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Oxidative and non-oxidative glucose metabolism in non-obese Type 2 (non-insulin-dependent) diabetic patients

A. Golay[1], R. A. DeFronzo[5], E. Ferrannini[6], D. C. Simonson[4], D. Thorin[1], K. Acheson[2], D. Thiébaud[1], B. Curchod[3], E. Jéquier[2] and J. P. Felber[1]

[1]Division of Endocrinology and Clinical Biochemistry, CHUV, [2]Institute of Physiology, Lausanne University, [3]Clinic La Source, Lausanne, Switzerland, [4]Department of Medicine, Yale University School of Medicine, New Haven, Connecticut, [5]Diabetes Division, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, USA, [6]University of Pisa, Italy

Summary. Insulin resistance is a common feature of Type 2 (non-insulin-dependent) diabetes mellitus. This defect in insulin-mediated glucose metabolism could result from a defect in either glucose oxidation or non-oxidative glucose disposal. To examine this question, euglycaemic insulin clamp studies were performed in 16 normal weight Type 2 and 11 age-matched control subjects. In Type 2 diabetic patients the fasting plasma glucose concentration, 8.39 ± 0.50 mmol/l, was allowed to decline (over 54 ± 6 min) to 5.33 ± 0.11 mmol/l before starting the insulin clamp. Total body glucose uptake was significantly decreased in Type 2 diabetic patients vs control subjects (148 ± 15 vs 264 ± 25 mg/min·m^2, $p < 0.001$). Both total glucose oxidation (59 ± 6 vs 89 ± 6 mg/min·m^2, $p < 0.005$) and non-oxidative glucose disposal (89 ± 15 vs 179 ± 24 mg/min·m^2, $p < 0.005$) were significantly reduced in the Type 2 diabetic patients. Basal glucose oxidation was also reduced in the Type 2 diabetic patients (22 ± 3 vs 38 ± 5 mg/min·m^2, $p < 0.01$). In conclusion, during the postabsorptive state and under conditions of euglycaemic hyperinsulinaemia, impairment of glucose oxidation and non-oxidative glucose disposal both contribute to the insulin resistance observed in normal weight Type 2 diabetic patients. Since lipid oxidation was normal in this group of diabetic patients, excessive non-esterified fatty acid oxidation cannot explain the defects in glucose disposal.

Key words: Insulin-mediated glucose uptake, glucose oxidation, non-oxidative glucose disposal, lipid oxidation, insulin resistance.

Patients with Type 2 (non-insulin-dependent) diabetes mellitus form a heterogeneous group in whom both insulin deficiency and insulin resistance contribute to the impairment in glucose utilisation [1]. With respect to the insulin resistance, defects in peripheral glucose uptake [2-11] and suppression of hepatic glucose metabolism [3, 4, 9, 12] have been described. The defect in insulin-mediated glucose metabolism has been documented with a variety of techniques, including the insulin clamp [2-5], forearm perfusion [6-8, 10], insulin tolerance test [13, 14] and insulin suppression test [5, 9]. All of these techniques primarily examine peripheral, muscle, glucose uptake [15, 16]. In the most general sense, the glucose which is taken up by peripheral tissues can have one of three major metabolic fates: (1) oxidation to carbon dioxide and water [15]; (2) storage, primarily in the form of glycogen [17]; or (3) conversion to lactate [15].

In *obese* Type 2 diabetic patients, defects in both oxidative and non-oxidative pathways of glucose utilisation have been described [18-21]. Further, it has been suggested that the impairment in glucose utilisation results from an increased rate of lipid oxidation [18, 19, 21]. Thus, according to the glucose/fatty acid cycle first proposed by Randle [22, 23], increased lipid oxidation impairs glucose oxidation by altering the redox potential of the cell and inhibiting several key enzymes in the glycolytic cascade. Experimental validation of this glucose/fatty acid cycle has been provided both in man [24, 25] and animals [26, 27].

In contrast to the obese diabetic patient, no previous study has examined whether the insulin resistance in *normal weight* Type 2 diabetic patients results from an impairment in oxidative versus non-oxidative glucose utilisation and whether such defects, if present, are related to an accelerated rate of lipid oxidation. In the absence of obesity one might not expect plasma non-esterified fatty acid levels and lipid oxidation to be increased.

In the present study we have employed the insulin clamp technique [28] in combination with indirect calorimetry to: (1) assess the relative contributions of

defects in glucose oxidation versus non-oxidative glucose disposal to the overall impairment in total body glucose utilisation in *normal weight* Type 2 diabetic patients, and (2) examine whether any of the observed defects in glucose disposal are related to an enhanced rate of lipid oxidation.

Subjects and methods

Subjects

Sixteen non-obese Type 2 diabetic patients (14 males/2 females; age = 54 ± 2 years) were compared to 11 age-matched non-obese, non-diabetic subjects (10 males/1 female; age = 53 ± 2 years). All subjects were within 25% of ideal body weight based on medium frame individuals from the Metropolitan Life Insurance Tables, 1959. The body mass index (25 ± 1 vs 23 ± 1 kg/m^2 respectively) was similar in the diabetic and control groups. There was no family history of obesity or diabetes mellitus in any of the control individuals and none was taking any medication. The fasting plasma glucose concentration in the diabetic patients on the day of study was 8.39 ± 0.50 mmol/l. Fasting glucose levels, determined on at least three occasions during the three months prior to study, agreed closely with this value. All of the diabetic patients were being treated with diet alone. None had ever received insulin or an oral hypoglycaemic agent. The mean duration of diabetes was 4 ± 1 years. None of the diabetic patients had any evidence of diabetic retinopathy or diabetic neuropathy on routine physical examination. No subject had evidence of diabetic nephropathy as evidenced by a negative urine test for albumin. There was no evidence of any other major organ system disease or endocrine abnormality other than diabetes on a routine history and physical examination. A physical activity history was obtained on all control subjects and diabetic patients; none were participating in any unusual exercise programs and none were excessively sedentary.

All subjects were consuming a weight-maintaining diet containing at least 250–300 g of carbohydrate per day for 3 days before each study. The distribution of calories, obtained by dietary history, was about 50% carbohydrate, 20% protein and 30% fat. Prior to their participation the nature, purpose and risks of the study were carefully explained to all subjects and their voluntary consent was obtained. The experimental protocol was submitted to and approved by the human investigation committee of the Department of Medicine of the University of Lausanne, Switzerland and by the Yale University School of Medicine, New Haven, Connecticut, USA. All studies were performed in Lausanne.

Experimental protocol

Studies were performed in the recumbent position at 08.00 hours following a 10–12 h overnight fast. A teflon catheter was inserted into an antecubital vein for the infusion of all test substances. For blood sampling, a second catheter was inserted retrogradely into a wrist vein and kept patent with an infusion of isotonic saline. The hand was inserted into a heated box (70° C) to achieve arterialisation of the venous blood [29]. Sixty min prior to beginning the insulin clamp studies, continuous respiratory exchange measurements were begun and continued throughout the duration of the experimental protocol.

Euglycaemic insulin clamp

After a 60-min equilibration period, a prime-continuous infusion of crystalline porcine insulin was administered. In control subjects the plasma glucose concentration was maintained constant at basal levels by determination of the plasma glucose concentration every 5 min and periodically adjusting a variable 20% glucose solution based on a negative feedback principle [28]. Under these steady state conditions of constant glycaemia, the rate of glucose infusion provides an index of total body glucose uptake since hepatic glucose production is suppressed by over 90–95% in control subjects and in diabetic patients with a comparable degree of fasting hyperglycaemia [2, 3, 30, 31]. The continuous insulin infusion was given at 40 mU/m^2·min in all subjects. This infusion rate was chosen because it produces portal insulin levels slightly less than observed during a standard oral glucose tolerance test (OGTT) [32] while peripheral insulin levels are slightly greater than observed during an OGTT [33, 34] or mixed meal [35]. In the control group the duration of each insulin clamp study was 120 min. In the Type 2 diabetic patients the glucose infusion rate was not begun until the plasma glucose had declined to approximately 5.5 mmol/l, at which level it was held constant. The mean time for the plasma glucose concentration to decline to euglycaemic levels was 54 ± 6 min. The duration of the insulin clamp from the time that euglycaemia was achieved was 120 min.

Respiratory exchange measurements

During the 60-min control period and throughout the 2-h insulin clamp study, substrate utilisation rates were determined by computerised open-circuit indirect calorimetry using a ventilated hood as previously described [17]. Briefly, a transparent plastic ventilated hood is placed over the subject's head and made airtight around the neck. To avoid air loss, a slight negative pressure is maintained in the hood. Ventilation was measured with a massic flowmeter (Setaram, Lyon, France). A constant fraction of the air flowing out of the hood was automatically collected for analysis. The oxygen content was continuously measured by a thermomagnetic analyser (Hartmann and Braun, Frankfurt, FRG) and carbon dioxide content by an infrared analyser (Hartmann and Braun). The non-protein respiratory quotient was calculated from calorimetric values and urinary nitrogen. Determination of carbohydrate oxidation rate was obtained as previously described [17]. The quantity of urinary nitrogen excreted during the entire period of calorimetry (i.e. basal plus insulin-stimulated state) was used to obtain an index of the amount of protein oxidised; this calculation assumes that protein oxidation was relatively constant throughout the entire study period. It should be noted, however, that this assumption does not substantially affect the calculation of carbohydrate or lipid oxidation [35].

Analytical procedures

Plasma glucose concentration was determined in duplicate by the glucose oxidase method on a Beckman Glucose Analyzer II (Beckman Instrument Inc., Fullerton, Calif, USA). Plasma immunoreactive insulin was determined by radioimmunoassay as described by Herbert et al. [36]. Plasma non-estrified fatty acids were extracted using the method of Dole and Meinertz [37] and determined according to the method of Heindel et al. [38]. Glucagon was determined by radioimmunoassay as described by Aguilar-Parada et al. [39]. Urinary nitrogen was measured by the method of Kjeldahl [40]. Plasma catecholamines were measured with a radioenzymatic method (Cata-Kit, Upjohn, Kalamazoo, Mich, USA).

Data analysis

During the 120-min insulin clamp study the glucose infusion rate was calculated at 20-min intervals and the mean value from 60–120 min is presented. All results are expressed in mg/m^2·min. The rationale for expressing the data per m^2 surface area is that lean

body mass (measured directly by the antipyrine technique) can be predicted from body weight and height with multiple correlation coefficients of 0.96 and 0.83, respectively, in males and females of normal body weight or moderate obesity [41]. The rate of total body glucose disposal presented in the text represents the mean glucose infusion rate during the 60–120 min time period of the euglycaemic insulin clamp. This calculation assumes that hepatic glucose production is completely suppressed in both control subjects and diabetic patients, and we have previously documented the validity of this assumption in a large group of normal weight Type 2 diabetic individuals [2, 3, 31].

The glucose oxidation rate was calculated from calorimetric measurements for 5-min intervals during the basal state and throughout the 120-min insulin clamp period. In the diabetic group basal glucose oxidation was calculated during the 45 min preceding the initiation of insulin infusion. Non-oxidative glucose utilisation was calculated by subtracting the rate of glucose oxidation during a given time period from the total rate of glucose uptake during the same time period. Presented values for glucose oxidation and non-oxidative glucose disposal represent the mean for the 60–120 min time interval and are expressed in $mg/m^2 \cdot min$. To calculate the steady state plasma insulin and glucose concentrations the mean of values (every 5 min for glucose and every 10 min for insulin) from 60–120 min was employed. The mean values during the 60–120 min time period were chosen to allow insulin to more fully exert its biological effects.

Statistical analysis

All values in the text represent the mean ± SEM. Comparisons between the Type 2 diabetic and control groups were performed with the unpaired Student's t-test.

Results

Plasma glucose, non-estrified fatty acid, insulin, and glucagon concentrations (Fig. 1)

The mean fasting plasma glucose concentration in the Type 2 group was 8.39 ± 0.50 mmol/l versus 5.17 ± 0.17 mmol/l in the control group ($p < 0.001$, Fig. 1). During the last 60 min of euglycaemic insulin clamp, the steady-state plasma glucose values were similar in diabetic patients, 5.33 ± 0.11 mmol/l, and control subjects, 5.17 ± 0.17 mmol/l. The stability of the plasma glucose concentration is reflected by the coefficients of variation which ranged between 2.5 and 5.5%.

The fasting plasma insulin concentration was significantly elevated in the non-obsese diabetic group compared to the control group, 16 ± 2 vs 11 ± 1 mU/l ($p < 0.05$). The steady-state plasma insulin levels during the 60–120 min time period were 97 ± 6 and 90 ± 6 mU/l, respectively, with coefficients of variation ranging from 6 to 14%.

The fasting plasma glucagon concentration was similar in the control, 91 ± 7 pg/ml, and diabetic, 98 ± 9 pg/ml, groups. During the insulin clamp the decline in plasma glucagon was similar in both groups, 77 ± 5 versus 67 ± 9 pg/ml respectively. The fasting epinephrine and norepinephrine levels were 43 ± 4 and 223 ± 13 pg/ml, respectively, in the diabetic groups. During the insulin clamp study neither the epinephrine (52 ± 6) nor norepinephrine (230 ± 14) concentrations changed significantly from baseline in the diabetic patients.

In the postabsorptive state the plasma non-esterified fatty acid (NEFA) concentration was similar in diabetic, 628 ± 45 μmol/l, and control, 575 ± 98 μmol/l, groups. Following hyperinsulinaemia the absolute plasma NEFA level was slightly greater in the Type 2 diabetic vs control group, 207 ± 21 vs 142 ± 8 μmol/l ($p < 0.05$); the decrement in plasma NEFA was similar in diabetic and control groups, 420 ± 47 vs 434 ± 92 μmol/l. Within the weight range examined there was no correlation between body mass index and the fasting plasma NEFA level or the basal rate of lipid oxidation in either diabetic or control groups.

Glucose metabolism (Table 1)

In the basal state the rate of glucose oxidation was reduced by approximately 40% ($p < 0.01$) in the Type 2 diabetic patients as compared to the control subjects. During the 60–120 min time period of the insulin clamp study, glucose oxidation rose about 2.5-fold in both diabetic and control groups ($p < 0.01$, Table 1). In the diabetic patients, however, the absolute total rate of glucose oxidation after insulin administration was still 34% lower than in the control subjects ($p < 0.01$), and the increment in glucose oxidation above basal values (suprabasal glucose oxidation) was reduced by 31% ($p < 0.02$). Likewise, the post-insulin rate of non-oxidative glucose disposal in diabetic patients was 50% lower than in control subjects ($p < 0.005$, Table 1). As a consequence, the total rate of glucose uptake during the clamp was reduced by 43% in Type 2 diabetic patients compared to control subjects ($p < 0.001$). The time course of change in total glucose uptake, glucose oxidation, and non-oxidative glucose disposal is displayed in Figure 2.

The estimated rate of glucose disposal in the Type 2 diabetic group during the initial falling glucose phase after starting insulin was 141 ± 14 $mg/m^2 \cdot min$. This was calculated by multiplying the rate of plasma glucose decline by the space of glucose distribution (assumed to be 25%). This estimated rate of glucose utilisation correlated well with the value, 148 ± 15 $mg/m^2 \cdot min$, observed during the insulin clamp ($r = 0.900$, $p < 0.001$).

Protein and lipid metabolism

The rate of protein oxidation was 39% greater in the Type 2 diabetic compared to the control group, $p < 0.05$ (Table 1). In the postabsorptive and insulin-stimulated states, the rate of lipid oxidation was similar in diabetic and control groups (Table 1). The basal

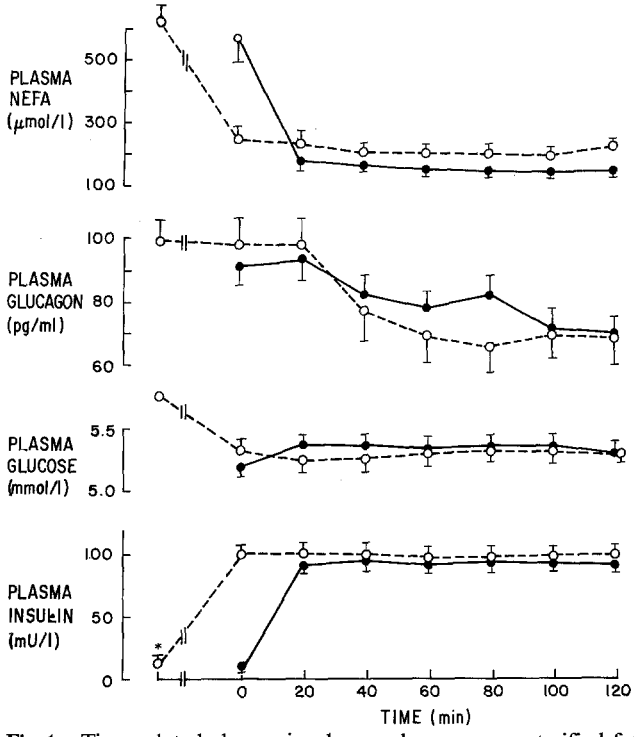

Fig. 1. Time related change in plasma glucose, non-esterified fatty acids (NEFA), insulin, and glucagon concentrations in control subjects (•) and normal weight Type 2 diabetic patients (o) during the insulin clamp. All values represent the mean ± SEM. In Type 2 patients the first point on the curve (t = zero) represents the basal value before insulin infusion

rate of lipid oxidation was inversely correlated with the basal rate of glucose oxidation in the Type 2 diabetic patients ($r = -0.667$, $p < 0.001$), in the control subjects ($r = -0.699$, $p < 0.01$) and in both groups combined ($r = -0.678$, $p < 0.001$). During the insulin clamp the rates of lipid and glucose oxidation also were inversely correlated in the Type 2 diabetic patients ($r = -0.624$, $p < 0.001$), control subjects ($r = -0.604$, $p < 0.01$), and both groups combined ($r = -0.638$, $p < 0.001$). No correlation was observed between the basal rate of lipid oxidation and total glucose uptake or non-oxidative glucose disposal during the insulin clamp. Similarly, there was no correlation between the rate of lipid oxidation during the insulin clamp and either the total rate of glucose uptake or non-oxidative glucose disposal during the insulin clamp.

Discussion

Under conditions of hyperinsulinaemia, hepatic glucose production is inhibited and glucose uptake by peripheral tissues is stimulated in a dose-dependent fashion [15, 42]. As we have previously shown, the major metabolic fate of the glucose that is taken up by cells involves: (1) oxidation to carbon dioxide and water [15]; (2) storage [15], presumably as glycogen [43]; and (3) conversion to lactate [15]. Although it is well established that the total amount of glucose taken up by the entire body in *normal weight* Type 2 diabetic patients is diminished following insulin infusion [1–5], no previous studies have examined whether this defect involves impaired glucose oxidation or impaired non-oxidative glucose uptake or both. The present results indicate that both processes are defective in Type 2 diabetes mellitus. When the plasma insulin concentration was raised by approximately 100 mU/l, the total amount of glucose metabolised by the diabetic patient was reduced by 44% compared to age- and weight-matched control subjects. This reduction is quantitatively similar to results previously reported [2–5] in Type 2 diabetic patients with similar degrees of fasting hyperglycaemia and was accounted for both by a decrease in insulin-mediated glucose oxidation and in non-oxidative glucose disposal.

In the postabsorptive state the rate of glucose oxidation also was significantly reduced in normal weight Type 2 diabetic patients by 42% ($p < 0.01$, Table 1). It is likely, however, that the true rate of glucose oxidation in the basal state is somewhat underestimated by the technique of indirect calorimetry since oxidation of glucose derived from amino acids through gluconeogenesis is not accounted for as glucose but as protein oxidation [44]. This is so since protein oxidation is subtracted from the total respiratory quotient for the calculation of the non-protein respiratory quotient. In the postabsorptive state it is unlikely that fuels, other than amino acids, could have contributed to an underestimation of glucose oxidation to any significant extent.

Recently, several papers have appeared in which glucose oxidation/storage have been examined in *obese* Type 2 diabetic patients [18–21]. Boden et al. [20] demonstrated normal rates of glucose oxidation in the postabsorptive state whereas Bogardus, Ravussin and

Table 1. Glucose, lipid and protein metabolism in normal weight Type 2 diabetic patients and control subjects (mean ± SEM)

	Glucose oxidation[a]			Total glusose uptake[a]	Non-oxidative glucose uptake[a]	Lipid oxidation[a]		Protein oxidation[a]
	Basal	Post-insulin	Increment			Basal	Post-insulin	
Type 2 diabetic patients	22 ± 3	59 ± 6	36 ± 4	148 ± 15	89 ± 15	36 ± 2	19 ± 2	32 ± 4
Control subjects	38 ± 5	89 ± 6	52 ± 6	264 ± 25	179 ± 24	36 ± 4	20 ± 4	23 ± 2
p value	< 0.01	< 0.01	< 0.025	< 0.001	< 0.005	NS	NS	< 0.05

[a] All data are expressed in mg/m^2 · min; NS = not significant

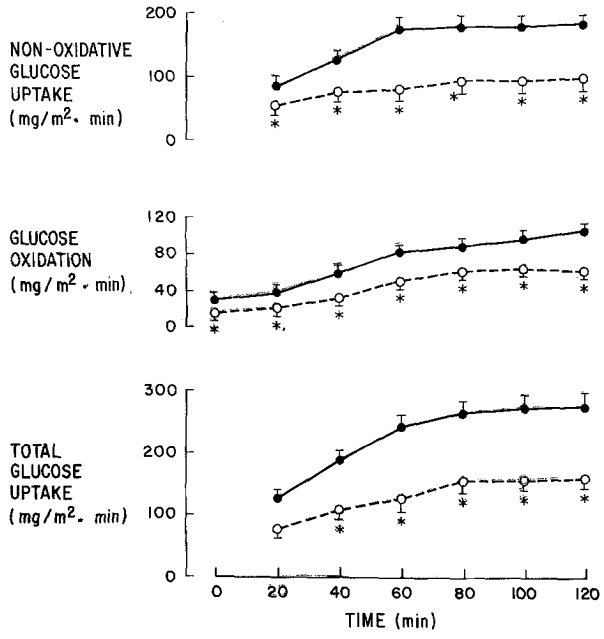

Fig. 2. Time related change in total body glucose uptake (assumed to be equal to the infusion rate of exogenous glucose) in control subjects (•) and in normal weight Type 2 diabetic patients (○) during the insulin clamp. All values represent the mean ± SEM. $*p<0.01$

coworkers [19, 21], in obese diabetic Pima Indians, found a significant decrease in basal glucose oxidation. The reason(s) for the difference in basal glucose oxidation rates between Boden et al. [20] and Bogardus et al. [19, 21], as well as ourselves, is not clear at present but may be explained by differences in the patient populations (i.e. our patients were normal weight; the patients in Boden were obese Caucasions; those of Bogardus were obese Pima Indians) or severity of diabetes. The results from all three groups [19–21, and ours] are consistent in that following hyperinsulinaemia the increase in glucose oxidation and non-oxidative glucose uptake both were significantly impaired.

In the postabsorptive state we found that the rate of lipid oxidation was strongly and inversely correlated to the rate of glucose oxidation in both diabetic and control groups (-0.678, $p<0.001$). Similarly, following hyperinsulinaemia the rates of insulin-mediated glucose and lipid oxidation were inversely correlated in both diabetic and control groups ($r=-0.638$, $p<0.001$). These results suggest that the rate of lipid oxidation is an important determinant of glucose oxidation in both control subjects and diabetic patients and that the normal insulin-mediated decline in lipid oxidation is, in part, responsible for the rise in glucose oxidation. Thus, our data provide evidence that the Randle cycle [22, 23] is operative in regulating glucose oxidation both in the postabsorptive state and following insulin administration. These results are consistent with observations that lipid infusion inhibits the ability of insulin to enhance glucose oxidation [24, 25, 45, 46]. It is important to point out, however, that neither the basal rate of lipid oxidation, nor the rate of lipid oxidation during the insulin clamp, were elevated in Type 2 diabetic patients compared to control subjects (Table 1). Since neither the basal rate of lipid oxidation nor the rate of lipid oxidation during the insulin clamp was elevated in our *normal* weight Type 2 patients, it is obvious that increased Randle cycle activity cannot explain the defects in glucose oxidation or non-oxidative glucose uptake observed during the postabsorptive and insulin-stimulated states. In contrast, in *obese* Type 2 diabetic patients lipid oxidation under basal conditions, as well as in the insulin-stimulated state, is increased and correlates inversely with the defect in glucose oxidation [18, 19, 21]. Similarly, Lillioja et al. [47] have reported an inverse relationship between basal and insulin-stimulated carbohydrate and lipid oxidation in *obese*, non-diabetic Pima Indians.

From a quantitative standpoint impaired glucose oxidation and non-oxidative glucose disposal contribute about 30% and 70%, respectively, to the defect in total body insulin-stimulated glucose metabolism in Type 2 diabetic patients (Table 1). In a previous study Meyer et al. [17] reported a similar decrease in glucose storage in the course of a 100 g OGTT. This was explained by a decrease in the plasma insulin response to the glucose load. In the present study, with comparable glucose and steady state plasma insulin levels of approximately 100 mU/l in diabetic and control groups, the lower rate of non-oxidative glucose uptake in Type 2 diabetic patients suggests that the ability of insulin to stimulate non-oxidative pathways of glucose metabolism is impaired in Type 2 diabetes mellitus.

It is well known that hyperglycaemia per se, by a mass action effect, enhances tissue glucose uptake and augments the rate of insulin-mediated glucose metabolism [42, 43, 48]. In addition, we have shown that the mass action effect of hyperglycaemia to stimulate glucose uptake is impaired in Type 2 diabetic patients [49]. Therefore, the present studies were performed after lowering the plasma glucose concentration to euglycaemic levels. In the diabetic group, it took 54 ± 6 min for the plasma glucose concentration to decline from 8.39 to 5.33 mmol/l. Thus, insulin had approximately one hour longer to exert its effect on glucose metabolism in the diabetic versus control group. Since the ability of insulin to stimulate glucose uptake and oxidation increases as a function of time during the second to third hour of an insulin clamp [50, 51], if anything, one would have expected a higher rate of glucose oxidation and uptake in the Type 2 diabetic group. Thus, it is likely that our experimental design could have underestimated the magnitude of the defect in glucose oxidation and total body glucose uptake in the diabetic patients. One additional factor that needs to be considered in interpreting the reduced rates of insulin-mediated glucose oxidation in the Type 2 diabetic patients is the possible release of

counterregulatory hormones as the plasma glucose concentration declined from hyperglycaemic to euglycaemic levels [52]. With regard to this concern neither plasma glucagon, epinephrine, or norepinephrine levels rose significantly during the 174-min insulin clamp study in the diabetic group. One additional observation suggests that release of counterregulatory hormones did not exert any effect on glucose metabolism during the insulin clamp. The rate of glucose uptake, estimated from the fall in plasma glucose concentration during the period after starting the insulin infusion, when no exogenous glucose was being infused, was strongly correlated with the rate of glucose uptake measured during the insulin clamp study. If a significant release of counterregulatory hormones had occurred and these hormones exhibited an insulin-antagonistic effect on glucose metabolism, such a strong correlation would not have been expected.

In summary, the present results indicate that insulin-mediated glucose metabolism is impaired in normal weight Type 2 diabetic patients and that this impairment results from a diminution in both glucose oxidation and non-oxidative glucose disposal. Basal lipid oxidation is not elevated in normal weight Type 2 diabetic individuals and suppresses normally in response to insulin.

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Dr. R. A. DeFronzo
Chief, Diabetes Division
Department of Medicine
University of Texas Health Science Center
7703 Floyd Curl Drive
San Antonio, Texas 78284
USA