THE EFFECT OF FOOD INTAKE ON INSULIN RECEPTOR IN MAN

By

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ABSTRACT

Insulin binding to circulating monocytes was studied in 22 normal volunteers before and 1, 3 and 5 h after a 1400 Kcal meal. Results indicate that 3 h after food intake there is an increase in the specific cell binding fraction ($P < 0.001$) with a change in receptor affinity. Data emerging from the present study demonstrate that there are rapid changes in insulin receptor properties during the day. These changes probably play a role in the regulation of the hormonal and metabolic pattern in normal subjects.

Insulin receptors have been extensively investigated both in animal (Freychet et al. 1971; Cuatrecasas et al. 1971; Cuatrecasas 1971) and man (Gavin et al. 1973; Olefsky & Reaven 1974; Olefsky et al. 1974; Haour & Bertrand 1974), and it has been demonstrated that many insulin resistant states have a decreased capacity to bind insulin due to changes in receptor affinity and/or concentration (Kahn et al. 1973; Goldstein et al. 1975; Beck-Nielsen et al. 1976; Olefsky 1976a,b; Bar et al. 1976).

In the present report we demonstrate that changes in receptor properties also occur along physiological variations in hormonal and metabolic patterns. Food intake is assumed to be a physiological stimulus capable of varying the hormonal and metabolic patterns.

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MATERIALS AND METHODS

Subjects

Twenty-two volunteer doctors or medical students (15 men and 7 women) were studied. All were normal as far as body weight (Geigy Table, 6th Ed.), metabolic parameters (blood glucose and the following plasma constituents: insulin, BUN, FFA, cholesterol, uric acid, total lipids, ketone bodies, or creatininc), physical examination, family history of diabetes and obesity, and had at no time taken any drug known to affect carbohydrate or insulin metabolism. Mean age, weight and height for male were: 25 years (range 22–26), 73 kg (range 66–82), and 180 cm (range 172–190), respectively; those for females: 27 years (range 21–30), 56 kg (range 53–60), 164 cm (range 161–167), respectively. All subjects led a normal life according to their usual habits refraining from any changes in food, sleep and activity for at least 7 days prior the test. On the day of the test all had a continental breakfast (250 Kcal; ≈ 70 % carbohydrate, 12 % protein, 18 % fat) at 8 a.m. and a meal of 1400 Kcal (≈ 45 % carbohydrate, 20 % protein, 35 % fat) at 1 p.m. The subjects were allowed to take their usual time to consume the meal. No other food or drinks were permitted except for water ad libitum. During the day of the study volunteers stayed in the hospital where they were allowed to read, study, walk etc.

It should be pointed out that this investigation did not include subjects who were in the habit of taking their meal at times different from those of the study.

Control studies

Fourteen of the 22 subjects were re-investigated for comparative purposes. Four ate a meal of 1400 Kcal (≈ 45 % carbohydrate, 20 % protein, 35 % fat) at 3 p.m. and blood samples were collected 15 min before and 1, 3 and 5 h after the meal; in 10 blood samples were collected at 1, 2, 4 and 6 p.m., these subjects having fasted between 8 a.m. and 7 p.m.

Preparation of cells

Fifty ml of blood was collected in tubes containing sodium citrate 3.8 % (8 ml) 15 min before and 1, 3 and 5 h after the meal. Each sample was diluted with isotonic saline to a total volume of 120 ml and then transferred to a Ficoll-Hypaque gradient for cell fractionation according to the method of Boyum (1968). The mononuclear cells were repeatedly washed and then re-suspended in Tris 25 mM, KCl 5 mM, glucose 10 mM, Na-EDTA 1 mM, NaCl 120 mM, Na-acetate 15 mM, MgSO4 · 7H2O 1.2 mM, bovine serum albumin 1 % buffer (pH 7.6) to a final concentration of 30 × 10^6 × ml⁻¹. Approximately 85 % of white cells consisted of lymphocytes, 14 % monocytes and 1 % granulocytes. Lymphocytes and monocytes were measured in all the samples and no marked differences in monocyte content (± 1.8 % of total white cells) were found between the 4 samples of each subject. Lymphocytes were stained with 0.1 % acridine orange whereas monocytes were measured by phagocytosis of latex beads and also by non-specific esterase staining (Yam et al. 1971). The Trypan Blue exclusion test always revealed more than 97 % viable cells. The concentration and viability of the cells were controlled at the end of each incubation period. It is possible from 50 ml of blood to obtain sufficient mononuclear cells to perform 6 samples of 30 × 10^6 × ml⁻¹.

Binding studies

Mononuclear leucocytes (30 × 10^6 × ml⁻¹) were incubated at 15°C with [125I]insulin (172 pmol/l) (110–130 μCi/μg, Sorin, Italy) with or without different amounts of native
insulin (Organon, Holland) for a total volume of 0.5 ml. After 100 min of incubation duplicate 200 μl samples were layered over 150 μl of Di-n-butyl phthalate (density 1.04, BDH Chemicals Ltd., England) (Andreasen et al. 1974) and centrifuged immediately at room temperature in a Beckman Microfuge for 1 min. The specific cell binding fraction, corrected for the monocyte content, was determined according to the method described by Beck-Nielsen et al. (1977). Non-specific binding was defined as the amount of [125I]insulin remaining “bound” in the presence of 7 μmol/l of native insulin.

**Statistical and computer analysis**

Student's t-test for paired data was employed for comparison of values between basal, 1, 3 and 5 h collections. Competition curve analysis was performed using the equation $y = ax^b$ for curve fitting (power regression).

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**Fig. 1.**

Post-prandial plasma glucose and insulin responses in 22 normal subjects (mean ± sd).

Dashed vertical lines indicate beginning and end of meal.
Analytic methods

Plasma glucose was determined by the glucose-oxidase method using a Beckman glucose analyzer. Plasma immunoreactive insulin was measured by the double-antibody method (Hales & Randle 1963) using a reagent kit from Sorin, Italy; the coefficient of inter-assay variation was 6.1 % and that of intra-assay was 4.2 %.

RESULTS

Blood insulin and plasma insulin concentration increased at 1 h returning to baseline values of about 3 h after food intake (Fig. 1).

The specific cell binding fraction was $2.1 \pm 0.43$ before the meal (basal value) and $2.19 \pm 0.57$ 1 h, $2.49 \pm 0.50$ 3 h, $2.03 \pm 0.56$ 5 h, respectively, following food intake (Fig. 2). Differences between values obtained under basal conditions and at 1 or 5 h were not significant. On the contrary significant differences were observed between basal values and those after 3 h ($P < 0.001$). A

![Graph showing specific cell binding fraction before and after food intake.](image)

Specific cell binding fraction before and 1, 3 and 5 h after food intake. $[^{125}]$insulin (172 pmol/l) was incubated with $4 \times 10^6 \times ml^{-1}$ monocytes for 100 min at 15°C with or without native insulin (7 µmol/l). Dashed vertical lines indicate beginning and end of meal. Data are the mean ± sd from 22 normal subjects.
Fig. 3.
Inhibiting effect of native insulin on labelled insulin binding before (●—●) and 3 h after (∆—∆) food intake. $4 \times 10^6 \times \text{ml}^{-1}$ monocytes were incubated with $[125\text{I}]$insulin (172 pmol/l) with or without increasing unlabelled insulin concentrations. Labelled insulin binding, expressed as a per cent of initial (i.e. maximal) binding, is plotted as a function of total insulin concentration. Data are corrected for non-specific binding and represent the mean from 22 normal subjects.

change in the competition curves, compared with basal values was observed 3 h after the meal (Fig. 3). Thus the amount of native insulin causing the 50% reduction of labelled insulin specific maximal binding, calculated using power regression, decreased from 0.94 to 0.62 nmol/l; $r^2$ of basal curve was 0.9983 whereas that of the 3rd h curve was 0.9997. This change coincided with a decrease in the non-specific binding from 30 to 23% (Fig. 4). Data obtained from samples collected at the 1st and 5th h are very similar to basal values and are therefore not shown.

In 4 of the subjects studied twice (first study food intake at 1 p.m. and the second study at 3 p.m.) the changes described occurred, in both instances, 3 h after the meal. In the 10 subjects re-investigated during fasting no changes were observed in the shape of the competition curve or non-specific binding and only very slight fluctuations above and below basal values were found at 2, 4 and 6 p.m. (Fig. 5).

Of the 22 subjects studied, one did not show any of the described changes and 3 had minimal changes. It should be stressed, however, that in none of
Fig. 4.
Inhibiting effect of native insulin on labelled insulin binding before (●—●) and 3 h after (△—△) food intake. Insulin labelled binding, expressed as per cent of initial (i.e. maximal) binding, is plotted as a function of total insulin concentration. Data are not corrected for non-specific binding and represent mean from 22 normal subjects.

Fig. 5.
Specific cell binding fraction at 1, 2, 4 and 6 p.m. in 10 subjects fasted between 8 a.m. and 7 p.m.

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the subjects studied after the meal was the specific cell binding fraction at the 3rd h less than the basal value, at the 1st and 5th h, on the other hand, values were frequently lower than under basal condition.

**DISCUSSION**

In the 22 subjects studied blood glucose and plasma insulin concentration rose after the intake of food in the same manner as in normal subjects (*Malherbe et al.* 1969; *Ahmed et al.* 1976). One and 5 h after food intake the specific cell binding fraction was either higher or lower than the basal value, depending upon the subject, with no change in receptor affinity and non-specific binding. On the contrary at 3 h all the samples except one showed an increase in the specific cell binding fraction (*P* < 0.001) revealing different shape in the competition curve. Furthermore, the non-specific binding in basal conditions, 1 and 5 h after food intake was always 30 %, as described by *Pedersen & Beck-Nielsen* (1976) in normal man under fasting conditions, while it was 23 % at 3 h. These phenomena were not related to spontaneous receptor variations during the day. In fact in the two types of controls studied there are no difference related to the time of food ingestion (1 or 3 p.m.), whereas fasting does not produce change in insulin receptor properties suggesting that the changes are related to food intake.

Changes in receptor affinity have recently been observed 5 h after ingestion of glucose (*Muggeo et al.* 1977). This result is in agreement with the present data even if the same changes are found at 3 h with a return to baseline values at 5 h. It should be pointed out that in another series of normal subjects studied during OGTT we observed an increase at the 5th h of receptor affinity (unpublished data) as described by *Muggeo et al.* (1977).

Data emerging from this study suggest that food intake induces an increase in the specific cell binding fraction with changes in receptor affinity and non-specific binding. These changes occur rapidly probably due to physiological variations in the hormonal and metabolic patterns.

It has been suggested that the hormone receptor interaction is the first step in the biological action of the hormones (*Roth* 1973; *Cuatrecasas* 1974). Regarding monocytes the exact role of the insulin receptor has not yet been revealed, but has been suggested to reflect what happens to the insulin receptor present on the cell membrane of other tissues (*Gavin et al.* 1973; *Olefsky* 1976). For these reasons, and in the light of the present data and those of others (*Muggeo et al.* 1977) it is tempting to suggest that the site of this first step (i.e. receptor) changes its properties upon certain “messages” which are at present unknown. It would be interesting to examine if this phenomenon is also present in those clinical states presenting an alteration in the receptor.
Note added in proof

After this paper was submitted for publication, Beck-Nielsen & Pedersen (1978) reported a study on diurnal variation in insulin binding to human monocytes. The reported results demonstrate that food intake provokes acute changes in insulin binding due to changes in receptor affinity, and shows that during the day there are conspicuous fluctuations in monocyte concentration in the cell suspension.

Like Beck-Nielsen & Pedersen (1978) we conclude that food intake provokes rapid changes in insulin binding and receptor affinity, but in contrast we do not find conspicuous changes in monocyte content during the 5 h studied. One possible explanation of this discrepancy could be due to the different dietary habits.

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