Dysregulation of Glucose Transport in Hearts of Genetically Obese *(fa/fa)* Rats

D. Zaninetti, M. Crettaz and B. Jeanrenaud

Laboratoires de Recherches Métaboliques, Geneva University Medical School, Geneva, Switzerland

Summary. Overall D-glucose metabolism and 3-0-methylglucose transport were measured in the perfused heart preparation of lean and genetically obese *(fa/fa)* rats. Absolute values of basal and insulin-stimulated glucose metabolism were decreased in hearts of 15-week-old obese rats when compared to lean age-matched controls. Basal and maximally stimulated (i.e., by the combined addition of insulin and increasing perfusion pressure) 3-0-methylglucose transport was normal in hearts from young obese rats (5-week-old). However, when only one stimulus was used (insulin or increasing perfusion pressure alone), 3-0-methylglucose transport was stimulated to values that were lower than those of lean rats. Basal 3-0-methylglucose transport was four times lower in hearts from older obese rats (15-week-old) than in lean ones of the same age. At this age, stimulation of 3-0-methylglucose transport by insulin alone, by increasing perfusion pressure alone or by the combination of both stimuli, reached values in obese rats that were only half those of lean animals. It is concluded that: (a) in the early phase of the syndrome, the basal glucose transport system in hearts of obese rats is normal, but its response to stimulation becomes abnormal and; (b) at a later phase of obesity, the glucose transport system becomes abnormal even under basal conditions and its responsiveness to various stimuli is markedly impaired.

Key words: Perfused heart; genetically obese rats; glucose transport; insulin; perfusion pressure.

Obesity in man and laboratory animals is usually associated with hyperinsulinaemia and insulin resistance [1]. It was initially thought that the major cause of insulin resistance in obese hyperinsulinaemic animals was the decreased ability of plasma membranes of liver [2-5], adipose tissue [6] and muscle [4, 7] to bind insulin, an abnormality that could be accounted for by a decrease in specific insulin receptor number [8]. Subsequent studies have suggested that additional defects unrelated to the insulin receptors (i.e. post-receptor defects) also contributed to insulin resistance [1, 9, 10]. In insulin-resistant muscles, several such post-receptor defects have been described [4, 7, 10-14]. In isolated soleus muscles of the genetically obese Zucker *(fa/fa)* rat the following post-receptor abnormalities have been shown: (a) increased utilization of endogenous fatty acids inhibitory to glycolysis [7]; (b) decreased uptake of the D-glucose analogue, 2-deoxy-D-glucose (2-DG) [4, 7, 12]. In the perfused hindquarter of obese hyperglycaemic *(db/db)* mice the existence of a post-receptor defect at the level of glucose transport (again measured with 2-DG) has been proposed to be the major cause of insulin resistance [15]. Thus the evidence that insulin resistance could be partly attributed to defectual glucose handling has been indirect, based either on overall glucose metabolism or on 2-DG glucose uptake.

Studies carried out with 2-DG have two major drawbacks: (a) 2-DG is not only taken up but phosphorylated [16]; (b) transport of 2-DG from some plasma membrane site to intracellular water is a rate-limiting step [17]. In contrast, the other D-glucose analogue, 3-0-D methylglucose (3-0-MG) has been reported to enter the membrane and to reach the cell interior from such membrane without passing via a rate-limiting step [17]. Furthermore, 3-0-MG is transported as D-glucose but is not metabolized [18]. It represents therefore a better index of actual transport of D-glucose.

The present experiments were undertaken to assess the existence, in hearts of genetically obese *(fa/fa)* rats, of a possible defect at the level of glucose transport per se. As glucose transport is symmetrical, once 3-0-MG is transported inside the cells, it is rapidly counter-transported out [17]: the measure of glucose transport was therefore made on the basis of its rate of efflux following periods of pre-loading heart with labelled 3-0-MG.

The choice of heart muscle as a model was made on the following basis: (a) skeletal muscles such as soleus are using 70%-90% of needed substrates as lipids [7],
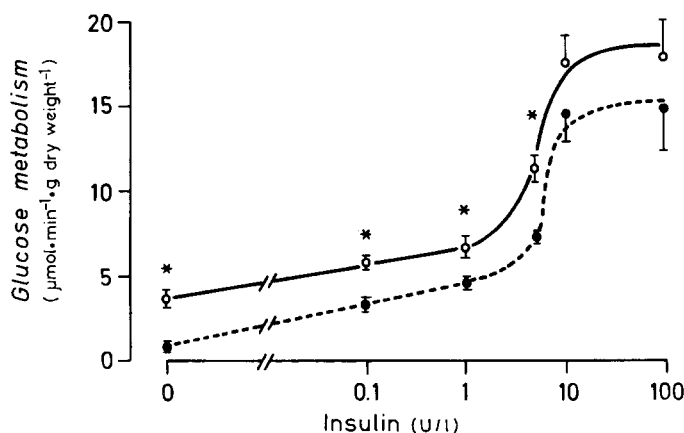
Fig. 1. Insulin dose response curve of glucose metabolism (i.e., glucose disappearance from medium) in perfused hearts of 15-week-old lean (O——O) and genetically obese *(fa/fa)* rats (● --- ●). All points are in absolute values which represent mean ± SEM of three to ten experiments. Perfusion time was 7 min, and the perfusion medium was Krebs-Ringer bicarbonate buffer containing 6 mmol/l glucose. *: $2p < 0.05$

making it difficult to investigate glucose uptake; (b) in contrast, heart, when perfused at 50 mmHg pressure and with 6 mmol/l glucose, utilizes 90%–100% of glucose as energy supply [19, 20].

Materials and Methods

Animals

Genetically obese *(fa/fa)* rats and their lean control *(FA/fa; FA/FA)* bred in our laboratories were used (at 5 or 15 weeks of age). They had free access to a standard laboratory chow (UAR, Epinay/Orge, France) and were maintained at a constant temperature (23 °C) in an animal house with a 12 h artificial light cycle.

Heart Perfusion

Rats were anaesthetized with pentobarbital (90 mg/kg). The heart was rapidly removed, placed in ice-cold 0.9% NaCl for 10 s, cannulated via the aorta and perfused, according to the Langendorff technique [20, 21] in which the perfusion medium is pumped into the left ventricle via the aorta just above the coronary arteries.

The perfusion medium used for measurement of glucose metabolism consisted of a Krebs-Ringer bicarbonate buffer (pH 7.4) with 6 mmol/l glucose. For measurement of 3-0-MG transport, glucose was replaced by pyruvate as energy source, as reported elsewhere [7].

Glucose Metabolism

To measure glucose metabolism, hearts were pre-perfused for 3 min (flow-through, wash-out period), then perfused for an additional 3 min with a recirculating medium, at which time a first sample of medium was taken. A second sample of medium was collected 7 min later. Glucose concentrations in the perfusion media so obtained were measured with a glucose analyser (Beckman, Fullerton, California, USA). This allowed determination of overall glucose metabolism by glucose disappearance from the medium. The glucose disappearance and lactate production were linear at least for 25 min. At the end of the perfusion (t = 7 min), hearts were freeze-clamped and used for subsequent measurements of metabolic intermediates and dry weights. Frozen hearts were homogenized (glass homogenizer, ABS, Geneva, Switzerland) in 5% (w/v) HClO$_4$ and centrifuged (2 000 g for 10 min). Supernatants were neutralized with K$_2$CO$_3$ and used for fluorimetric measurements of citrate [22], fructose-6-phosphate and

glucose-6-phosphate concentrations [23]. Results were expressed per gram dry weight [19].

3-0-MG Uptake as Measured by Its Efflux Rate

To measure glucose transport per se, hearts were pre-perfused with or without insulin (10 min) with a recirculating medium containing 6 mmol/l (0.3 μCi/ml) ^{14}C-3-0-methyl-D-glucose, 3-0-MG and 6 mmol/l (0.3 μCi/ml) ^{3}H-L-glucose (loading period). Ten minutes were needed for the perfusion to reach a steady-state level. Labelled L-glucose was used to calculate the extracellular and free diffusion components. At the end of the loading period, perfusion was continued without recirculation while switching to a medium containing no sugar. Fractions were collected every 12 s in a fraction collector (Gilson TDC 220, Villier le Bel, France). Vials obtained from the collector each contained 12s fractions of medium with 3-0-MG and L-glucose arising from the heart. 3-0-MG was in the intra- and the extracellular space, L-glucose mainly in extracellular space and to some extent (via simple diffusion) in intracellular space. 3-0-MG counter-transported via facilitated diffusion (efflux) could be calculated by subtracting, in each vial, total labelled L-glucose content from total labelled 3-0-MG content. The plot of cumulative effluxes as a function of time was used to obtain kinetics of efflux. Various tests of linearity (e.g. 0, 1, 2 order kinetics) indicated that cumulative efflux curves fitted the first order kinetic formula: $A(t) = A_0(1-e^{-kt})$, where A was the quantity of effluent hexose(s) at given time (t); A_0 the maximal quantity of effluent hexose at infinite time; e = base of natural logarithm; k: the kinetic constant. The rate of efflux (V) during a small period of time was: $V = dA/dt = kA_0e$ (kt). As initial rate of efflux mimicked glucose transport, the rate was calculated with a t value equal to 0 s. The initial speed (V_i), thus, was: $V_i = kA_0$, expressed as μmol · min^{-1} · heart^{-1}.

When the effect of insulin was studied, the hormone was added to both pre- and perfusion media. When basal glucose metabolism or efflux rates were investigated (without added insulin) a pre-perfusion period of 10 min was allowed to wash away endogenous insulin.

Statistical Analysis

Statistical analysis was performed using the Student's t-test for unpaired data (2P).

Results

Basal and Insulin-Stimulated Glucose Metabolism in Hearts from 15-Week-Old Lean and Obese Rats

The dose response of insulin-stimulated glucose metabolism is shown in Figure 1. In normal hearts, total glucose uptake and metabolism (i.e., glucose disappearance from the medium) reached maximal values at a high insulin concentration (10 U/l), half-maximal response being obtained at 5.5 U/l. The low sensitivity of perfused heart to insulin was not due to degradation of insulin: insulin concentration in the medium before and after passage through the heart was measured and no significant change was detected (data not shown). As can also be seen in Figure 1, basal and insulin-stimulated glucose metabolism was clearly lower (expressed in absolute values) in hearts of obese than in control rats. In particular, basal glucose metabolism was four times lower in hearts from obese than control rats. When results were expressed in absolute increases of insulin-stimulated glucose over baseline values, both sensitivity (half-maximal effect of the hormone) and the responsiveness (maximal effect of the hormone) were similar

in hearts of lean and obese rats. This indicated that the major defect of glucose handling by heart from obese rats was at the level of *basal* glucose metabolism.

Intracellular Glucose Metabolism in the Heart of 15-Week-Old Lean and Obese Rats

Previous studies carried out in skeletal (soleus) muscle of obese rodents have shown the existence of an increased fatty acid utilization which, via citrate accumulation, inhibits glycolysis at the level of phosphofructokinase level, with resulting accumulation of glucose-6-phosphate, fructose-6-phosphate and glycogen [24]. To determine whether such inhibition would be present in heart of obese rats and be responsible for the absolute decreases in basal and insulin-stimulated glucose metabolism, intracellular concentrations of glucose-6-phosphate, fructose-6-phosphate and citrate were measured. Basal, as well as insulin-stimulated values, of glucose-6-phosphate, fructose-6-phosphate and citrate were not different in hearts from lean and obese rats (data not shown).

Basal and Insulin-Stimulated 3-0-Methyl Glucose Uptake (as Measured by Efflux Rates) in Hearts of 15-Week-Old Lean and Obese Rats

As decreased *basal* glucose metabolism (without insulin) appeared to be the major defect in the heart of obese rats, actual 3-0-MG transport was assessed by measuring efflux rates as described above. In normal rats (Fig. 2) insulin stimulated 3-0-MG efflux fourfold when compared to basal values. 3-0-MG efflux was complete within 3 min. The $t_{1/2}$ was 36 s for basal and 18 s for insulin-stimulated 3-0-MG efflux rates. The insulin-stimulated 3-0-MG efflux process was completely abolished by superimposed addition of cytochalasin b, a drug which specifically binds to glucose transporter units [25]. In absolute terms and as shown by Figure 3 A, basal and insulin-stimulated 3-0-MG efflux by the heart of obese rats was significantly lower than that of controls. Taking into account the observation that basal 3-0-MG efflux was decreased in hearts of obese rats (when results obtained with insulin were expressed as absolute increases over basal values), it was found that the sensitivity (half-maximal response to the hormone) of heart from obese rats was similar in both groups of animals, but that an impairment in the maximal response to insulin was present in obese animals (Fig. 3 B). Upon plotting data of 3-0-MG transport (Fig. 3 A) against those of glucose actually metabolized (data of Fig. 1), the resulting slopes were 1.40 ± 0.03 for lean rats (correlation coefficient of 0.99) and 0.89 ± 0.04 (correlation coefficient of 0.96) for obese rats, respectively. As the estimated k_m for glucose transport is 7 mmol/l [24] and for glucose metabolism is 9 mmol/l [26], the ratio of glucose transport to glucose metabolism should be around 0.80 if glucose transport were to represent the rate-limiting step of glucose metabolism.
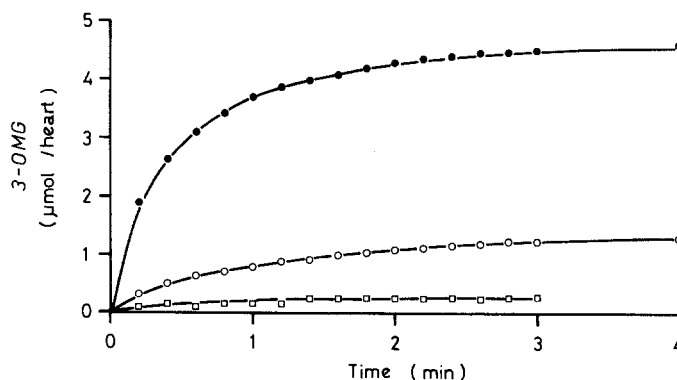

Fig. 2. Cumulative 3-0-methyl glucose (3-0-MG) efflux in perfused hearts of 15-week-old lean rats in the absence (O — O) or the presence (● --- ●) of insulin (10 U/l) or in the presence of insulin + cytochalasin b (100 μmol/l) (□ --- □). The data shown are taken from a representative experiment repeated at least four times
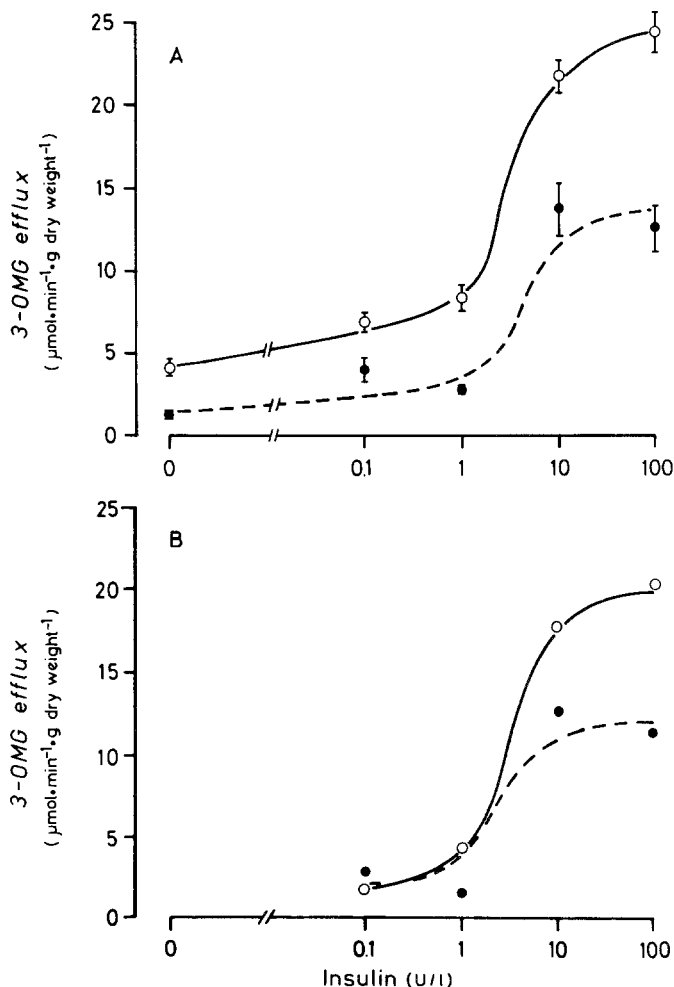

Fig. 3 A and B. This figure represents an insulin dose response curve of 3-0-methyl glucose (3-0-MG) efflux in perfused heart of 15-week-old lean (O——O) and genetically obese *(fa/fa)* rats (● --- ●). Upper panel (A): data plotted as absolute values (mean ± SEM) of three to four experiments; lower panel (B): data shown as absolute increases over basal values. All the points of lean versus obese are statistically significant: $2p < 0.02$
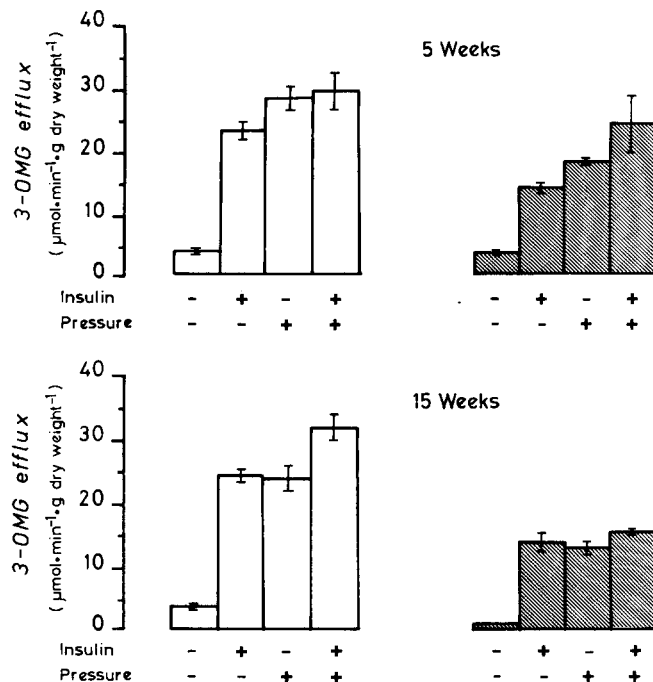
Fig.4. 3-0-methyl glucose efflux (3-0-MG) measured in perfused heart of 5- and 15-week-old lean (□) and genetically obese *(fa/fa)* rats (▨). When present (+), insulin was at a concentration of 10 U/l. Pressure (−) refers to heart perfused at low 50 mmHg perfusion pressure; pressure (+) refers to heart perfused at 100 mmHg pressure. Each bar is the mean ± SEM of three to four experiments. All the differences of lean versus obese are statistically significant (2*p* < 0.005), except lean versus obese of 5 week-old rats, in which basal and insulin plus pressure stimulated, are not statistically different

Assuming analogous K_m values in obese rats this would suggest that glucose transport would be rate-limiting in hearts of these animals (observed ratio of 0.89) at all insulin concentrations used, while such would not be the case for normal controls (observed ratio of 1.40).

Basal, Insulin- and Pressure-Stimulated 3-0-Methyl Glucose Uptake (Measured by Efflux Rates) in Hearts of 5 and 15-Week-Old Lean and Obese Rats

As glucose transport appeared to be the major defect in the hearts of obese rats and as the pathology of insulin-resistant animals often changes with the duration of the syndrome [7], 5-week as well as 15-week-old rats were studied. Workload is known to be a major regulator of glucose utilization at physiological concentrations of insulin [21] so the effect of varying perfusion pressure was also tested. As shown by Figure 4, in heart from normal 5 or 15 week-old rats, insulin or increasing perfusion pressure markedly stimulated 3-0-MG efflux. When combined, insulin and higher perfusion pressure had no significant additive effect. Basal 3-0-MG efflux was also normal in heart of young obese (5 weeks old) when compared to normal rats (Fig. 4). Despite this, supramaximal concentrations of insulin alone (tested at 50 mmHg pressure), or increased perfusion pressure alone, (i.e., 100 mmHg in the absence of insulin) were

not able to stimulate 3-0-MG efflux to values as high as those obtained in normal hearts of the same age. When the two stimuli were applied together, 3-0-MG efflux from hearts of 5-week-old obese rats was further increased, reaching values as high as those of the respective controls. In older obese rats (15 weeks), basal 3-0-MG efflux was fourfold lower than that of controls (Fig. 4, lower panel). This defect could not be overcome by the addition of insulin, by increasing perfusion pressure or by the combination of insulin and higher perfusion pressure, the respective effect of these stimuli always remaining half that seen in hearts of normal rats (Fig. 4, lower panel).

Discussion

The present study shows that glucose metabolism (glucose disappearance from perfusion medium) by normal rat heart is poorly sensitive to insulin (as shown by the dose response curve), the first one to be carried out in this organ. Taegmeyer et al. [20] have reported such an effect at a concentration of 10 U/l, a result comparable to that obtained in our work. Insulin degradation is not responsible for the low sensitivity of the heart to the hormone. These data, as well as others [20, 21] indicate that insulin is not the main regulator of glucose metabolism in normal hearts, in contrast to work load which is a potent stimulator of glucose metabolism.

When data are expressed in absolute values, basal and insulin-stimulated glucose metabolism in heart of 15-week-old genetically obese rats is decreased compared to that of lean controls. Decreased basal glucose metabolism and the inability of insulin in absolute terms, to overcome this defect is not due to increased intracellular lipid utilization inhibitory to glycolysis (i.e., to an increased glucose fatty acid cycle), as reported for soleus muscle [7]. Given that basal glucose metabolism in hearts of 15-week-old obese rats was fourfold lower than in controls, this defect has to be taken into consideration vis a vis the insulin effect. Thus, when data obtained with insulin are expressed as absolute increases over baseline, insulin-stimulated glucose metabolism in hearts of obese rats is superimposable upon those of controls. Hearts from 15-week-old obese rats are therefore not strictly speaking, insulin-resistant but their main defect appears to be located at the level of glucose transport per se. This is supported by the data on 3-0-methyl D-glucose transport as assessed by 3-0-MG efflux rates. This measure is a valid measurement of the D-glucose transport system as it is stimulated by high insulin concentrations and is abolished by cytochalasin b, a drug that binds to glucose transport units. In 15-week-old obese rats 3-0-MG efflux is four times lower than that of normal controls, and is the likely cause of the decreased basal glucose metabolism observed in these animals. For 3-0-MG transport, even when data are expressed as absolute increases over baseline, this process is insulin-resistant in hearts of 15-

week-obese animals since supramaximal concentration of the hormone cannot reach values obtained by such concentrations added to normal hearts (decreased insulin responsiveness).

The defective basal 3-0-MG transport in hearts of obese rats is of late occurrence: it is absent at 5 weeks but becomes evident at 15 weeks of age. However, at 5 weeks of age, the regulation of this process is already abnormal: insulin or increased perfusion pressure alone stimulates glucose transport to values that are only half of those seen in normal hearts. Only when combined can these stimuli enhance glucose transport to normal values.

As the obesity syndrome evolves, basal 3-0-MG transport becomes abnormal and is four times lower than that seen in normal rats. The regulation of the process is now markedly altered since insulin and high perfusion pressure, singly or combined, are only able to increase the transport process to values half of those observed in controls.

To summarize, these data demonstrate the existence of an abnormal glucose transport system per se in the hearts of genetically obese *(fa/fa)* rats. This "post-insulin receptor" defect is also abnormally regulated by insulin, by perfusion pressure, or by the combination of the two stimuli; it worsens with increasing duration of the obesity syndrome. Whether such abnormal regulation is due to altered translocation of glucose transport systems (triggered by one of the other of these stimuli) from some intracellular pool to the plasma membrane, translocation known to occur in normal muscle [25] and adipose tissue [27], or to altered synthesis of the glucose transport system remains to be established.

References

1. Assimacopoulos-Jeannet F, Jeanrenaud B (1976) The hormonal and metabolic basis of experimental obesity. Clin Endocrinol Metab 5: 337–365
2. Kahn CR, Neville DM, Roth J (1973) Insulin-receptor interaction in the obese-hyperglycemic mouse. J Biol Chem 248: 244–250
3. Soll AH, Neville DM, Kahn CR (1975) Insulin binding to liver plasma membranes in the obese hyperglycemic (ob/ob) mouse. J Biol Chem 250: 4702–4707
4. Le Marchand-Brustel Y, Jeanrenaud B, Freychet P (1978) Insulin binding and effects in isolated soleus muscle of lean and obese mice. Am J Physiol 234: E348–E358
5. Lockwood DH, Hamilton CL, Livingston JN (1979) The influence of obesity and diabetes in the monkey on insulin and glucagon binding to liver membranes. Endocrinology 104: 76–81
6. Olefsky JM (1981) Insulin resistance and insulin action on in vitro and in vivo perspective. Diabetes 30: 148–162
7. Crettaz M, Prentki M, Zaninetti D, Jeanrenaud B (1980) Insulin resistance in soleus muscle from obese Zucker rats. Involvement of several defective sites. Biochem J 186: 525–534
8. Kahn CR (1980) Role of insulin receptors in insulin resistant states. Metabolism 29: 455–466
9. Cushman SW, Zarnowski MJ, Franznsoff AJ, Salans LB (1978) Alteration in glucose metabolism, and its stimulation by insulin in isolated adipose cells during the development of genetic obesity in the Zucker fatty rat. Metabolism 27 (Suppl 2): 1930–1940
10. Czech MP, Richardson DK, Becker SG, Walters CG, Glitomer W, Heinrich J (1978) Insulin response in skeletal muscle and fat cells of the genetically obese Zucker rat. Metabolism 12 (Suppl 2): 1967–1981
11. Berger M, Hagg SA, Goodman MN, Ruderman NB (1976) Glucose metabolism in perfused skeletal muscle. Effects of starvation, diabetes on glucose uptake and disposition. Biochem J 158: 191–202
12. Cuendet GS, Loten EG, Jeanrenaud B, Renold AE (1976) Decreased basal, non-insulin-stimulated glucose uptake and metabolism by skeletal soleus muscle isolated from obese hyperglycemic *(ob/ob)* mice. J Clin Invest 58: 1078–1088
13. Kemmer FW, Berger M, Herberg L, Gries A, Wirdeir A, Becker K (1979) Glucose metabolism in perfused skeletal muscle. Insulin resistance in the obese Zucker rat. Biochem J 178: 733–741
14. Le Marchand-Brustel Y, Freychet P (1978) Studies of insulin insensitivity in soleus muscles of obese mice. Metabolism 27: 1982–1993
15. Chan TM, Dehaye JP (1981) Hormone regulation of glucose metabolism in the genetically obese-diabetic mouse *(db/db)*. Diabetes 30: 211–218
16. Kipnis DM, Cori CF (1960) Studies of tissues permeability. IV. The penetration and phosphorylation of 2-deoxyglucose in the diaphragm of diabetic rats. J Biol Chem 235: 3070–3075
17. Foley JE, Foley R, Gliemann J (1980) Glucose-induced acceleration of deoxyglucose transport in rat adipocytes. Evidence for a second barrier to sugar entry. J Biol Chem 255: 9674–9678
18. Whitesell RR, Gliemann S (1979) Kinetic parameters of transport of 3-0-methylglucose and glucose in adipocytes. J Biol Chem 254: 5276–5283
19. Randle PJ, England PJ, Randle RM (1970) Control of the tricarboxylate cycle and its interactions with glycolysis during acetate utilization in rat heart. Biochem J 117: 677–695
20. Taegmeyer H, Hems R, Krebs HA (1980) Utilization of energy-providing substrates in the isolated working rat heart. Biochem J 186: 701–711
21. Neely JR, Liebermeister H, Battersby EJ (1967) Effect of pressure development on oxygene consumption by isolated heart. Am J Physiol 212: 804–814
22. Passonneau JV, Brown JG (1974) Citrat. In: Bergmeyer HU (ed). Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim, pp 1611–1615
23. Lang G, Gerhard M (1974) Glucose-6-phosphate and fructose-6-phosphate. In: Bergmeyer HU (ed). Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim, pp 1283–1287
24. Cheung JY, Conover C, Regen DM, Whitfield CF, Morgan HE (1978) Effect of insulin on kinetics of sugar transport in heart muscles. Am J Physiol 234: E70–E78
25. Wardzala LJ, Jeanrenaud B (1981) Potential mechanism of insulin action on glucose transport in the isolated rat diaphragm. J Biol Chem 256: 7090–7093
26. Czech MP (1980) Insulin action and the regulation of hexone transport. Diabetes 29: 399–409
27. Cushman SW, Wardzala LJ (1980) Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. J Biol Chem 255: 4758–4762

Professor B. Jeanrenaud
Laboratoires de Recherches Métaboliques
Faculté de Médecine
Université de Genève
64 avenue de la Roseraie
CH-1205 Genève
Switzerland