IMPAIRMENT OF INSULIN BINDING TO THE FAT CELL PLASMA MEMBRANE IN THE OBESE HYPERGLYCEMIC MOUSE

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1. Introduction

The specific insulin binding systems developed with mono [^{125}I]insulin and purified plasma membranes of target cells [1, 2] provide a useful tool to study directly the insulin–receptor interaction in pathological states of altered sensitivity to insulin. The obese hyperglycemic (ob/ob) mouse represents a recessively inherited trait whose syndrome includes a marked resistance to both endogenous and exogenous insulin [3]. Simultaneously to the findings reported here, some of us [4] have observed that liver membranes of ob/ob mice bind only 16–35% as much [^{125}I]insulin as membranes of the thin mice; control studies suggest that this alteration may be due, at least in part, to an impairment of the insulin receptor in the liver. We studied here the binding of [^{125}I]insulin to fat cell purified plasma membranes of ob/ob mice. Under identical conditions of preparation and incubation, membranes of obese mice bound only 25% as much [^{125}I]insulin as membranes of their non-obese littermates. Binding studies strongly suggest that affinity of membranes for insulin is identical or very close in both situations, whereas the insulin binding capacity of membranes of the obese animals is 4–7 times less than that of membranes of their thin littermates. It is as yet unclear whether this striking difference represents a primary defect or a secondary phenomenon. Our data, as well as those obtained with liver membranes [4], suggest that the high circulating insulin level observed in the obese mice is not likely to be responsible for the reduced insulin binding. These findings afford direct evidence of the possible implication of the insulin receptor in the pathophysiology of this syndrome.

2. Materials and methods

Obese hyperglycemic (ob/ob) mice and their non-obese (thin) littermates, 7–9 weeks of age (Bar Harbor strain), were obtained from "Centre d'Elevage du C.N.R.S." (45-Orléans-La Source - France) and fed ad libitum (unless stated otherwise) on mouse chow. Purified plasma membranes were prepared from epididymal isolated fat cells as described previously [5]. Membranes were prepared from the obese animals and from their thin littermates under identical conditions throughout the entire procedure, and were stored in liquid nitrogen until use. Protein concentrations were measured by the method of Lowry [6] using bovine serum albumin (BSA, fraction V, Pentex) as standard; the protein concentration that had been determined on aliquots of each membrane preparation was measured again on the dilution that was used in each binding experiment.

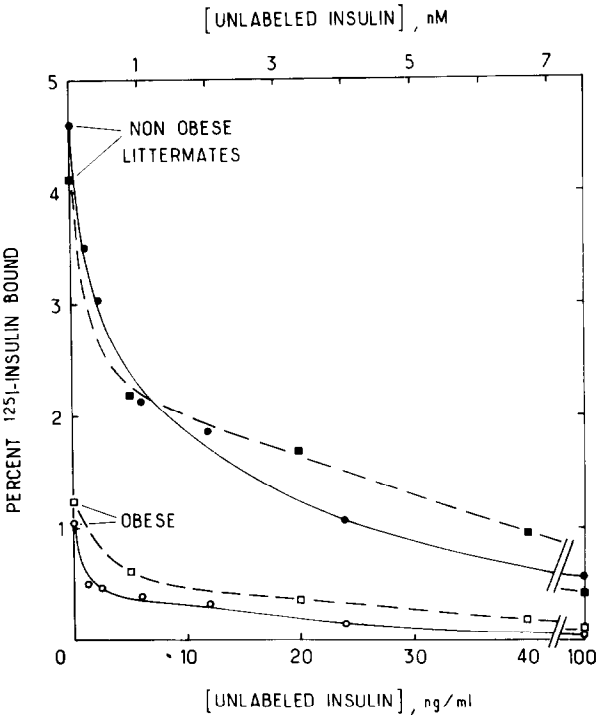

Fig. 1. Specific binding of [^{125}I]insulin (0.06 nM) to purified fat cell plasma membranes of obese mice and their non-obese littermates, as a function of concentration of unlabelled insulin added to incubation media. Results of two separate experiments with different preparations of membranes are depicted by solid lines and dashed lines. Solid lines represent data obtained, within the same experiment, with membranes prepared from obese mice (open circles, membrane protein: 0.08 mg/ml) and from their non-obese littermates (closed circles, membrane protein: 0.11 mg/ml). Dashed lines represent data obtained, within a second experiment, with another preparation of membranes from obese mice (open squares, membrane protein: 0.14 mg/ml) and from their non-obese littermates (closed squares, membrane protein: 0.16 mg/ml). Each experimental point is the mean of duplicate aliquots (see Methods) of a single determination.

Purified "mono component" porcine insulin (MCS 970, 27.2 U/mg), kindly supplied by Dr. Schlichtkrull (Novo Research Institute, Copenhagen, Denmark), was used for iodination and as native hormone in the binding experiments. [^{125}I]insulin was prepared as described elsewhere [7] and purified on DEAE cellulose as described previously [1, 7], except that urea was omitted from the eluting buffer. Specific activities of 250–350 μCi/μg (~ 1600–2000 Ci/nmole) were achieved.

Studies of binding of [^{125}I]insulin to membranes were performed at 30° for 30 min [2, 8] in Krebs–Ringer phosphate buffer, pH 7.5, that contained, in a final volume of 160–250 μl per incubation tube, 1.5% BSA and concentrations of insulin and membranes as indicated in fig. 1. The membrane-bound [^{125}I]insulin was isolated by centrifugation of duplicate aliquots from each incubation tube as previously described [2]. Data are reported as specific binding: this is obtained by subtracting from the total radioactivity of each pellet that amount of radioactivity that is not displaced by a large excess (50 μg/ml medium) of native insulin, a hormone concentration that largely exceeds the insulin binding capacity in liver [2] and fat cell [8] membranes. This "non specific" binding represents 15–20% of the total binding with fat cell purified plasma membranes from the non-obese mice, and 20–30% of the total binding with membranes from the obese animals.

Plasma insulin concentration was measured by radioimmunoassay with the use of [^{131}I]human insulin and a guinea pig anti-human insulin serum [9]; the results are expressed in terms of a rat insulin standard, kindly supplied by Dr. A.E. Lambert (Louvain, Belgium).

3. Results and discussion

Membranes of obese mice bound only 20–25% as much [^{125}I]insulin as membranes of their non-obese littermates in two experiments that were conducted, under identical conditions of incubation, with two different membrane preparations of obese mice and two different membrane preparations of the non-obese littermates (fig. 1). Unlabelled insulin inhibited the specific binding of [^{125}I]insulin to membranes of both obese and non-obese mice; half-maximal inhibition of binding was observed with unlabelled insulin in the range of 2 to 5 ng/ml (0.33 to 0.83 nM) for both types of membranes (fig. 1). Scatchard analysis of the data suggests that a reduced affinity for insulin is not likely to be responsible for the reduced insulin binding observed with membranes of obese mice, whereas the insulin

Table 1
Apparent equilibrium constants and insulin binding capacities for fat cell membranes of obese mice and their non-obese littermates (calculated from the data shown in fig. 1).

	Equilibrium constant (M^{-1} at 30°)		Binding capacity (picomole insulin/mg membrane protein)	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Obese	2.4×10^9	1.4×10^9	0.05	0.07
Non-obese	1.4×10^9	1.4×10^9	0.35	0.30

Table 2
Plasma insulin levels and insulin binding capacities of fat cell membranes in ob/ob mice fed ad libitum, food restricted ob/ob mice, and their non-obese littermates.

	Animals (number)	Weight range (g)	Plasma insulin* (μU/ml)	Insulin binding capacity (picomole/mg)
Experiment 1	Obese (11)	39–45	1300	0.05
	Food restricted (11)	21–26	200	0.06
	Non-obese (35)	20–25	80	0.35
Experiment 2	Food restricted† (11)	21–27	95	0.09
	Non-obese (33)	20–25	100	0.46

* Blood was collected by decapitation and pooled for each group of animals.
† Plasma insulin was 3500 μU/ml in the corresponding group of obese mice fed ad libitum.

binding capacity appears to be 4–7 times lower with membranes of obese mice than with those of their thin littermates (table 1).

To investigate the possibility that this reduced binding capacity might be due to occupancy of binding sites by the high levels of endogenous insulin in the obese mice [3], we measured plasma insulin concentration and binding capacity of fat cell membranes in obese mice fed ad libitum and in obese mice given a restricted diet (2.5 g chow/day) for 15 days. Table 2 shows that, despite a dramatic reduction in the plasma insulin level of food restricted animals, the binding capacity of their membranes remains well below that of membranes of non-obese mice.

The exact nature of the observed alteration is, as yet, unclear. The fact that obesity involves an increase in fat cell size does not allow to readily interpret data that are obtained with the plasma membrane fraction. However, the obese mice used in these studies were in the onset phase of obesity; at this phase, the nitrogen content of their adipose tissue is only 30% that of non-obese mice [10]. On the other hand, the fat cell volume is about 3 times greater in obese than in non-obese mice [11]. It thus appears reasonable to consider that, at this phase of obesity, 1 mg of membrane protein may correspond to roughly the same number of cells in both the obese and the non-obese mice (assuming that membrane protein represents about the same fraction of the total cell protein in both situations). That this might well be the case is suggested by the fact that, in order to get approximately the same amount of membrane protein in preparing the membranes of obese and non-obese mice, one had to start, in obese, from 3 times as much adipose tissue as in non-obese animals. It is also of considerable interest that a reduced insulin binding is observed in a very similar manner with the liver plasma membrane of obese mice [4]; in studies with liver membranes, control experiments suggest that the observed alteration is not due to the high circulating endogenous insulin levels nor to a major difference in membrane purification. Likewise, the data presented here (table 2) do not suggest that site occupancy by endogenous insulin might be responsible for an "apparent" reduction in the binding capacity of membranes of obese mice. Although further evidence is required to elucidate the precise significance of these findings in the pathophysiology and the pathogenesis of the obese hyperglycemic syndrome, the data reported here represent the first direct measurement of insulin–receptor interaction in the plasma membrane of fat cells in the obese hyperglycemic mouse.

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