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The role of non-esterified fatty acids in the deterioration of glucose tolerance in Caucasian subjects: results of the Paris Prospective Study

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Summary Although an increased plasma non-esterified fatty acid (NEFA) concentration has been shown to increase insulin resistance (Randle cycle), decrease insulin secretion and increase hepatic gluconeogenesis, the effect of NEFA on the deterioration of glucose tolerance has not been studied prospectively in Caucasian subjects. Therefore, we investigated whether plasma NEFA may be regarded as predictors of deterioration of glucose tolerance in subjects with normal (NGT, $n = 3671$) or impaired (IGT, $n = 418$) glucose tolerance who were participants in the Paris Prospective study. The subjects were first examined between 1967 and 1972 and underwent two 75-g oral glucose tolerance tests 2 years apart with measurements of plasma glucose, insulin and NEFA concentrations. Glucose tolerance deteriorated from NGT to IGT or non-insulin-dependent diabetes (NIDDM) in 177 subjects and from IGT to NIDDM in 32 subjects. In multivariate analysis, high fasting plasma NEFA in NGT subjects and high 2-h plasma NEFA and low 2-h plasma insulin concentrations in IGT subjects were significant independent predictors of deterioration along with older age, high fasting and 2-h plasma glucose concentrations and high iliac to thigh ratio. When subjects were divided by tertiles of plasma NEFA concentration at baseline, there was an increase in 2-h glucose concentration with increasing NEFA in the subjects who did not deteriorate, but no effect of plasma NEFA in those who deteriorated. In subjects with IGT who deteriorated compared with those who did not 2-h plasma insulin concentration was lower but there was no evidence that this resulted from an effect of plasma NEFA. Our data suggest that a high plasma NEFA concentration is a risk marker for deterioration of glucose tolerance independent of the insulin resistance or the insulin secretion defect that characterize subjects at risk for NIDDM. [Diabetologia (1997) 40: 1101–1106]

Keywords Non-insulin-dependent diabetes mellitus, impaired glucose tolerance, non-esterified fatty acids, insulin resistance, insulin secretion, epidemiology.

There are several ways by which an increased non-esterified fatty acid (NEFA) concentration could alter glucose tolerance. The first was proposed more than 30 years ago by Randle et al. [1] who suggested that an increase in NEFA, by inhibiting glucose uptake and oxidation in muscle cells, explains the insulin resistance of subjects with non-insulin-dependent diabetes mellitus (NIDDM). The second is an interaction between glucose and NEFA metabolism within the liver. An increased rate of delivery of NEFA to the liver is a stimulus for gluconeogenesis [2, 3] and increased plasma NEFA prevents normal suppression of hepatic glucose production by insulin [4, 5]. The third potential mechanism was revealed more recently with the hypothesis of a lipotoxicity on the beta cells [6]. When exposed chronically to high concentrations of NEFA, the ability of islet cells

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Abbreviations: NEFA, Non-esterified fatty acid; NIDDM, non-insulin-dependent diabetes mellitus; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; ITR, iliac to thigh ratio; OGTT, oral glucose tolerance test.

to secrete insulin in response to glucose is reduced, a defect that can be prevented by inhibitors of fatty acid oxidation [6–8].

Although it has been known for years that subjects with NIDDM have high NEFA concentrations [3, 9], only one longitudinal study, in Pima Indians, has evaluated whether NEFA were implicated in the development of this disease [10]. This study concluded that a high NEFA concentration was a risk marker for NIDDM, independent of percent body fat, fat distribution, and insulin mediated glucose uptake, which may be explained by an inhibition of insulin secretion.

Pima Indians are characterized by a high prevalence of obesity and NIDDM [11]. Therefore, to evaluate whether similar results hold in subjects with a lower risk of NIDDM, we examined the predictive effect of NEFA in the conversion from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and from IGT to NIDDM, over 2 years, in male subjects enrolled in the Paris Prospective Study.

Subjects and methods

Study design and subjects. All the subjects were participants in a longitudinal epidemiologic study of cardiovascular risk factors, the Paris Prospective Study, which was initiated in 1967. The detailed methodology of this study has already been published [12, 13]. Male employees of the Paris Police, born in France between 1917 and 1928 were invited to participate in the study which included five annual clinical and biological examinations.

The subjects underwent, every year for 5 years, a clinical examination with measurements of height, weight in light clothes without shoes. Iliac (at the iliac crest level) and left thigh (at the midlevel between trochanter and lateral femoral condyle) circumferences were only measured at the first examination. The body mass index (BMI: weight (kg) divided by height (m) squared) and the iliac to thigh ratio (ITR) were used as indices of respectively, obesity and central fat distribution.

A glucose tolerance test (OGTT), in which 75 g of glucose was administered orally, was performed at the second and fourth examinations. The biological diagnoses of IGT and NIDDM were based on the 2-h glucose concentration according to World Health Organisation (WHO) criteria for epidemiological studies [14].

Of the 7540 subjects who attended the second clinical examination, 5137 were born in continental France, were free of NIDDM (either previously known or diagnosed by the OGTT) and of patent cardiovascular disease and had plasma glucose [15], insulin [16], triglyceride [17] and NEFA [18] concentrations measured at fasting and 2 h following the first OGTT. Of those, the 4089 subjects included in this analysis had their glucose tolerance status known 2 years later either by the second OGTT or because of diabetes diagnosed by a physician during follow-up. In the analysis, the baseline variables corresponded to the variables recorded at the second examination of the study, except for ITR which was measured 1 year before. All the biological measurements were done at the time of the OGTT.

Statistical analysis. Two-sided unpaired *t*-tests and chi-square tests with the Mantel-Haenszel statistic for linear trend were used for comparison of, respectively, continuous and categorical variables between groups. Because of skewed distributions, a logarithmic transformation was used for insulin and triglyceride concentrations. Correlations between continuous variables used Spearman coefficients. Adjustment for age and BMI were by analysis of covariance. Risk markers for deterioration to IGT and NIDDM were analysed by multivariate stepwise logistic regression with a p value for entry of less than 0.10 and age forced into the model. All analyses were done with the SAS software (SAS Institute Inc, Cary, N.C., USA) on a IBM 7013-J40 computer.

Results

Of the 4089 subjects included in the analysis, 3671 had NGT and 418 IGT at the first OGTT.

Glucose tolerance deteriorated over 2 years in 177 (4.8%) of the subjects who initially had NGT: 27 (0.7%) subjects were diagnosed as diabetic and 150 (4.1%) subjects were classified as having IGT by the second OGTT. Thirty-two (7.6%) of the subjects with IGT at baseline had developed NIDDM at the time of the second OGTT, 273 (65.3%) had reverted to NGT and 113 (27.0%) had remained IGT. Within each group of glucose tolerance at baseline, fasting and 2-h plasma glucose, fasting plasma insulin, triglyceride and fasting and 2-h plasma NEFA concentrations were higher in subjects whose glucose tolerance deteriorated (Table 1). The mean 2-h plasma insulin concentration was significantly higher in the subjects with NGT who deteriorated compared with those who did not, but the highest concentrations were observed in IGT subjects who did not deteriorate during follow-up. The subjects who developed NIDDM from IGT had an intermediate mean concentration which however did not differ statistically from that of the other IGT subjects.

At baseline, fasting plasma NEFA concentrations correlated positively with 2-h plasma glucose concentrations (in NGT: $r = 0.20$, $p < 0.001$; in IGT: $r = 0.13$, $p < 0.01$). Therefore, a multivariate analysis was performed to evaluate whether a high plasma NEFA concentration at baseline was associated with a deterioration of glucose tolerance independent of plasma glucose concentration. In NGT subjects, when age, fasting and 2-h plasma glucose concentrations and iliac to thigh ratio were accounted for, a higher concentration of fasting plasma NEFA contributed significantly to an increased risk of developing IGT or NIDDM. An increase of 0.12 mmol/l (one SD) in fasting plasma NEFA was associated with a 30% increase in the risk of deterioration of glucose tolerance ($p < 0.001$) (Table 2). In contrast, neither BMI nor fasting or 2-h plasma insulin concentrations added a significant predictive value once glucose concentrations were in the model. In IGT subjects, both low 2-h plasma insulin and high 2-h plasma NEFA concentrations were independently and significantly

Table 1. Comparison of the baseline characteristics of the subjects who did or did not deteriorate their glucose tolerance according to baseline glucose tolerance status

Baseline status	NGT		IGT	
2-year follow-up glucose tolerance	Not deteriorated	Deteriorated	Not deteriorated	Deteriorated
n	3494	177	386	32
Age (years)	48.9 ± 1.8	49.1 ± 1.6	48.7 ± 1.8	48.8 ± 1.8
BMI (kg/m^2)	26 ± 3	27 ± 4[b]	27 ± 4	27 ± 4
Iliac thigh ratio	1.7 ± 0.1	1.8 ± 0.1[b]	1.8 ± 0.1	1.9 ± 0.1[d]
Fasting plasma glucose (mmol/l)	5.5 ± 0.5	5.9 ± 0.6[b]	6.0 ± 0.6	6.6 ± 0.8[d]
2-h plasma glucose (mmol/l)	5.2 ± 1.1	6.1 ± 1.1[b]	8.8 ± 0.8	9.3 ± 0.9[d]
Fasting plasma insulin (pmol/l)	68 (19–248)	83[b] (21–311)	86 (22–345)	107 (25–457)
2-h plasma insulin (pmol/l)	222 (48–1021)	302[b] (71–1280)	509 (132–1962)	479 (113–2028)
Fasting plasma triglyceride (mmol/l)	1.2 (0.4–3.2)	1.4[b] (0.4–4.3)	1.4 (0.5–4.3)	1.5 (0.6–3.9)
Fasting plasma NEFA (mmol/l)	0.29 ± 0.12	0.35 ± 0.14[b]	0.40 ± 0.17	0.42 ± 0.14
2-h plasma NEFA (mmol/l)	0.12 ± 0.07	0.14 ± 0.06[a]	0.14 ± 0.07	0.16 ± 0.06[c]

Mean ± SD or geometric mean (95% confidence interval). From NGT [a] $p < 0.01$, [b] $p < 0.001$. From IGT [c] $p < 0.10$, [d] $p < 0.001$

Table 2. Significant predictors of deterioration of glucose tolerance from normal or impaired glucose tolerance by stepwise logistic regression

From normal glucose tolerance						
Steps		I	II	III	IV	
Variables	Age[c]	Fasting glucose	2-h glucose	ITR	Fasting NEFA	
Odds ratio[a]	1.1	1.7	1.7	1.4	1.3	
95% CI[b]	1.0–1.3	1.5–2.0	1.4–2.0	1.2–1.6	1.1–1.4	
From impaired glucose tolerance						
Steps		I	II	III	IV	V
Variables	Age[c]	Fasting glucose	2-h glucose	ITR	2-h insulin	2-h NEFA
Odds ratio[a]	1.1	2.6	1.7	1.9	0.6	1.4
95% CI[b]	0.7–1.8	1.7–3.8	1.2–2.5	1.3–2.8	0.4–1.0	1.0–2.1

[a] Odds ratio for an increase of 1 SD in the population of subjects with NGT or IGT. [b] 95% confidence interval of the odds ratio [c] forced into the model

associated with an increased risk of NIDDM in IGT subjects, after fasting and 2-h plasma glucose concentration and ITR were accounted for (Table 2). The increase in risk associated with a variation of 0.07 mmol/l (one SD) in 2-h plasma NEFA was 42% in IGT subjects. In both models of deterioration of glucose tolerance, from normal or impaired glucose tolerance, fasting NEFA could be interchanged with 2-h NEFA with close predictive value. The results were similar when the area under the insulin curve was proposed for entry into the models in place of fasting and 2-h insulin concentrations. A low area under the insulin curve was predictive of deterioration in IGT subjects and a high 2-h NEFA concentration had a significant and independent predictive value.

To further illustrate the relationships between plasma NEFA concentrations and glucose metabolism, the 4089 subjects were categorized according to tertiles of fasting plasma NEFA concentrations at baseline (cut-off points: 0.23 and 0.33 mmol/l). More than half of the subjects with IGT (62%) were in the upper tertile of fasting plasma NEFA concentration. The rate of deterioration of glucose tolerance from NGT were, from the lower to the upper tertiles of fasting NEFA, 3.3 (44/1320), 3.5 (44/1254) and 8.1% (89/1097) (Mantel-Haenszel test for trend: $p < 0.001$). From IGT, they were 3.3 (2/60), 5.1 (5/99) and 9.7% (25/259) (Mantel-Haenszel test for trend: $p < 0.06$). In the subjects who maintained NGT, the increase in fasting plasma NEFA was associated with a progressive increase of 2-h plasma glucose concentration (Fig. 1, left panel) and the age and BMI adjusted mean difference between fasting and 2-h plasma glucose concentrations decreased from 0.47 to 0.05 mmol/l ($p < 0.001$). In contrast, in subjects whose glucose tolerance deteriorated from NGT, the 2-h plasma glucose concentration was already elevated in the lower tertile of fasting plasma NEFA

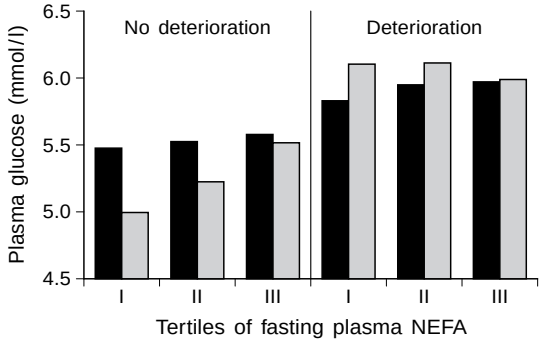

Fig. 1. Age and BMI adjusted mean fasting and 2-h plasma glucose concentrations according to tertiles of fasting plasma NEFA in subjects who did or did not deteriorate their glucose tolerance from normal

concentration and it was not significantly influenced by the plasma NEFA concentration (Fig. 1, right panel).

The subjects with IGT who deteriorated had lower 2-h insulin concentrations than those who did not deteriorate (Table 1). All but seven of these subjects were in the highest tertile of NEFA concentration (78%). However, there was no significant effect of fasting plasma NEFA on either fasting or 2-h plasma insulin concentrations in subjects with NGT whose glucose tolerance deteriorated: from the lowest to the highest tertile of fasting NEFA concentrations, the age and BMI adjusted mean fasting and 2-h insulin concentrations were respectively 78, 91, 79 and 309, 372, 281 pmol/l.

Discussion

This study demonstrates for the first time in a large-scale epidemiologic study of Caucasian subjects that a high plasma NEFA concentration is a risk marker for the deterioration of glucose tolerance from both NGT and IGT.

A relationship between glucose and NEFA metabolism has been known since Randle's first description in 1963 of a "glucose-fatty acid cycle" in perfused rat hearts and diaphragms [1]. Randle postulated a substrate competition between NEFA and glucose for the production of energy in muscle cells, in which an increased NEFA oxidation inhibited glucose uptake and oxidation. Although the exact mechanism is still debated [19, 20] an operative "glucose-fatty acid cycle" has been confirmed in healthy humans: several investigators, using hyperinsulinaemic glucose clamps, have found that increasing plasma NEFA concentration by infusion of a triglyceride emulsion, had an inhibitory effect on total body glucose uptake [5, 21, 22] and therefore was able to produce an insulin resistant state in healthy humans. Our study is in accordance, showing, in subjects who maintained NGT, a reduced ability to lower glucose concentration after an oral glucose load, when plasma NEFA concentration increased (Fig. 1, left panel).

In subjects whose glucose tolerance deteriorated from NGT, however, fasting and 2-h glucose concentrations were elevated and an increase in plasma NEFA concentration had no obvious effect. Thus, in these subjects, the "glucose-fatty acid cycle" was not shown. Similarly, Eriksson and collaborators [23] found an inverse correlation between plasma NEFA concentration and total glucose disposal during a euglycaemic hyperinsulinaemic clamp in control subjects but no correlation in first degree relatives of subjects with NIDDM.

In subjects with NIDDM and also in their first degree relatives, the increased availability of NEFA cannot be held responsible for the insulin resistance, which is mainly accounted for by an impaired non-oxidative glucose metabolism [24–26]. In this study, the subjects whose glucose tolerance deteriorated from either NGT and IGT had higher plasma glucose concentrations despite higher fasting plasma insulin concentrations, compared with those who did not deteriorate. A high fasting plasma insulin concentration has been shown to be a predictor of the development of NIDDM in this population [27] and is considered to be a reflection of a higher degree of insulin resistance [28]. The fasting plasma insulin concentration did not change with increasing plasma NEFA concentration in subjects whose glucose tolerance worsened from NGT. Therefore, our results show, in agreement with the literature, that the insulin resistance which characterizes the subjects at high risk of NIDDM is not explained by their higher plasma NEFA concentration.

Recently, the theory of lipotoxicity on beta cells has been developed following in vitro and in vivo animal experimentations [6]. According to this theory, their chronic exposure to high concentrations of NEFA decreases glucose-induced insulin secretion [7, 8]. In Zucker diabetic fatty rats, the plasma NEFA concentration starts to increase before the plasma glucose concentration. At the time NIDDM develops, there is a ten-fold increase in islet triglyceride content and a loss of glucose-induced insulin secretion [29]. These anomalies can be prevented by reducing plasma NEFA concentration [6]. In our study, we also found that an increase in NEFA concentration preceded the deterioration of glucose tolerance (Table 2) but we did not find any evidence that it was, at this stage, associated with a decrease in glucose-stimulated insulin secretion, as measured by the 2-h insulin concentration or the area under the insulin curve after a 75-g glucose load. In the Pima Indian study, the acute insulin response to intravenous glucose was measured. In that study, the risk ratio for NIDDM in subjects over the 90th percentile compared with those below the 10th percentile of plasma

NEFA concentration decreased only from 2.1 (95% confidence interval: 1.1–4.2) to 2.0 (0.7–5.3) when the acute insulin response was accounted for, in addition to sex, percent body fat and insulin sensitivity measured by a euglycaemic hyperinsulinaemic clamp [10]. It is likely that the loss of significance of the NEFA effect was due to the smaller number of subjects with data on acute insulin response, than to a real confounding effect. In our study, a high plasma NEFA concentration and a decreased 2-h plasma insulin concentration were independent significant predictors of deterioration to NIDDM in IGT subjects (Table 2). However, it is noticeable that 78% of the 32 subjects who develop NIDDM from IGT were in the highest tertile of fasting NEFA concentration. This might actually be a consequence of their reduced insulin secretion, leading to a reduced suppression of lipolysis by insulin [30]. Reaven [30] suggested that the increase in plasma NEFA concentration that occurs when insulin resistant individuals can no longer maintain a state of compensatory hyperinsulinaemia, is primarily responsible for the rapid increase in blood glucose concentration in subjects who develop NIDDM, through a stimulation of hepatic glucose production by the increased NEFA flux to the liver.

Therefore, with two different (although imperfect) means of assessing insulin secretion in vivo, the Pima study and the present study suggest that the subjects whose glucose tolerance deteriorates do not exhibit major defects in glucose-induced insulin secretion in relation to their higher NEFA concentration when they are at the stage of either NGT or IGT. However, it cannot be excluded that the high NEFA concentration potentiates the insulin secretion defect at a later stage in the development of the disease, for example when NIDDM occurs, as seems to be the case in Zucker rats [29].

In conclusion, a high NEFA concentration is a predictor of conversion from NGT to IGT and from IGT to NIDDM. This was also the conclusion of the Pima study of men and women in a younger age range. The concordance of the two studies allows some generalization of the results. It emphasizes the need to develop actions or treatments not only directed towards lowering of glucose but also of lipid concentration for the prevention of NIDDM.

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