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Non-esterified fatty acids do not contribute to insulin resistance in persons at increased risk of developing Type 2 (non-insulin-dependent) diabetes mellitus

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Summary. The mechanisms underlying insulin resistance in Type 2 (non-insulin-dependent) diabetes mellitus are not fully understood. An enhanced lipid/non-esterified fatty acid oxidation could provide an explanation. To test this hypothesis we examined the relationship between glucose and lipid metabolism in 44 first-degree relatives (28 glucose-tolerant and 16 glucose-intolerant) of Type 2 diabetic patients and in 18 healthy control subjects. Total body glucose disposal was impaired among both glucose-tolerant and glucose-intolerant relatives compared with control subjects (36.3 ± 3.8 and 30.4 ± 2.7 vs 47.7 ± 3.4 μmol · kgLBM^{-1} · min^{-1}; $p < 0.05$). The impairment in glucose disposal among the relatives was primarily accounted for by impaired non-oxidative glucose metabolism (14.8 ± 3.0 and 12.5 ± 1.8 vs 25.3 ± 3.1 μmol · kgLBM^{-1} · min^{-1}; $p < 0.05$). Plasma non-esterified fatty acid concentrations were similar in both glucose-tolerant and glucose-intolerant relatives and control subjects (646 ± 36, 649 ± 43 and 615 ± 41 μmol/l) and showed the same degree of suppression by insulin (99 ± 8, 86 ± 7 and 84 ± 9 μmol/l). Basal lipid oxidation was similar in all groups (1.29 ± 0.09, 1.52 ± 0.13 and 1.49 ± 0.21 μmol · kgLBM^{-1} · min^{-1}). Furthermore, insulin suppressed lipid oxidation to the same degree in glucose-tolerant, glucose-intolerant relatives and control subjects (0.65 ± 0.13, 0.88 ± 0.15 and 0.59 ± 0.09 μmol · kgLBM^{-1} · min^{-1}). An inverse correlation between plasma non-esterified fatty acid concentration and total body glucose disposal was observed in the group of control subjects ($r = -0.540$; $p < 0.05$), but not among the relatives ($r = 0.002$; p = N.S.). In conclusion the present data challenge the view that the "glucose-fatty acid cycle" contributes to the insulin resistance seen in first-degree relatives of patients with Type 2 diabetes.

Key words: Type 2 (non-insulin-dependent) diabetes mellitus, insulin sensitivity, peripheral glucose utilisation, non-esterified fatty acids, risk group.

Insulin resistance is a characteristic feature of Type 2 (non-insulin-dependent) diabetes [1, 2]. The underlying causes are not known but genetic factors seem to play a major role. About 40% of first-degree relatives of Type 2 diabetic patients will develop diabetes before the age of 80 years [3]. First-degree relatives of patients with Type 2 diabetes are also characterized by insulin resistance which can be observed despite normal oral glucose tolerance [4]. Several mechanisms could explain the genetic insulin resistance: a decreased number and affinity of the insulin receptors, impaired suppression of hepatic glucose production by insulin, impaired peripheral glucose uptake and activation of the "glucose-fatty acid cycle" [5–7].

A number of studies have shown that elevated rates of non-esterified fatty acids (NEFA)/lipid oxidation are associated with a reduced rate of glucose metabolism in patients with manifest Type 2 diabetes as well as in non-diabetic subjects [8–14]. However, it is not known whether enhanced lipid oxidation represents a primary defect or compensates for a defect in glucose oxidation. If primary, the enhanced rate of lipid oxidation should be observed in insulin-resistant first-degree relatives with normal rate of glucose oxidation.

To test this hypothesis we examined the relationship between NEFA/lipid oxidation and oxidative and non-oxidative pathways of glucose metabolism in insulin-resistant first-degree relatives of Type 2 diabetic patients.

Subjects and methods

Forty-four first-degree relatives of patients with Type 2 diabetes and 18 healthy control subjects without family history of Type 2 diabetes participated in the study. Sixteen of the first-degree relatives had impaired glucose tolerance (IGT) [15]. The clinical characteristics of the study groups are given in Table 1. Informed consent was obtained from all subjects and the study protocol was approved by the ethical committee of Helsinki University Hospital.

Table 1. Clinical characteristics of the study population

	Control subjects	Glucose-tolerant relatives	Glucose-intolerant relatives
Number	18	28	16
Male/Female	10/8	15/13	7/9
Age (years)	47 ± 3	45 ± 2	47 ± 2
Weight (kg)	72 ± 3	74 ± 3	78 ± 3
Lean body mass (kg)	53 ± 2	51 ± 3	55 ± 4
Body mass index (kg/m^2)	23.7 ± 0.6	25.4 ± 0.7	26.9 ± 0.8[a]
Plasma glucose (mmol/l)	4.9 ± 0.1	5.0 ± 0.1	5.4 ± 0.1
Insulin (pmol/l)	50 ± 7	68 ± 6	75 ± 8
Cholesterol (mmol/l)	5.6 ± 0.2	6.1 ± 0.2	6.8 ± 0.2[a]
HDL-Cholesterol (mmol/l)	1.35 ± 0.07	1.34 ± 0.06	1.41 ± 0.09
Triglycerides (mmol/l)	1.2 ± 0.1	1.4 ± 0.1	2.0 ± 0.2[a]
HbA_1 (%)	6.3 ± 0.2	6.2 ± 0.1	6.6 ± 0.2

[a] $p < 0.05$ vs control subjects

Metabolic studies

All the subjects participated in two experiments: (1) an oral glucose tolerance test (OGTT), and (2) a euglycaemic, hyperinsulinaemic clamp. The metabolic studies were performed starting at 07.30 hours after a 12 h overnight fast. The clamp studies were performed with the patient in the recumbent position.

Oral glucose tolerance test (OGTT). After a 12 h overnight fast the subjects were given a 75 g oral glucose load. Venous blood samples were taken at −15, 0, 30, 60, 90 and 120 min after glucose ingestion for determination of plasma glucose concentrations.

Euglycaemic insulin clamp. Insulin sensitivity was measured by the euglycaemic hyperinsulinaemic clamp technique [16]. Substrate oxidation and energy production were estimated by indirect calorimetry [17]. After obtaining three basal samples for glucose, insulin and non-esterified fatty acids (NEFA), a primed constant infusion of short-acting human insulin (Actrapid, Novo Industry, Copenhagen, Denmark) was administered at a rate of 323 pmol/min · m^2 of body surface area for 2 h. The plasma glucose concentration was determined at 5 min intervals and a variable infusion of 20% glucose was adjusted to maintain the plasma glucose concentration constant. The duration of the clamp was 120 min in all subjects. At unchanged plasma glucose concentration, the amount of glucose required to maintain euglycaemia equals whole-body glucose disposal, provided that there is no entry of glucose from the liver. Hepatic glucose production was measured by the isotope dilution technique using [^{3}H-3]-glucose (Amersham Inc, Amersham, UK) administered as a primed (25 μCi)-constant (0.25 μCi/min) infusion for 150 min and continued throughout the experiment. Blood samples for determination of insulin, NEFA, and [^{3}H-3]-glucose specific activity were obtained in the basal state and at 15 min intervals throughout the clamp. The mean insulin concentrations during the clamp did not differ between the groups.

Indirect calorimetry was employed in the basal state and during the last 60 min of the insulin clamp to estimate net rates of carbohydrate and lipid oxidation [17]. A computerized, open-circuit system was used to measure gas exchange through a transparent 25 liter PVC plastic canopy (Deltatrac, Datex, Helsinki, Finland) [18]. The monitor has a precision of 2.5% for oxygen consumption and of 1.0% for carbon dioxide production. Protein oxidation was calculated from the urinary urea nitrogen excretion obtained before and during the insulin clamp.

Analytical determinations

Plasma glucose was assayed with a glucose oxidase method adapted for Beckman glucose analyzer II (Beckman Instruments, Fullerton, Calif, USA). Glycohaemoglobin concentration in blood was measured by microcolumn cation exchange chromatography (Isolab, Akron, Ohio, USA). The reference level for the assay was 5–7%. The insulin concentration in serum was measured by a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden). [^{3}H-3]-glucose specific activity was measured in duplicate on supernatants of 1 N perchloric acid extracts of plasma samples, after evaporation of radiolabelled water. Plasma NEFA concentrations were measured by a microfluorometric method [19]. Lean body mass was determined with the tritiated water dilution technique [20]. Briefly, 40 μCi of tritiated water (Amersham Inc, Amersham, UK) diluted in NaCl 0.9% was given as an iv injection. Blood samples for determination of tritiated water specific activity in plasma were drawn before and 80, 100 and 120 min after injection.

Calculations

Basal hepatic glucose production was calculated by dividing the [^{3}H-3]-glucose infusion rate by the steady-state plateau of [^{3}H-3]-glucose specific activity achieved during the last 30 min of the basal tracer infusion period. After administration of insulin and glucose, a non-steady-state condition in glucose specific activity exists, and the rate of glucose appearance was calculated by a two compartment model [21]. This model, as well as the classic model of Steele, is known to produce negative estimates of hepatic glucose production at high rates of glucose infusion [22, 23]. Negative rates of endogenous glucose production were only observed during the second hour of the insulin clamp. These values were taken to indicate that hepatic glucose production was completely suppressed. The infusion rate of cold glucose was integrated over 20 min intervals and subtracted from the total rate of glucose appearance to obtain the hepatic glucose production rate. Total body glucose metabolism was calculated by adding the mean rate of hepatic glucose production during the last 60 min of each insulin clamp step to the mean glucose infusion rate during the same period. Non-oxidative glucose metabolism was calculated as the difference between total body glucose uptake and glucose oxidation, as determined by indirect calorimetry.

Net glucose and lipid oxidation rates were calculated from indirect calorimetric measurements in the basal state and during the last 60 min of each insulin clamp. The constants to calculate glucose, lipid and protein oxidation from gas exchange data have been shown previously [17].

Total body water was calculated from steady-state kinetic data assuming that 1 ml of plasma contains 93% water. Lean body mass (LBM) was obtained by dividing total body water by 0.73, since the lean body mass contains approximately 73% water. Fat mass was calculated as the differences between body weight and lean body mass.

Statistical analyses

All data are expressed as means ± SEM. A BMDP statistical package was used for the statistical analyses [24]. One-way analysis of variance or the Welch test was used to test the equality of group means. Scheffe's method was used for multiple comparisons between group means. Correlations were tested by linear regression analyses. A p-value less than 0.05 was considered statistically significant.

Results

Glucose tolerance

All control subjects had normal oral glucose tolerance. Sixteen relatives of Type 2 diabetic patients had impaired glucose tolerance (IGT) while 28 had normal oral glucose tolerance.

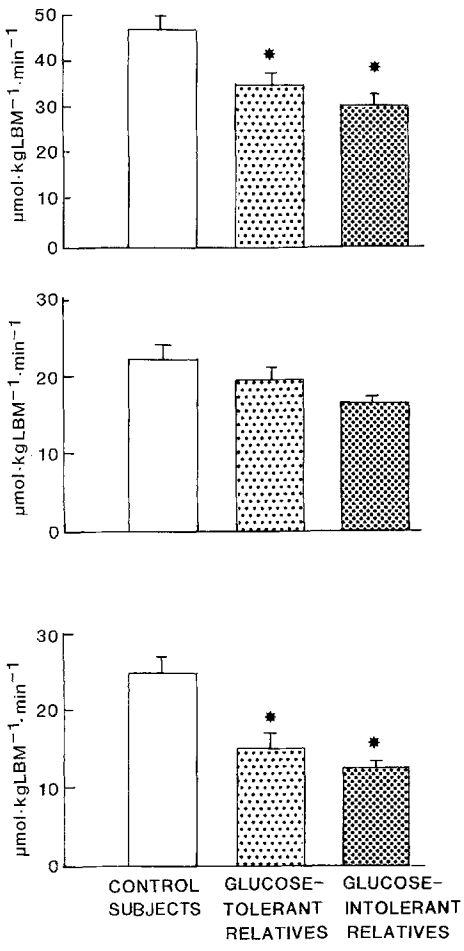

Fig. 1. Rates of insulin-stimulated total glucose disposal (upper panel), glucose oxidation (middle panel) and non-oxidative glucose metabolism (lower panel) in 18 control subjects, 28 glucose-tolerant and 16 glucose-intolerant first-degree relatives of patients with Type 2 (non-insulin-dependent) diabetes. Values are mean ± SEM. * $p < 0.05$ vs control subjects. LBM = lean body mass

Glucose metabolism

Total body glucose disposal was impaired among both glucose-tolerant and glucose-intolerant relatives (36.3 ± 3.8 and 30.4 ± 2.7 μmol · kgLBM^{-1} · min^{-1}) compared with the control subjects (47.7 ± 3.4 μmol · kgLBM^{-1} · min^{-1}; $p < 0.05$; Fig. 1).

Basal rate of glucose oxidation did not significantly differ between glucose-tolerant, glucose-intolerant relatives and control subjects (11.9 ± 0.8, 9.2 ± 1.3 and 10.3 ± 0.6 μmol · kgLBM^{-1} · min^{-1}). Furthermore, insulin-stimulated glucose oxidation was virtually similar in the three groups (21.4 ± 1.9, 17.8 ± 2.1 and 22.4 ± 1.8 μmol · kgLBM^{-1} · min^{-1}; p = N.S.; Fig. 1).

The impairment in glucose disposal among the relatives was primarily accounted for by impaired non-oxidative glucose metabolism (14.8 ± 3.0, 12.5 ± 1.8 vs 25.3 ± 3.1 μmol · kgLBM^{-1} · min^{-1}; $p < 0.05$; Fig. 1).

Hepatic glucose production. The basal rate of hepatic glucose production was similar in glucose-tolerant and glucose-intolerant relatives compared with control subjects (806 ± 24, 798 ± 26 and 775 ± 37 μmol/min). In both first-degree relatives and control subjects insulin administration inhibited hepatic glucose production by more than 90% without difference between the groups.

Lipid metabolism

First-degree relatives did not differ from control subjects with respect to basal NEFA levels (646 ± 36, 649 ± 43 vs 615 ± 41 μmol/l; Fig. 2). Insulin suppressed NEFA levels by over 80% in all groups (99 ± 8, 86 ± 7 and 84 ± 9 μmol/l; Fig. 2). No statistically significant correlations were observed between fat mass and NEFA concentrations in the individual groups.

Basal rate of lipid oxidation was similar in glucose-tolerant (1.29 ± 0.09 μmol · kgLBM^{-1} · min^{-1}), glucose-intolerant relatives (1.52 ± 0.13 μmol · kgLBM^{-1} · min^{-1}) and control subjects (1.49 ± 0.21 μmol · kgLBM^{-1} · min^{-1}). Net lipid oxidation was suppressed by insulin in both groups of relatives as well as in control subjects to the same degree (0.65 ± 0.13, 0.88 ± 0.15 and 0.59 ± 0.09 μmol · kgLBM^{-1} · min^{-1}; Fig. 2).

Lipid oxidation correlated with NEFA level in the combined group of relatives ($r = 0.400$; $p < 0.01$), while no significant correlation was observed among the control subjects ($r = 0.250$; p = N.S.). When the relatives were divided into two groups, i.e. glucose-tolerant and glucose-intolerant relatives, the correlation was strong in the glucose-intolerant group ($r = 0.701$; $p < 0.01$), but not valid among the glucose-tolerant relatives.

Fat mass correlated positively with lipid oxidation ($r = 0.494$; $p < 0.01$) in the group of relatives, but not in the control subjects ($r = 0.274$; p = N.S.). The positive correlation was valid only in the group of glucose-tolerant relatives ($r = 0.432$; $p < 0.05$).

Relationship between lipid and glucose metabolism

An inverse correlation was observed between plasma NEFA concentration and total glucose disposal during the insulin clamp in the group of control subjects ($r = -0.540$; $p < 0.05$), while no such correlation was found in the combined group of relatives ($r = -0.002$; p = N.S.), nor when the relatives were studied as two separate groups ($r = 0.069$ and $r = -0.240$; p = N.S.). NEFA concentration correlated inversely with the rate of glucose oxidation in the first-degree relatives ($r = -0.488$; $p < 0.001$) but not in the control subjects ($r = -0.288$; p = N.S.). The inverse correlation was observed both in glucose-tolerant ($r = -0.490$; $p < 0.01$) and glucose-intolerant relatives ($r = -0.595$; $p < 0.01$).

In the control subjects NEFA concentration correlated inversely with the rate of non-oxidative glucose metabolism ($r = -0.469$; $p < 0.05$). Among the relatives, on the other hand, NEFA concentration showed a positive correlation with the rate of non-oxidative glucose disposal ($r = 0.379$; $p < 0.05$). This correlation was statistically significant only in the group of glucose-tolerant relatives ($r = 0.492$; $p < 0.01$).

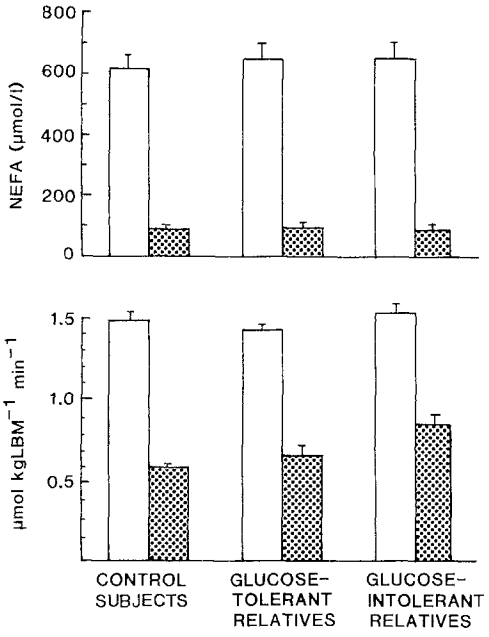

Fig. 2. Plasma non-esterified fatty acid (NEFA) concentrations (upper panel) and rates of lipid oxidation (lower panel) in the basal state (open bars) and during insulin clamp (filled bars) in 18 control subjects, 28 glucose-tolerant and 16 glucose-intolerant first-degree relatives of patients with Type 2 (non-insulin-dependent) diabetes. Values are mean ± SEM. * $p < 0.05$ vs control subjects

Rates of lipid and glucose oxidation correlated inversely in all groups ($r = -0.515$, $r = -0.460$ and $r = -0.509$; $p < 0.05$). No significant correlation was observed between rates of lipid oxidation and glucose disposal in the groups, nor between lipid oxidation and non-oxidative glucose metabolism.

Discussion

The glucose-fatty acid cycle first described in rat heart muscle by Randle and co-workers has been suggested as a cause of insulin resistance in obesity and Type 2 diabetes [7]. It should, however, be kept in mind that no definite proof for an operative Randle cycle in skeletal muscle, the major site of insulin resistance in Type 2 diabetes and obesity, has been presented.

First-degree relatives of patients with Type 2 diabetes are characterized by insulin resistance, and particularly by impaired non-oxidative glucose metabolism, despite normal glucose tolerance [4]. Storage of glucose as glycogen accounts for the major part (> 90%) of the non-oxidative glucose metabolism, as recently shown using in vivo nuclear magnetic resonance technique [25]. In contrast to patients with manifest diabetes, relatives of patients with Type 2 diabetes show normal rates of glucose oxidation both in the basal state and after insulin stimulation. Furthermore, we could not observe any defect in hepatic glucose production or in the suppression of hepatic glucose production by insulin in neither glucose-tolerant nor glucose-intolerant first-degree relatives. Decreased sensitivity of the liver to insulin could theoretically have been masked by the insulin concentration used. However, Gulli and coworkers have shown a > 90% suppression of hepatic glucose production rate with an insulin infusion rate of 20 mU/min · m^2, i.e. half the infusion rate employed in our study [26]. Enhanced basal hepatic glucose output in first-degree relatives of Type 2 diabetic patients has also been reported. Consequently, fasting plasma glucose was significantly higher among the relatives compared to control subjects in the study by Osei [27]. This finding, compared to ours, probably reflects the differences in different ethnic and racial groups studied. The possibility that the finding of Osei is a reflection of the heterogenity of Type 2 diabetes cannot be ruled out.

The present findings argue against a role for NEFA in the pathogenesis of insulin resistance in relatives of Type 2 diabetic patients. Basal NEFA concentrations were normal and they were normally suppressed by insulin. In addition, the rate of lipid oxidation was not enhanced in the groups of relatives and could be normally suppressed by insulin.

Indirect calorimetry measures net lipid oxidation i.e. oxidation of both plasma NEFA – which are derived from lipolysis in adipose tissue – and oxidation of intracellular lipids, which are not in equilibrium with the plasma NEFA pool [28]. The plasma NEFA concentration is the major determinant of the plasma NEFA oxidation and accounts for about 40–50% of total lipid oxidation [29]. Therefore, relatively large changes in plasma NEFA concentrations are required to significantly influence the rate of lipid oxidation as measured by indirect calorimetry.

Only in the group of glucose-tolerant relatives, net lipid oxidation – but not the NEFA concentrations – correlated positively with the fat mass. This suggests that a major part of the fat mass is not equilibrated with the plasma NEFA pool, i.e. does not per se result in elevated plasma NEFA concentrations. However, in control subjects and glucose-intolerant relatives, no significant correlations were observed between the fat mass and lipid oxidation.

NEFA concentrations and net lipid oxidation correlated only in glucose-intolerant relatives. Whether this means that a larger part of net lipid oxidation is accounted for by oxidation of plasma NEFA in glucose-intolerant relatives than in control subjects and glucose-tolerant relatives is not known. Given this information, it is evident that plasma NEFA concentrations and net lipid oxidation do change in concert or exert the same effect on e.g. glucose metabolism.

The fact that plasma NEFA concentrations and the rate of lipid oxidation were normal and normally suppressed by insulin does not exclude the possibility that they still could influence glucose metabolism within the physiological range. To further elaborate a possible interaction between plasma NEFA and glucose metabolism in control subjects and relatives we plotted NEFA concentrations and the rate of lipid oxidation against different parameters of glucose metabolism in the groups. In control subjects, there was an inverse relationship between plasma NEFA and glucose disposal which was primarily due to an inhibitory effect of NEFA on non-oxidative glucose metabolism. In contrast, in both groups of relatives, plasma NEFA concentrations as well as the rate of net

lipid oxidation correlated inversely with the rate of glucose oxidation. In glucose-tolerant relatives NEFA concentration showed a positive correlation with the rate of non-oxidative glucose metabolism. Theoretically, the difference between the groups could be a reflection of different rates of glucose metabolism. It has been proposed that NEFA has a restraining effect on glucose metabolism only at high rates of glucose metabolism, i.e. during exercise and in the well-insulinized state [30, 31]. This could, in turn, result in storage of energy as lipids rather than as glucose. In support of this NEFA has been shown to stimulate glycogen synthesis in the rat [31]. Increase of circulating NEFA to 2 mmol/l in the presence of insulin stimulated the activity of glycogen synthase and doubled the rate of glycogen synthesis. A similar increase of NEFA with only basal insulin replacement resulted in a marked reduction in the rate of glycogen synthesis. More recently increasing concentrations of palmitate were shown to inhibit insulin-stimulated glycogen synthesis in human skeletal muscle strips [32]. However, the positive correlation between NEFA and non-oxidative glucose metabolism was observed only in the glucose-tolerant relatives and not in the equally insulin-resistant, glucose-intolerant relatives, meaning that the difference observed between the groups does not seem to be a reflection of different rates of glucose metabolism. Caution is, however, warranted in the interpretation of the data because of the low level of correlation observed between the variables.

Data in humans on the relationship between plasma NEFA and non-oxidative glucose metabolism are inconsistent. Both Thiebaud et al. and Ferrannini et al. showed that supraphysiological increases in the plasma NEFA concentrations induced by Intralipid can inhibit both the oxidative and non-oxidative pathway of glucose metabolism [9, 33]. In contrast, increasing the plasma NEFA concentrations in obese or Type 2 diabetic subjects during hyperinsulinaemic conditions did not result in inhibition of non-oxidative glucose metabolism [34, 35]. Similarly, when the plasma NEFA concentration was maintained unchanged with infusion of heparin, insulin could normally stimulate the rate of glucose storage in healthy subjects [28].

In conclusion, the data challenge the view that the glucose/NEFA cycle contributes to the development of insulin resistance in Type 2 diabetes. However, the glucose/NEFA cycle appears to be operating in both control subjects and relatives but it may at best have only a partial role in producing insulin resistance in individuals at increased risk of developing Type 2 diabetes.

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