Diabetologia 5, 300–303 (1969)

Insulin Sensitivity of Adipose Tissue and of Diaphragm in Rats Adapted to Periodic Hyperphagia

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Received: October 16, 1969

Summary. In the present paper the authors have investigated the sensitivity of adipose tissue and diaphragm to insulin in rats adapted to periodic hyperphagia. Insulin was administered by the intraperitoneal route in amounts that did not affect the blood sugar level. The incorporation of labelled glucose, administered simultaneously with insulin, into lipids of parametrial adipose tissue and into glycogen of the diaphragm was investigated, together with the glycogen content of the diaphragm. Moreover, the authors investigated the influence of insulin added to the medium on the glycogen content of the isolated diaphragm. It was found that adipose tissue of adapted rats responded to insulin at all levels used, more sensitively than adipose tissue of control rats fed *ad libitum*. On the other hand, no differences were found in the sensitivity of the diaphragm to insulin either *in vivo* or *in vitro*. The relationship between morphological and functional changes in the adipose tissue of adapted rats and the increased reactivity of this tissue to insulin is discussed.

Sensibilité à l'insuline du tissu adipeux et du diaphragme de rats adaptés à l'hyperphagie périodique

Résumé. Dans cet article les auteurs ont étudié la sensibilité à l'insuline du tissu adipeux et du diaphragme de rats adaptés à l'hyperphagie périodique. L'insuline était administrée par voie intrapéritonéale en quantités ne modifiant pas le taux de la glycémie. L'incorporation du glucose marqué, administré en même temps que l'insuline, dans les lipides du tissu adipeux paramétrial et dans le glycogène du diaphragme, a été étudiée en même temps que le contenu en glycogène du diaphragme. En outre, les auteurs ont étudié l'influence de l'addition d'insuline au milieu sur le contenu en glycogène du diaphragme isolé. On a constaté que le tissu adipeux des rats adaptés répondait à l'insuline à tous les taux utilisés, d'une manière plus sensible que le tissu adipeux des rats témoins nourris *ad libitum*. D'autre part on n'a trouvé aucune différence dans la sensibilité du diaphragme à l'insuline *in vivo* ou *in vitro*. La relation entre les variations morphologiques et fonctionnelles dans le tissu adipeux des rats adaptés et la réactivité augmentée de ce tissu à l'insuline est discutée.

Insulinempfindlichkeit des Fettgewebes und Zwerchfells von Ratten nach Adaptation an stoßweise Nahrungszufuhr

Zusammenfassung. Die Autoren überprüften die Empfindlichkeit des Fettgewebes und des Zwerchfells gegenüber Insulin bei Ratten, die sie an stoßweise Nahrungszufuhr gewöhnt hatten. Insulin wurde dabei in nicht blutzuckerwirksamen Mengen i.p. injiziert. Es wird über den Einbau von gleichzeitig verabreichter Radioglucose in die Lipide des parametranen Fettgewebes und das Zwerchfellglykogen und über den Glykogengehalt des Zwerchfells berichtet. Ferner untersuchten die Autoren die Wirkung von Insulinzusätzen auf den Glykogengehalt des isolierten Zwerchfells. Es zeigte sich, daß das Fettgewebe adaptierter Ratten auf alle verwandten Insulinkonzentrationen stärker ansprach als das Gewebe der Kontrolltiere mit freiem Zugang zum Futter. Andererseits wies die Insulin-Ansprechbarkeit des Zwerchfells weder *in vivo* noch *in vitro* Unterschiede auf. Die Beziehungen zwischen morphologischen und funktionellen Veränderungen des Fettgewebes adaptierter Ratten und seiner erhöhten Insulin-Empfindlichkeit werden besprochen.

Key-words: Insulin sensitivity, feeding pattern, adipose tissue.

In previous work we found that in rats adapted to periodic hyperphagia the sensitivity to insulin changes. Adapted animals responded to exogenous insulin by a greater drop of the blood sugar level than control animals fed *ad libitum*. Marked differences were also found in the sensitivity of target organs to insulin. The sensitivity of adipose tissue to insulin judged from the stimulation of glycogen synthesis and lipogenesis was much higher in adapted rats than in controls fed *ad libitum*. On the other hand, muscle tissue of adapted rats responded to insulin by a smaller increase of the glycogen content than did the tissue of controls. These findings were obtained using doses of insulin that lower the blood glucose level (Braun et al., 1967).

Insulin administered by the intraperitoneal route influences in a marked way the metabolism of insulin-sensitive tissues in the abdominal cavity, even when the doses of insulin used are 10—100 times smaller than those needed to influence the blood sugar level. The glycogen content of diaphragm increases; and when, concomitantly with insulin, labelled glucose is administered, the effect of insulin can be observed on the diaphragm (incorporation of labelled glucose into glycogen), as well as on adipose tissue in the abdominal cavity (incorporation of labelled glucose into lipids and glycogen). This approach thus permits the simultaneous investigation of the effect of insulin on these two tissues *in vivo* and without affecting the blood sugar level (Rafaelsen, 1964; Rafaelsen et al., 1965).

The object of the present work was to investigate the reactivity of tissues of adapted rats to insulin *in vivo*, using insulin doses that do not affect the blood sugar level, i.e. under conditions where probably counterregulating factors are not involved and where the availability of glucose is not the limiting factor.

In addition the reactivity of the diaphragm to insulin was investigated *in vitro*.

Methods

Female rats of the Wistar strain, weighing 180—220 g and fed a laboratory diet (FÁBRY, 1959) were used. The animals were fed for 2 h a day (7 to 8 a.m.) for a period of six weeks; the controls were fed *ad libitum*. All animals had free access to water. Before the experiment all animals were fasted for 24 h.

Physiological saline containing 250 mg of bovine serum albumin/100 ml and uniformly labelled ^{14}C-glucose[1] (spec. activity 75 mCi/mmol) in an amount corresponding to 2 μCi/100 g body wt., was injected intraperitoneally. When insulin was used it was included in the injected fluid in the following amounts: 125, 500 and 2000 μU/100 g body wt. Tissue specimens were collected under pentobarbital anaesthesia 150 min after the intraperitoneal injection. The diaphragms were frozen on a block of solid carbon dioxide and weighed; and the glycogen was then extracted by boiling in 30% KOH for a period of 60 min and subsequently reprecipitated three times with 60% ethanol. After dissolving the precipitated glycogen in distilled water, part of the solution was used for assessing the glycogen content by means of the anthrone reagent (CARROLL et al., 1956), and part was used for measuring its radioactivity by scintillation counting in Bray's medium (BRAY, 1960). Parametrial adipose tissue was homogenized in methanol, and the total lipids were extracted using Folch's method (FOLCH et al., 1957). Protein in adipose tissue was estimated by Lowry's method (LOWRY et al., 1951). The radioactivity of the glycogen obtained from the diaphragm and of the lipids from the adipose tissue was measured using a scintillation spectrometer (Nuclear Chicago).

The sensitivity of the diaphragm to insulin was also investigated *in vitro*. The rats were killed by decapitation after a 24-hour fast. One hemidiaphragm from each animal was incubated in an insulin-free medium (the control), and the other in a medium containing insulin. The incubation medium was Krebs-Ringer bicarbonate buffer (pH 7.4), equilibrated with 95% O_2/5% CO_2 at 37°C in a Dubnoff metabolic shaker (90 cycles/min). The incubation medium contained bovine serum albumin (250 mg/100 ml) to prevent adsorption of insulin on glass. The concentrations of glucose and insulin are given in Table 3. After incubation for 120 min, the glycogen content of the diaphragms was assessed.

Results

It is apparent from Table 1 that insulin in all doses used stimulated the incorporation of labelled glucose into lipids of parametrial adipose tissue considerably more in adapted rats than in control rats fed *ad libitum*. No differences were found between control and adapted animals in those groups where the administered saline did not contain insulin. The incorporation of labelled glucose into the lipids of adipose tissue was, with all the doses of insulin used, 2—3 times greater in the adapted rats than in the control rats fed *ad libitum*. The greater sensitivity to insulin of adipose tissue from adapted rats was particularly marked with a dose of 125 μU of insulin/100 g, where there was a doubling of the incorporation of labelled glucose into lipids of adipose tissue, whereas the adipose tissue of rats fed *ad libitum* did not respond to this dose.

Table 1. *Effect of crystalline insulin on lipogenesis from U-^{14}C-glucose by adipose tissue in rats adapted to periodic hyperphagia and in control rats fed* ad libitum. *Incorporation of ^{14}C-radioactivity into lipids is expressed as counts/min. mg protein. Each value is the mean for six animals ± standard error of mean*

	Insulin administered (μU/100 g body wt.)			
Feeding pattern	0	125	500	1000
Ad libitum	785.5 ± 88.7	802.1 ± 80.0 [a]	1181.1 ± 299.3 [b]	4675.0 ± 547.9 [a]
2 h/day	736.6 ± 88.6	1580.1 ± 137.5 [a]	2064.5 ± 449.1 [b]	12134.8 ± 1122.4 [a]

[a] $P < 0.001$ [b] $P < 0.2$

Table 2. *Effect of crystalline insulin on the glycogen content of diaphragm and on the incorporation of U-^{14}C-glucose into diaphragm glycogen in rats adapted to periodic hyperphagia and in rats fed* ad libitum. *Each value is the mean for six animals ± standard error of mean*

	Insulin administered (μU/100 g body wt.)							
Feeding	0	125	500	2000	0	125	500	2000
pattern	glycogen content (mg/100 g wet wt.)				^{14}C-incorporation (counts/min. mg glycogen)			
Ad libitum	299.8 ± 20.4	359.3 ± 7.0	519.1 ± 23.1	658.3 ± 26.3	905.0 ± 227.6	5365.0 ± 905.2	12330.0 ± 1713.6	15669.0 ± 990.4
2 h/day	321.0 ± 6.2	381.8 ± 22.8	495.0 ± 10.5	664.5 ± 26.9	644.0 ± 197.3	5619.0 ± 843.1	13196.0 ± 1224.0	14657.0 ± 2235.0

[1] Supplied by Institute for Research, Production and Users of Radioisotopes, Prague, Czechoslovakia.

On the other hand, we did not find any difference between adapted and control rats as regards the sensitivity of the diaphragm to insulin (Table 2). It is obvious from the data in Table 2 that there are no significant differences between adapted and control rats, whether or not the administered saline contained insulin, as regards the glycogen content of the diaphragm or the incorporation of labelled glucose into diaphragm glycogen.

Table 3. *Effect of crystalline insulin on the glycogen content of incubated diaphragms isolated from rats adapted to periodic hyperphagia and from rats fed* ad libitum. *Each value is the mean for six diaphragms ± standard error of mean*

	Feeding pattern	Glycogen content of diaphragm (mg/100 g wet weight)	
		Without insulin	With insulin
Exp. I	*Ad libitum*	241.0 ± 17.6	342.7 ± 16.7
	2 h/day	270.0 ± 18.9	327.0 ± 28.6
Exp. II	*Ad libitum*	317.5 ± 20.8	528.2 ± 31.9
	2 h/day	305.0 ± 31.9	570.5 ± 24.0

In experiment (I) diaphragm was incubated for 120 min in 3 ml KRB buffer, containing 15 μmoles glucose and 7.5 mg bovine serum albumin without hormone or with 10000 μU insulin per ml.

In experiment (II) diaphragm was incubated for 120 min in 3 ml KRB buffer, containing 60 μmoles glucose and 7.5 mg bovine serum albumin without hormone or with 100000 μU insulin per ml.

The reactivity of the diaphragm to insulin *in vitro* (Table 3) was similar. We did not find significant differences between adapted and control rats, either in the absence or in the presence of insulin, in experiments using two different concentrations of glucose and insulin in the incubation medium.

Discussion

Adipose tissue of adapted rats compared with that of controls fed *ad libitum* responded more sensitively to small doses of insulin given intraperitoneally, acting locally and not affecting the blood sugar level, just as had been shown using hypoglycaemic doses of insulin. Muscle tissue, on the other hand, responded to insulin under these conditions in the same way in adapted rats as in controls fed *ad libitum*. A rather similar position as regards the enhanced sensivity of adipose tissue to insulin was described by STAUFFACHER in mice with hereditary obesity; in that model, however, the sensitivity of muscle tissue to insulin declines further (STAUFFACHER et al., 1965).

In addition to many metabolic changes which are typical of adipose tissue of rats adapted to periodic hyperphagia (FÁBRY, 1967), this tissue also differed from the adipose tissue of controls fed *ad libitum* in its composition (BRAUN et al., 1965; BRAUN et al., 1967) and morphological aspects (BRAUN et al. 1965). Compared with adipose tissue of controls, it contained more RNA and was more cellular, as suggested by the higher DNA content per mg tissue as well as per fat body, and by the histological appearance of the tissue. Fat cells of adapted rats were smaller than fat cells of controls, and thus per unit of weight or volume there was also a greater cell surface. This altered tissue was more sensitive to the lipogenetic stimulus of insulin, and also to lipolytic stimuli, e.g. adrenaline (BRAUN et al., 1966).

In the increased sensitivity of adipose tissue for insulin, humoral factors that potentiate the effect of insulin on adipose tissue probably do not participate; in other experiments, where we investigated the serum insulin-like activity of adapted rats by the effect on adipose tissue, we did not find differences in the serum insulin activity between rats adapted to periodic hyperphagia and controls fed *ad libitum* (VRÁNA et al., 1968).

If we consider the role of insulin in accentuated lipogenetic and other anabolic processes in adipose tissue of rats adapted to periodic hyperphagia, we must consider in addition to the reactivity of the target organ to the hormone also the secretory capacity of the endocrine pancreas and the serum insulin concentration. When investigating the secretory response of the insular apparatus to administered glucose, using as the criterion the serum insulin concentration after a glucose load, we did not find any differences between rats adapted to periodic hyperphagia and controls (VRÁNA et al., 1968). Owing to ingestion of large amounts of food within a short period of time and owing to accentuated intestinal absorption of the main physiological stimulus for insulin secretion, glucose-adapted rats develop a prompt, short-term rise in serum insulin concentration, a periodic hyperinsulinaemia (VRÁNA et al., 1968).

In view of the fact that adipose tissue of adapted rats responds much more sensitively to insulin even in the doses corresponding to a hormone concentration in the medium to which the tissues are exposed, and in view of the high concentration of serum insulin in the absorption stage of digestion, we may assume an important role of insulin in the mechanism of adaptive hyperlipogenesis in rats adapted to periodic hyper-

phagia. By interplay of regulatory changes at the level of hormone secretion as well as at the level of the target tissue reactivity, a situation develops which is from the energetic aspect favourable for the infrequently fed animal. In the absorption stage anabolic processes in adipose tissue are potentiated, their final effect being the maximum conversion of carbohydrate into lipids and their deposition.

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