diabetics the rectus muscles of healthy individuals and of rat diaphragm muscles both consumed glucose, whereas the diabetic's own muscle in the identical plasma was unable to take up an appreciable quantity of glucose.

Field[2] has reported on the temporarily decreased glucose utilization of the fatty tissue of diabetic patients. Thus, it seems that in some cases the reason for insulin resistance (or perhaps even diabetes) is due to the fact that the patient's tissues are unresponsive to both endogenous and exogenous insulin.

That form of diabetes where besides insulin resistance, insulin-like activity in the patient's plasma can be demonstrated and his muscle tissue is still unable to take up glucose (in vitro and presumably also in vivo) has been designated *achrestic diabetes.*[4]

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