Insulin Action and Binding in Isolated Hepatocytes from Fasted, Streptozotocin-Diabetic, and Older, Spontaneously Obese Rats

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Insulin binding and basal and insulin-stimulated uptake of α -aminoisobutyric acid were measured in isolated hepatocytes from young control rats as well as from older spontaneously obese, 72 h-starved, and nonketotic streptozotocin-diabetic rats. Isolated hepatocytes from older spontaneously obese rats are similar to those from younger smaller rats in size, maximal insulin responsiveness, the dose-response relationship for insulin-stimulated aminoisobutyrate uptake, and the number and affinity of insulin receptors. Hepatocytes from 72 h-fasted rats have similar numbers of insulin receptors per cell as cells from young control animals, but are significantly smaller, have an enhanced basal rate of aminoisobutyrate uptake, and are insulin resistant with regard to maximal insulin-stimulated uptake of aminoisobutyrate at 0.1 mM-aminoisobutyrate. Because of the decreased maximal response to insulin, the concentration of insulin that elicits a half-maximal response of aminoisobutyrate uptake is decreased. Hepatocytes from diabetic animals, like those from starved rats, have significantly greater basal rates of aminoisobutyrate uptake; whereas the maximal absolute insulin response is the same as control cells, the percentage response is smaller. These cells bind significantly more insulin than do control cells. The increase in insulin binding is reflected in a shift to the left of the dose-response curve for insulin-stimulated uptake of aminoisobutyrate. These studies indicate that there is no insulin resistance with regard to uptake of aminoisobutyrate in hepatocytes from older obese rats. Furthermore, the insulin resistance observed in hepatocytes from starved rats occurs despite an increase in the number of receptors per unit surface area and cannot be explained by alterations in the interaction between insulin and its receptor. The enhanced insulin binding per unit surface area, however, is reflected in the shift to the left of the dose-response curve for insulin. This is also true for hepatocytes from diabetic animals, in which insulin binding per cell is increased.

Previous attempts to investigate the cellular basis of the insulin resistance observed in obese, fasted and diabetic people and animals have concentrated on cellular and subcellular preparations that are unresponsive to insulin (Archer *et al.*, 1973; Olefsky & Reaven, 1976; Davidson & Kaplan, 1977; DeFronzo *et al.*, 1978), or that are relatively unimportant in total insulin action (Livingston *et al.*, 1972; Freychet *et al.*, 1972; Amatruda *et al.*, 1975; Olefsky, 1976a,b; Czech *et al.*, 1977; Kasuga *et al.*, 1978). As a result, with the exception of the adipocyte, most studies have evaluated the insulin-receptor interaction only and have attempted to draw conclusions concerning insulin sensitivity based on these data. Others have evaluated the insulin-receptor interaction in one tissue and bio-

logical responsiveness in others (DeFronzo *et al.*, 1978). It has been demonstrated, however, that the modulation of the number of available receptors in different tissues may respond differently to the same metabolic alteration (Olefsky *et al.*, 1975). Conversely, the same alteration in insulin receptor number may manifest itself differently in different tissues (LeMarchand *et al.*, 1977). Also, previous studies in adipocytes from spontaneously obese older rats, starved rats, and diabetic rats indicate that while receptor number per unit surface area tends to vary inversely with ambient insulin concentrations, the defect in insulin action lies subsequent to the insulin-receptor interaction (Czech, 1976; Olefsky, 1976a; Czech *et al.*, 1977; Kasuga *et al.*, 1978). It is for these reasons that it is important

to examine both insulin binding and insulin action in the same cellular preparation. Because of the importance of the liver in total insulin action and the recent advances in our ability to isolate insulin-responsive hepatocytes, we have examined the insulin-receptor interaction and insulin action in hepatocytes from spontaneously obese, starved, and streptozotocin-diabetic rats.

Experimental

Animals

Male Sprague-Dawley rats were used for all studies, and with the exception of the 72h-starved rats, were fed *ad libitum*. The weight (mean $\pm$ S.E.M.) of the rats in each group was 209 ± 15 ($n=8$), 414 ± 10 ($n=8$), 190 ± 6 ($n=8$) and 197 ± 12 ($n=8$) g for the control, obese, starved, and diabetic groups respectively. Control, starved, and streptozotocin-induced diabetic rats were approx. 2 months old and obese rats were approx. 4–5 months old. Diabetes was induced in fed rats by a single injection of streptozotocin (85 mg/kg body wt.) via the left femoral vein under light diethyl ether anaesthesia. Liver cells were isolated within 5–7 days from animals that had glycosuria of at least 20 mg/ml and no ketonuria. Serum insulin values while anaesthetized at the time of death were (mean $\pm$ S.E.M.) 39 ± 3 ($n=4$), 60 ± 5 ($n=4$), 5 ± 1 ($n=4$) and 15 ± 2 ($n=4$) μ -i.u./ml in the control, obese, starved, and diabetic groups respectively ($P < 0.005$ for all three groups compared to control rats).

Preparation of hepatocytes

Liver cells were isolated by the method of Feldhoff *et al.* (1977) with minor modifications. The rats were anaesthetized with sodium pentobarbital, 8 mg/100 g body wt. Liver perfusion was performed according to the method of Exton (1975). The perfusion solution was Krebs-Ringer bicarbonate buffer (Krebs & Henseleit, 1932) free of calcium and albumin and supplemented with 5 mM-glutamate, 11 mM-glucose, 5 mM-sodium pyruvate, and 10% (v/v) washed outdated human erythrocytes. The erythrocytes were washed three times with 0.9% (w/v) NaCl. After equilibration of the perfusion medium for 30 min at 37°C with O₂/CO₂ (19:1, v/v), livers were perfused in a non-circulating system for 15 min at 14 ml/min. The system was then set to recirculate and 20 mg of collagenase (Worthington CLS II, 48H141, 152 units/mg) was added to the remaining 125–150 ml of perfusion medium. After 35 min the liver was removed, minced gently with scissors, suspended in 50 ml of perfusion buffer (without erythrocytes) and filtered through nylon. The cells were centrifuged at 50 g in 50 ml conical centrifuge tubes in a Dynac table top centrifuge and

washed three times with perfusion medium without erythrocytes but containing 1 g of bovine serum albumin/100 ml, 2.4 mM-CaCl₂, and normal plasma concentrations of amino acids (Tolman *et al.*, 1973). All buffers were maintained at 37°C and equilibrated with O₂/CO₂ (19:1, v/v). After three washes, cells were suspended in the same buffer containing 3 g of albumin/100 ml to yield 3.0×10^6 – 6.0×10^6 cells/ml and were placed in 250 ml polycarbonate Erlenmeyer flasks with a maximum of 60 ml of suspended cells in one flask. After 30 min of incubation at 37°C with a constant flow of the O₂/CO₂ mixture, the cells were centrifuged again and resuspended at 3.0×10^6 – 6.0×10^6 cells/ml to measure biological response at 37°C, or at 1.5×10^5 – 3.0×10^5 cells/ml to measure insulin binding at 30°C. Flasks were placed in an orbital water bath shaking at 150–250 rev./min with continuous gassing with humidified O₂/CO₂ (19:1, v/v). Cells were counted in a haemocytometer at 1:20 dilution.

Uptake of aminoisobutyrate by hepatocytes

To measure uptake of aminoisobutyrate in freshly isolated hepatocytes, amino[¹⁴C]isobutyrate (0.1 mM, 1.4 mCi/nmol) was added to the Erlenmeyer flasks containing the cells prepared and suspended as described above in the absence and presence of insulin. All assays were performed in triplicate. After the desired period of incubation the reaction was terminated by the oil separation method used for mitochondria (Amatruda *et al.*, 1978). Briefly, 0.3 ml of incubation mixture was layered over 0.1 ml of dibutylphthalate oil in a Beckman microcentrifuge tube. The tube was centrifuged for 2 min at 10000 g in a Beckman Microfuge during which time the cells sedimented through the oil and were separated from the incubation medium. There was no radioactivity associated with the oil phase. The Microfuge tube was cut at the oil-medium interphase, the hepatocyte pellet was dislodged with a plastic disposable pipette tip and the hepatocytes, pipette tip, and bottom portion of the Microfuge tube were placed in 10 ml of Scintiverse and the radioactivity in the cell pellet was determined. Uptake of aminoisobutyrate was linear for at least 1 h in all cell groups (Jefferson, 1976). Insulin responsiveness was first observed at 15 min with a maximum effect achieved at 1 h. Uptake rates are therefore calculated on the basis of the first hour of incubation. The intracellular water space was determined by incubating the cells with 3-O-[¹⁴C]methyl-D-glucose for 30 min at 37°C (Kletzien *et al.*, 1975). The cells were isolated and digested as above and the radioactivity associated with them was determined. At equilibrium the intracellular and extracellular concentrations are equal and the water space of the cells can be determined after adjusting for the trapping of

extracellular water, which is determined by inulin space measurements (Amatruda *et al.*, 1978).

Binding of ^{125}I -labelled Insulin

Insulin was iodinated with chloramine-T according to the method of Cuatrecasas (1971). When prepared in this fashion, the ^{125}I -labelled insulin was at least 95% precipitable by trichloroacetic acid and retained full biological activity. Insulin binding was measured at 30°C and is expressed as specific binding, i.e. the ^{125}I -labelled insulin bound after subtraction of the insulin that remains bound in the presence of a large excess of unlabelled hormone (5 µg/ml). Studies of ^{125}I -labelled insulin binding to freshly isolated hepatocytes were performed at a labelled insulin concentration of 0.185 ng/ml in the presence of increasing concentrations of native insulin in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 3 g of bovine serum albumin/100 ml and supplemented with 11 mM-glucose, 5 mM-sodium pyruvate and normal plasma concentrations of amino acids. Previous studies, as well as our own preliminary data indicated that maximum binding is reached within 30 min at 30°C (Terris & Steiner, 1975). Non-specific binding represents 10.1 ± 2.1 , 10.7 ± 1.4 , 5.9 ± 0.8 and $4.7 \pm 1.3\%$ of total binding in hepatocytes from control ($n = 5$), older obese ($n = 4$), starved ($n = 5$), and diabetic ($n = 4$) rats respectively. Binding of ^{125}I -labelled insulin was assessed by the oil-separation method outlined above for uptake of amino[^{14}C]isobutyrate. Under the conditions utilized to measure insulin binding, the degradation of insulin, measured by precipitation in 5% (w/v) trichloroacetic acid, was less than 10% in all cell groups.

Statistical treatment of results

Statistical analyses for the comparison of groups were performed by the unpaired Student's *t* test, and for comparisons within groups for insulin-treated and non-insulin-treated cells by the paired Student's *t* test. All data are expressed as the mean $\pm$ S.E.M. followed by the number of values used in calculating the mean.

Materials

Carrier free Na^{125}I , [methoxy- ^3H]inulin, (186.4 mCi/g) and α -amino[1- ^{14}C]isobutyric acid (51.6 mCi/nmol) were obtained from New England Nuclear, Boston, MA, U.S.A. 3-*O*-[^{14}C]Methyl-D-glucose (58.9 mCi/nmol) was obtained from Amersham-Searle, Arlington Heights, IL, U.S.A.; crude collagenase came from Worthington Biochemical Corp., Freehold, NJ, U.S.A., fraction V bovine serum albumin from Reheis Chemical Co., Kankakee, IL, U.S.A., α -aminoisobutyric acid from Calbiochem, San Diego, CA, U.S.A., and all natural amino acids from Sigma. Crystalline porcine insulin

was kindly provided by Dr. Ronald Chance of Eli Lilly and Co., Indianapolis, IN, U.S.A., and streptozotocin by the Upjohn Company, Kalamazoo, MI, U.S.A. Scintiverse was purchased from Fisher Scientific Co., Pittsburgh, PA, U.S.A.

Results

Liver cells from obese animals were similar in size to cells from control animals [mean $\pm$ S.E.M. = 4.4 ± 0.2 ($n = 4$) versus 5.2 ± 0.5 ($n = 4$) µl cell water/ 10^6 cells, respectively], and had a similar basal uptake of aminoisobutyrate [525 ± 19 ($n = 8$) versus 523 ± 19 ($n = 8$) pmol/h per 10^6 cells respectively]. In contrast, hepatocytes from starved animals were significantly smaller [3.1 ± 0.2 ($n = 4$) µl/ 10^6 cells, $P < 0.01$] and had a higher basal rate of uptake of aminoisobutyrate [718 ± 87 ($n = 8$) pmol/h per 10^6 cells, $P < 0.02$] when compared to the control and obese groups. The water space of hepatocytes from diabetic animals (5.82 ± 0.45 ($n = 4$) µl/ 10^6 cells) was similar to that of hepatocytes from control and obese animals, yet the basal rate of uptake of aminoisobutyrate was enhanced (1010 ± 38 ($n = 8$) pmol/h per 10^6 cells, $P < 0.001$).

The insulin dose–response relationships for uptake of aminoisobutyrate are illustrated in Fig. 1. Hepatocytes from obese 450 g rats, diabetic rats, and control 200 g rats responded significantly to insulin at insulin concentrations of 10^{-10} – 10^{-6} M in hepatocytes from obese ($P < 0.05$ to $P < 0.001$ by paired *t* analysis) and diabetic ($P < 0.02$ to $P < 0.001$ by paired *t* analysis) rats and 10^{-11} – 10^{-6} M in hepatocytes from control 200 g rats ($P < 0.01$ to $P < 0.001$ by paired *t* analysis). Hepatocytes from starved rats responded to insulin from 10^{-10} to 10^{-6} M ($P < 0.02$ to $P < 0.001$ by paired *t* analysis) but this response was significantly less than that of hepatocytes from control animals ($P < 0.05$ to $P < 0.01$ by unpaired *t* analysis) at insulin concentrations of 10^{-8} – 10^{-6} M. With one exception [hepatocytes from diabetic versus control 200 g rats at 10^{-8} M-insulin ($P < 0.05$ by unpaired *t* analysis)], the maximum increase in aminoisobutyrate uptake due to insulin was the same for control, older obese, and diabetic animals. From these curves the concentration of insulin that elicits a half-maximal response in hepatocytes from control, obese, diabetic, and starved animals is 8.5×10^{-10} , 9.3×10^{-10} , 5.5×10^{-10} and 2.3×10^{-10} M respectively.

There is again no difference between hepatocytes from control 200 g and older 450 g rats when the dose–response curves for insulin-stimulated uptake of aminoisobutyrate are expressed as a percentage increase above basal. The maximal insulin effect was $45 \pm 4\%$ and $47 \pm 4\%$ above basal for control and older, obese animals respectively. Hepatocytes from diabetic rats had an enhanced basal rate of amino-

Fig. 1. Dose-response curves for insulin-stimulated uptake of aminoisobutyrate in hepatocytes from control 200g rats (●), older 450g rats (Δ), streptozotocin-diabetic rats (○) and 72 h-starved rats (□)

The data are expressed as the increment in aminoisobutyrate uptake due to insulin measured at 1 h after the addition of insulin and amino-¹⁴Cisobutyrate as discussed in the Experimental section. The data represent the means $\pm$ S.E.M. of eight separate experiments for each group of animals. The basal rates of uptake and the *P* values are stated in the text. Asterisks indicate values significantly different from controls and the arrows indicate the concentration of insulin that elicits a half-maximal response.

Fig. 2. Scatchard analysis of insulin binding to freshly isolated hepatocytes from control (●), 72 h-starved (□), obese (Δ), and streptozotocin-diabetic (○) rats

The conditions of cell isolation and measurement of insulin binding are described in the Experimental section. The data represent the mean of triplicate determinations of four to five separate experiments in each group. *, Bound/free and bound significantly greater than control ($P < 0.02$ to $P < 0.005$ by unpaired *t* test).

isobutyrate uptake and a similar change in uptake due to insulin (Fig. 1) as compared with control 200g rats. As a result, the percentage increase in uptake due to insulin was significantly less ($26 \pm 3.5\%$, $P < 0.001$ by unpaired *t* test) than that in hepatocytes from control rats. Owing to both an enhanced basal rate of uptake and decreased responsiveness, the percentage increase in uptake due to insulin was also significantly less in hepatocytes from starved animals ($21 \pm 6\%$, $P < 0.05$ by unpaired *t* test).

Fig. 2 illustrates Scatchard analyses of the insulin-binding data in hepatocytes from the four groups of rats. At the lowest insulin concentration investigated, 0.185 ng of ¹²⁵I-labelled insulin/ml, the bound/free ratios were 0.038 ± 0.003 , 0.041 ± 0.007 , 0.047 ± 0.004 , and 0.082 ± 0.014 in the control, obese, starved, and diabetic groups. Only the cells from diabetic animals bound significantly more insulin ($P < 0.02$) although there was a tendency for the cells from starved animals to bind more at low concentrations of hormone. If consideration is given to the 28% decrease in the surface area of cells from starved rats, these hepatocytes bound more insulin

per unit surface area of membrane. Hepatocytes from diabetic animals bound significantly more insulin and had significantly greater bound/free ratios at all insulin concentrations except 1 and 500 ng/ml ($P < 0.02$ to $P < 0.005$). From these curves, if one assumes a two-site model for insulin binding, the mean high-affinity dissociation constant, K_d , can be calculated by linear regression of the first portion of the Scatchard analyses. The K_d values for insulin binding were the same in all four groups [means $\pm$ S.E.M. = $(1.2 \pm 0.2) \times 10^{-9}$ ($n = 5$), $(1.0 \pm 0.1) \times 10^{-9}$ ($n = 4$), $(1.2 \pm 0.2) \times 10^{-9}$ ($n = 5$), and $(0.8 \pm 0.1) \times 10^{-9}$ ($n = 4$) M in the control, obese, starved, and diabetic groups respectively]. Whereas hepatocytes from diabetic rats had a lower K_d of binding, this did not achieve statistical significance ($P < 0.2$). This indicates that the enhanced binding in diabetic animals is mainly due to an increased number of binding sites and not to an altered affinity for insulin. Also, while it appears that the total number of insulin receptors is decreased in cells from older and starved animals, these differences did not achieve statistical significance because of the larger error in binding determinations at high concen-

trations of hormone. Under the conditions used to measure insulin binding, insulin degradation was minimal in all cell groups (see the Experimental section) and cannot explain the observed differences in insulin binding.

Discussion

The present studies were designed to determine the relationship between alterations in the insulin-receptor interaction and changes in insulin sensitivity and maximal insulin responsiveness in hepatocytes from rats with altered metabolic states. These studies indicated that hepatocytes from older obese rats are similar to those from younger rats with regard to cell size, basal levels of aminoisobutyrate uptake, maximal insulin responsiveness (Fig. 1) and the dose-response relationship for insulin-stimulated uptake of aminoisobutyrate (Fig. 1). In addition, the insulin-binding studies demonstrated similar receptor numbers and affinities in cells from older obese and younger rats (Fig. 2). In contrast, hepatocytes from non-ketotic streptozotocin-diabetic rats demonstrate increased basal rates of aminoisobutyrate uptake and enhanced sensitivity to insulin, as manifested by a shift to the left of the dose-response curve for insulin-stimulated uptake of aminoisobutyrate (Fig. 1). The maximal response to insulin is, however, the same as that in hepatocytes from non-diabetic control animals. These data are consistent with the observed increase in insulin receptor number in hepatocytes from diabetic animals (Fig. 2) as reflected in the enhanced sensitivity to insulin. Hepatocytes from 72 h-starved rats have enhanced basal rates of aminoisobutyrate uptake, as do those from diabetic animals. However, hepatocytes from starved animals are smaller than those from control 200g animals, and demonstrate a decreased ability to respond maximally to insulin (Fig. 1). This decreased maximal response is demonstrated in the presence of an enhanced sensitivity to insulin, as manifested by a decrease in the concentration of insulin needed for a half-maximal response (Fig. 1). This shift in sensitivity must be qualified, however, as it is present because of decreased maximal responsiveness to insulin and not because of enhanced sensitivity at low insulin concentrations. Thus, in starvation, coupling between insulin receptors and insulin-induced uptake of aminoisobutyrate appears to be intact, while the maximal responsiveness to insulin at 0.1 mM-aminoisobutyrate is impaired. The apparent increased sensitivity to insulin in starvation may occur as the result of the observed increase in the number of insulin receptors per unit surface area of the hepatocytes. It is likely that the decrease in maximal responsiveness is due to alterations in post-receptor events.

Although insulin binding to hepatocytes from older obese rats has not been previously evaluated, our data are similar to those in liver plasma membranes (Lockwood & East, 1978) and by some (Livingston *et al.*, 1972) but not all (Olefsky, 1976c) investigators using adipocytes, and demonstrate similar receptor numbers and affinities in cells from larger older rats. Our data from hepatocytes from streptozotocin diabetic animals are also in agreement with those of others who have demonstrated enhanced insulin binding to hepatic plasma membranes (Davidson & Kaplan, 1977), and adipocytes (Kasuga *et al.*, 1978). Insulin binding during starvation appears to be more complex, and has been reported to be increased due to changes in numbers of receptors (DeFronzo *et al.*, 1978; Olefsky & Reaven, 1975), and changes in affinity (DeFronzo *et al.*, 1978; Olefsky & Kobayashi, 1978; Bar *et al.*, 1976). In one study, (Almira & Reddy, 1979) however, whereas receptor affinity was increased in liver plasma membranes, receptor number was decreased in hepatocytes from 72 h-starved rats. Our data indicate equal numbers of receptors per cell in 72 h-starved rats. If expressed per unit of surface area, however, hepatocytes from starved rats would appear to bind more insulin than hepatocytes from control animals. As previously stated, this may be responsible for the decrease in the insulin concentration required for half-maximal insulin-stimulated uptake of aminoisobutyrate (Fig. 1).

Several studies have examined insulin binding to plasma membranes derived from the liver and adipose tissue of animals in various metabolic states. From these studies in animals it is generally accepted that insulin binding per unit of plasma membrane surface area and plasma insulin concentrations are inversely related. This has been demonstrated in liver plasma membranes from diabetic rats (Davidson & Kaplan, 1977) as well as diabetic (Soll *et al.*, 1975) and obese (Freychet *et al.*, 1972; LeMarchand *et al.*, 1977, 1978; Kahn *et al.*, 1973a,b) mice. The present studies in isolated hepatocytes confirm these findings that insulin binding in hepatocytes from starved animals is increased on the basis of membrane surface area. Binding to hepatocytes from diabetic animals is increased per cell as well as per membrane surface area. Both diabetic and fasted animals had low insulin levels. However, as reported for rat liver plasma membranes (Lockwood & East, 1978), thymocytes (Goldfine, 1975) and adipocytes (Livingston *et al.*, 1972) from adult obese animals insulin binding is unchanged despite an increase in plasma insulin concentrations in these animals. We (Amatruda *et al.*, 1975) and others (Misbin *et al.*, 1979) have reported a similar finding in human obesity. It is not clear at this time why such a dissociation between insulin binding and circulating

insulin concentrations occurs. Also, while the use of older rats as a model of spontaneous obesity with regard to adipocyte metabolism seems justified on the basis of enlargement of cell size with aging and the demonstration that fat-cell size, and not age, is the major determinant of insulin sensitivity (Czech *et al.*, 1977), the use of this model of obesity with regard to hepatocyte function has not been established. It is important to recognize that our data in hepatocytes from older, spontaneously obese animals may reflect hepatocyte function with aging and not obesity. Kahn *et al.* (1973a) have demonstrated that aging has no effect on specific insulin binding by mouse liver membranes, although genetic obesity in mice is associated with a decrease in insulin receptor numbers (Kahn *et al.*, 1973a,b).

In many cases, the ability of a tissue to respond to insulin is altered independently of qualitative or quantitative changes in the insulin-receptor interaction. Examples of this include adipocytes from older spontaneously obese rats (Livingston *et al.*, 1972; Olefsky, 1976c), from rats fed high-carbohydrate diets (Cushman & Salans, 1975), from starved rats (Olefsky, 1976a), from streptozotocin-diabetic rats (Kasuga *et al.*, 1978), and from the 4–12-week-old genetically obese (*ob/ob*) mouse (Freychet *et al.*, 1972; LeMarchand *et al.*, 1977; Czech *et al.*, 1977). In addition, LeMarchand *et al.* (1977) demonstrated an increase in insulin receptor number in liver, muscle, and adipose-cell membranes of the genetically obese (*ob/ob*) mouse after starvation and streptozotocin treatment, both of which lower plasma insulin concentrations. However, although the increase in number of insulin receptors in muscle membranes was accompanied by an increase in muscle-tissue sensitivity to insulin, this enhanced sensitivity to insulin was not observed in liver and adipose tissue. These authors state that the varying relationships between insulin binding and tissue responsiveness most likely reflect the multiplicity of factors involved. A similar conclusion was reached concerning the gold-thioglucose-treated obese mouse (LeMarchand *et al.*, 1978). Our data indicate that explanations other than alterations in the insulin-receptor interaction must be invoked to explain the decreased maximal responsiveness to insulin of hepatocytes from starved rats as well.

Finally, our data indicate that basal rates of uptake of aminoisobutyrate in hepatocytes from starved and diabetic rats are significantly higher than those of hepatocytes from control animals. This has been reported previously by Fehlmann *et al.* (1979) in hepatocytes from 48h-starved animals, and is attributed to the appearance, during starvation, of a high-affinity transport component with properties of a pure 'A' system. Our data indicate that this may be true for hepatocytes from diabetic animals as well, and that this component may play a regulatory role

in the control of gluconeogenesis in starvation and diabetes.

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