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Insulin resistance and decreased insulin response to glucose in lean type 2 diabetics

(early diabetes/pathogenesis)

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ABSTRACT **In an attempt to determine the mechanism of decreased glucose tolerance in lean type 2 diabetics, glucose turnover in such subjects and controls was studied under basal conditions and during hyperglycemia induced by intravenous administration of glucose. The diabetics had decreased intravenous glucose tolerance and a fasting plasma glucose of 6–8 mM (108–144 mg/dl). Glucose was infused for 2 hr at 2 mg/kg per min in the controls ($n = 16$) and diabetics ($n = 9$). Furthermore, 11 healthy subjects were infused also with glucose at 4 mg/kg per min to match the glycemia of the diabetics. Glucose production, utilization, and metabolic clearance were assessed by the primed constant tracer infusion technique. In the basal state, diabetics showed normal plasma insulin, C peptide, and glucagon concentrations. Their increased basal plasma glucose levels were associated with normal rates of glucose production and utilization, but the metabolic glucose clearance was 21% lower than in the controls ($P < 0.001$), indicating decreased sensitivity to insulin. During infusion of glucose at 2 mg/kg per min, the hyperglycemia attained in the diabetics (170 mg/dl) was higher than that in controls (115 mg/dl) but comparable to that of the controls exposed to the higher glucose load. With the lower glucose load, metabolic clearance rate decreased more markedly in diabetics, again suggesting insulin resistance. This was further substantiated by the fact that, at the same insulin levels, glucose utilization did not increase more in the diabetics than in the controls, although the glycemia reached was considerably higher in the diabetics. With the lower glucose load, glucose production was suppressed to the same degree in the controls and diabetics, although the attained glycemia was much more marked in the latter. Because both insulin and hyperglycemia can suppress glucose production, some defect in the regulation of glucose production of the diabetics is also indicated. The insulin and C peptide levels were much higher in the controls than in the diabetics at the same levels of glycemia, demonstrating the inadequacy of insulin response to glycemia of the diabetics. Glucagon concentration was equally suppressed in all groups. In conclusion, impaired glucose tolerance of mild type 2 diabetics resulted both from inadequate insulin response and from decreased sensitivity to insulin. The insulin resistance could mainly be ascribed to inadequate glucose uptake, but a defect in glucose-induced suppression of glucose production may also have contributed.**

During infusion of glucose in normal humans, inhibition of endogenous glucose production and increase in glucose uptake are factors that limit the changes in glucose concentration. At a small glucose load, the effect of inhibition of glucose production is a predominant one, whereas changes in peripheral glucose uptake may not be apparent (1–4). At a larger glucose load, an increase in peripheral glucose uptake also plays an important role in glucose homeostasis (4). In the present study, the effect of glucose infusion on glucose turnover was studied in lean type 2 diabetic subjects and matched controls in order to see whether the decreased glucose tolerance in the diabetics could be ascribed to inadequate insulin response, decreased sensitivity to insulin, or both. The diabetics were selected according to our previous criteria (5). With respect to the recently proposed classification (6) most of them may be considered as subjects with impaired glucose tolerance.

It is well known that type 2 diabetes is a heterogeneous disease, and the majority of diabetics of this type are obese. Therefore, conclusions from our study are restricted to a population of type 2 diabetics with normal body weight.

MATERIALS AND METHODS

Subjects. This study was approved by the Human Study and the Isotope Committees of the Karolinska Hospital, Stockholm, and informed consent was obtained from all subjects. None was on any kind of medication for at least 2 weeks prior to the study. The group of healthy volunteers consisted of 16 men and 4 women with a mean (±SEM) body weight of 91.9 ± 1.6% of the ideal (Metropolitan Insurance Company tables) and a mean age of 42.9 ± 2.0 years; 7 men were included both in groups A and B as described below (Table 1). They were in good general health and consuming an ordinary Swedish diet, which contains carbohydrates at around 40% of the caloric intake. All had a fasting plasma glucose of less than 6 mM in repetitive determinations, and normal intravenous and oral glucose tolerance tests (IVGTT and OGTT, respectively) according to Ikkos and Luft (7) and Reaven and Olefsky (8), respectively.

Three of the subjects with mild diabetes were women and six were men, with a mean body weight of 97.8 ± 3.8% of ideal and a mean age of 50.7 ± 2.6 years (Table 1). Their fasting plasma glucose was 6–8 mM (108–144 mg/dl) and all had a decreased IVGTT (K value < 1.0). All were outpatients and kept on a controlled diet. The body weights of the diabetic and the control groups were not different, and their ages were matched, although the mean age of the diabetics was 8 years older.

Infusions. All tests were performed when the subjects were in the postabsorptive state at 8 a.m. after a 12-hr overnight fast. An indwelling catheter was placed in a cubital vein of each arm, one for infusion of unlabeled or isotopically labeled glucose and the other for blood sampling. The subjects remained in recumbency during the experiments.

After cannulation, a primed constant infusion of sterile and nonpyrogenic [3-^{3}H]glucose (New England Nuclear) in isotonic

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Abbreviations: IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; MCR, metabolic clearance rate; R_a, rate of appearance of glucose; R_d, rate of disappearance of glucose.

Table 1. Clinical data for the control subjects and those with mild diabetes

Group	n	Sex ratio, M/F	Age, yr	Body weight, % of ideal	Fasting plasma glucose mg/dl	Fasting plasma glucose mM	OGTT, 1.75 g/kg	IVGTT, *K* value
A	16	14/2	42.1 ± 2.2	93.1 ± 2.0	<108	<6	Normal	1.97 ± 0.14
B	11	9/2	43.8 ± 2.3	93.2 ± 2.2	<108	<6	Normal	2.48 ± 0.30
C	9	6/3	50.7 ± 2.6	97.8 ± 3.8	108–144	6–8	—	0.72 ± 0.05

Results are expressed as mean ± SEM. OGTT was not done in group C.

saline was started; it was continued throughout the experiment at a rate of 0.1 ml/min (0.083–0.150 μCi/min; 1 Ci = 3.7×10^{10} becquerels). The priming dose of [3-^{3}H]glucose was the same as the amount infused during 120 min. After an equilibrium period of 120 min in which the labeled glucose was infused, an infusion of unlabeled 15% glucose was started; it was continued for 120 min with the help of a Tecmar pump. The concentration of glucose in the infusing solution was measured on each occasion with the same assay as the plasma samples.

In study A, 16 healthy subjects received glucose at 2 mg/kg per min and, in study B, 11 healthy subjects received glucose at 4 mg/kg per min. The rationale for this second group of experiments with normal subjects was to increase plasma glucose concentration to levels comparable to those attained in diabetics, who were infused with the lower glucose load only. Seven subjects were included in both study A and study B, but at least 2 months had elapsed between the experiments. Finally, nine subjects with mild diabetes received glucose at 2 mg/kg per min (study C).

Blood Sampling. During the control period, blood samples were taken at 0, 90, 100, 110, and 120 min. At the start of the infusion of unlabeled glucose the clock was reset to zero and samples were drawn every 10 min of the experiment.

Analytical Procedures. Heparinized blood was kept on ice until centrifuged at 4°C. A plasma sample was used for determination of blood glucose in triplicate, and the rest was frozen and kept at −20°C for later analyses of insulin, C peptide, glucagon, and specific activity of plasma glucose. Glucose was determined by the glucose oxidase method (9). Insulin was measured by radioimmunoassay using antibodies raised in guinea pigs against porcine insulin. Human insulin served as a standard (10). C peptide was determined by radioimmunoassay with a commercially available kit (Novo Research, Bagsværd, Denmark). Blood samples for glucagon were collected in prechilled tubes containing Trasylol and EDTA; the radioimmunoassay was based on the method of Faloona and Unger and used the 30 K antibody (11).

Tracer Methods and Calculations. Measurements of specific activity of glucose were performed on deproteinized plasma. Under reduced pressure, the supernatant was evaporated to dryness at 40°C and, after addition of 1 ml of water and liquid scintillation solution, its radioactivity was measured in a beta scintillation counter with the use of an external standard for quenching. All samples and standards were counted for 50 min, and the counts for the samples were at least 4 times higher than for the background samples. Tritium counting efficiency averaged 34%.

The rates of glucose production (R_a, rate of appearance) and utilization or uptake (R_d, rate of disappearance) were determined by the method of primed constant tracer infusion (12). This method is based on a modified single-compartment analysis of glucose turnover, in which it is assumed that rapid changes in the specific activity and concentration of glucose do not occur uniformly within the entire glucose pool. To compensate for this nonuniform mixing, a term of the non-steady-state equation was multiplied by a correction factor (pool fraction) of 0.65 (13, 14). A sliding fit technique employing three consecutive values of glucose concentration and specific activity was also used in the calculations as described previously (15). This approach for calculating non-steady-state kinetics of glucose turnover was validated *in vivo* and confirmed (14). In addition, glucose metabolic clearance rate (MCR) was also calculated in order to compensate in part for the mass effect that glucose concentration has on glucose uptake (16). Glucose clearance can be calculated as R_d/c, in which c is glucose concentration. It can also be directly derived from the original tracer data in the same way as R_a if the concentration of labeled glucose is used in the equation instead of specific activity; glucose clearance derived in this way is as valid and directly derived as is R_a (14, 17). We realize that glucose clearance is not fully independent of prevailing glycemia and, therefore, is not entirely equivalent to insulin-dependent changes in glucose uptake, particularly when insulin is basal or subbasal (18–20). During glucose infusions the endogenous rate of glucose production was calculated by subtracting the rate of infusion of exogenous glucose from the tracer-determined total rate of glucose appearance.

Statistical Methods. Results are expressed as mean ± SEM, and Student's *t* test was performed for nonpaired and paired data.

RESULTS

In all three experimental series, an isotopic plateau was reached during the last 30 min of the equilibrium period (Fig. 1). Plasma glucose was also stable before infusion of unlabeled glucose (−30 to 0 min, Fig. 2), implying that all preexperimental data were obtained during steady-state conditions for labeled and unlabeled glucose.

The patterns of plasma glucose in studies A, B, and C are shown in Fig. 2. During the first 60 min of the infusion, plasma glucose continuously increased. At the end of the infusion, a plateau was reached in the controls and, in the diabetics, glucose concentration approached a plateau as well. The rise in plasma glucose in the healthy subjects receiving glucose at 4 mg/kg per min was similar to that in the diabetics receiving 2 mg/kg per min, the maximal glucose values being 160 and 170 mg/dl, respectively. On the other hand, during the administration of glucose to the normal subjects, at 2 mg/kg per min, plasma glucose levels plateaued at about 115 mg/dl.

Basal insulin and C peptide of the diabetics (Fig. 2) were not different from mean values of all controls. The low-dose glucose infusion significantly enhanced the release of C peptide in the controls and the diabetics. However, peripheral insulin concentration did not show consistent, significant increases during glucose infusion at this rate. The high-dose glucose infusion, administered only in the controls, induced a further and marked increase in the C peptide and insulin release. Basal glucagon was of the same magnitude in the controls and the diabetics. The infusion of glucose suppressed glucagon concentration in the diabetics and in the controls to almost the same extent.

In the healthy subjects, the rate of endogenous glucose pro-

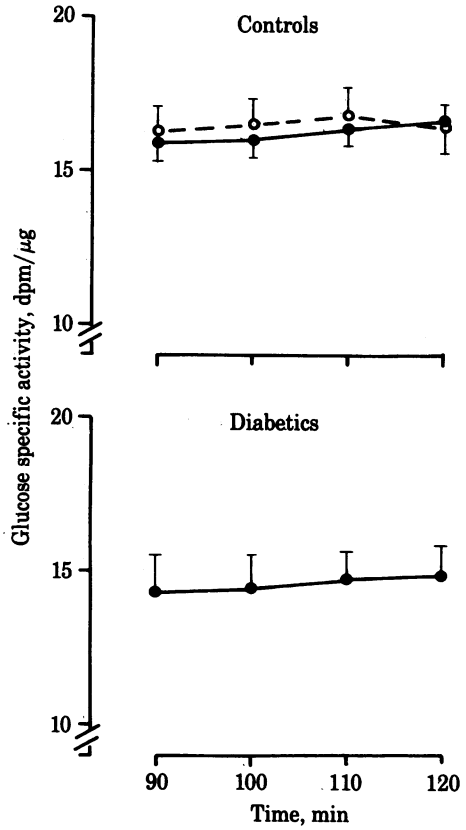

FIG. 1. Specific activity at the end of the equilibrium period. Glucose was infused at a rate of 4 (○) or 2 (●) mg/kg per min. Specific activity was normalized for tracer infusion rate. Data are shown as mean ± SEM. At the higher dose, $n = 11$; at the lower dose, $n = 16$ for controls and 9 for diabetics.

duction (R_a) during the control period was 2.04 (study A) and 2.13 (study B) mg/kg per min, and in the diabetics (study C) it was 2.17 mg/kg per min. The variation among individuals was small, as was the day-to-day variation in each subject, the mean coefficient of variation being 8.5%. The low-dose glucose infusion markedly decreased R_a, both in the controls and in the diabetics, the degrees of suppression being almost identical (Fig. 3). R_a was completely suppressed when the higher glucose load was applied to the controls.

The lower glucose load in the controls significantly stimulated glucose uptake (R_d) throughout the infusion period, and the high-dose glucose infusion doubled it. In contrast, in the diabetics, in spite of marked hyperglycemia, R_d was increased minimally and only at the end of the experiment.

Notably, the basal MCR of glucose in the diabetics was 21% lower than in the controls ($P < 0.001$), although insulin and C peptide were of the same magnitude and plasma glucose was only moderately increased. Administration of glucose in the diabetics was accompanied by a further average decrease in MCR by 19%. In the healthy subjects, the low-dose glucose load decreased MCR by approximately 8%, and the high one increased it by 11%.

DISCUSSION

It is generally accepted that heredity plays an important role in the development of maturity-onset diabetes (for review, see ref. 21). In many cases of manifest type 2 diabetes, the disease has developed slowly from prediabetes and through impaired glucose tolerance. For a long time, we have favored the idea that the primary defect in this type of diabetes is impaired rec-

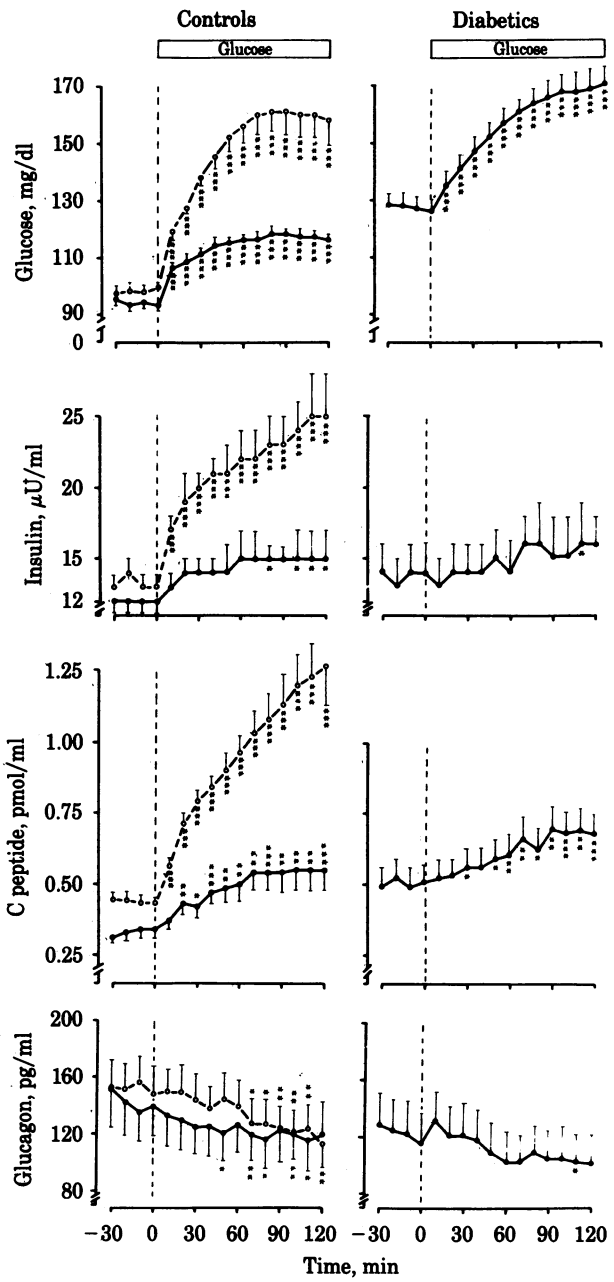

FIG. 2. Effect of glucose infusion on plasma concentration of glucose, insulin, C peptide, and glucagon in controls (*Left*) and subjects with mild type 2 diabetes (*Right*). U, international unit. Glucose was infused at a rate of 4 (○) or 2 (●) mg/kg per min. Data are shown as mean ± SEM. Numbers of subjects are as for Fig. 1. Probabilities, compared with basal concentrations for that group: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

ognition of the glucose stimulus by the beta cells, resulting in relative hypoinsulinemia (22). Others have argued that insulin resistance (8) plays a more important role in this connection.

In one study in obese subjects, decreased glucose tolerance was associated with insulin resistance (as assessed by tracer-determined glucose kinetics), but the insulin response to glucose and glucagon was considered to be normal (23). On the other hand, in a variety of lean diabetic patients, glucose intolerance was invariably characterized both by defective insulin response to oral and intravenous glucose and by insulin resistance (5). The latter study did not clarify the site of insulin resistance. In order to shed some light on this question, we compared here the effect

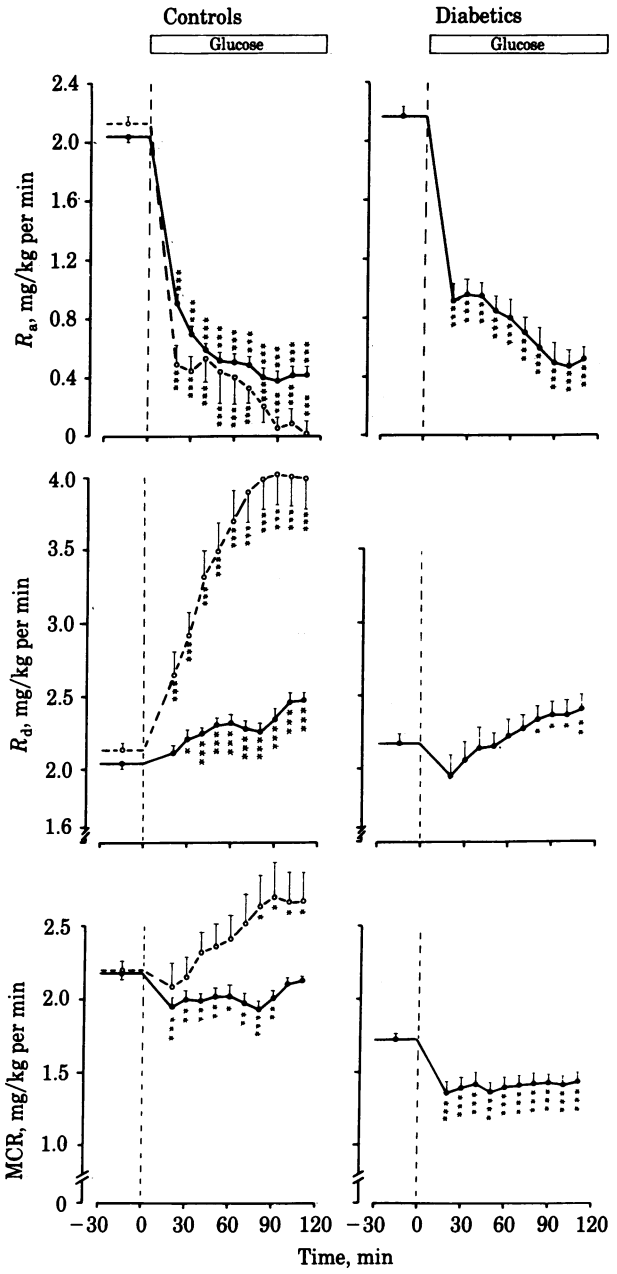

FIG. 3. Effect of glucose infusion on R_a, R_d, and MCR in controls (*Left*) and subjects with mild type 2 diabetes (*Right*). Glucose was infused at a rate of 2 (●) or 4 (○) mg/kg per min. Data are shown as mean ± SEM. See legend of Fig. 2 for numbers of subjects and *P* values.

of hyperglycemia on glucose production in the liver and on total glucose uptake in healthy subjects and in subjects with mild manifest type 2 diabetes. Because body weight (24) and, possibly, also age (25) play a role in insulin sensitivity, only nonobese subjects were included in the study, and we tried to match the two groups of subjects for weight and age as closely as possible.

The kinetics of glucose turnover was calculated by using a primed constant infusion of [3-^{3}H]glucose. Because the ^{3}H in position 3 of glucose is lost to body water and cannot recirculate (26), its use eliminates the underestimation that might otherwise result from recycling of the label. The overestimate of glucose production that can arise from loss of label as the result of futile cycling (27) is also minimal when [3-^{3}H]glucose is used, as demonstrated in dogs (26) and rabbits (28).

In our control subjects, hyperglycemia was induced by infusing glucose at a dose of 2 or 4 mg/kg per min. As in previous studies (4), R_a was suppressed by 80% with 2 mg/kg per min, presumably as a result of hyperinsulinemia. The higher glucose load suppressed R_a completely. This presumably did not result from more marked insulin release only, hyperglycemia being also a contributing factor (29, 30). With glucose at 2 mg/kg per min, R_d rose moderately while MCR decreased. The decrease in MCR confirms previous findings that hyperglycemia induced a decline of MCR (19). A hyperglycemia-induced decrease of MCR by 38% was noted in a normal man, in whom insulin was suppressed by somatostatin and glycemia was maintained at 195 mg/dl (10.8 mM) with a hyperglycemic clamp (20). At the higher glucose load, R_d increased markedly and, despite a further rise in glucose concentration, MCR increased significantly as well. Previous observers did not notice a similar rise in MCR unless insulin was added to the glucose infusion (4), possibly because of different dietary regimens or body weights.

The nonlinearity of MCR with increasing glucose concentrations was attributed to the fact that insulin-independent tissues such as the brain have a relatively fixed glucose uptake (glucose transport is already saturated), whereas other tissues have glucose transport systems that take up glucose at a rate proportional to its concentration (20).

The only defect in glucose turnover noted in the postabsorptive hyperglycemic diabetic subjects was that MCR was 21% lower than in the controls. R_a and R_d remained normal. Although it is possible that the decrease in MCR could in part result from hyperglycemia, this effect at best would be small at a postabsorptive glycemia of 130 mg/dl (7.2 mM). Because peripheral insulin and C peptide concentrations in the diabetics were not lower but, if anything, were increased, the decrease in MCR may be considered to indicate insulin resistance. Further proof of insulin resistance in the diabetics was obtained during glucose infusion. First, R_d did not increase more than in the controls despite the more pronounced hyperglycemia, while insulin and C peptide responses were similar. Second, the decrease in MCR during glucose infusion was much more pronounced than in the normal subjects, suggesting that the fraction of glucose metabolized in non-insulin-dependent tissues was larger than in the controls (20). Thus, these data complement the previous ones (19, 20) by demonstrating that not only insulin lack but also insulin resistance enhances the suppressing effect of hyperglycemia on MCR.

Because the suppression of R_a in the diabetics was almost the same as that in the controls, the exaggerated hyperglycemic response of mild diabetes to glucose infusion must have resulted only from a defective glucose uptake. However, this observation does not imply that liver sensitivity to the combined effects of hyperinsulinemia and hyperglycemia is unaltered in diabetics. As revealed by the C peptide data, the liver of the diabetics was exposed to the same insulin concentration as in normals when glucose was infused at 2 mg/kg per min. Because the hyperglycemia was higher in the diabetics, and hyperglycemia *per se* has a suppressive effect on R_a, a more marked R_a suppression would have been expected in our diabetics. This was not the case, implying—although the evidence is indirect—that the sensitivity of the liver to insulin was presumably also affected. A more direct proof of decreased insulin sensitivity of the liver in impaired glucose tolerance and in type 2 diabetes was provided by Kolterman *et al.* (31).

The diabetics in the basal state demonstrated normal peripheral insulin and C peptide levels. However, they showed markedly diminished insulin response to hyperglycemia. A quantitative view of this impairment is given by a comparison of the degree of insulinemia in the two groups at almost identical

levels of hyperglycemia (the normals receiving glucose at 4 mg/kg per min and the diabetics receiving 2 mg/kg per min). The present work is concerned only with decreased insulin response to an intravenous glucose load. However, in our previous studies on subjects with decreased IVGTT a decreased insulin response to both standardized oral and intravenous glucose loads was found.

In conclusion, in normal subjects with moderate hyperglycemia, in whom the increase in peripheral insulinemia is only marginal, glucose homeostasis is maintained mainly through suppression of R_a. With more marked hyperglycemia, when insulin secretion is markedly enhanced, both suppression of R_a and the ability of some tissues to take up more glucose play an important role. In lean subjects with mild type 2 diabetes, glucose intolerance can be ascribed both to an impairment of insulin release and to insulin resistance. The major cause of the latter defect seems to be the impairment of glucose uptake in the periphery or in the liver. A decreased responsiveness of the R_a to the combined suppressive effects of hyperinsulinemia and hyperglycemia could also be a contributing factor.

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