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High free fatty acid concentration: an independent risk factor for hypertension in the Paris Prospective Study

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Background An inconsistent relationship has been reported between insulin and hypertension incidence. Free fatty acids are related to insulin-resistance and may have a direct effect on hypertension. We examined the effect of free fatty acids on hypertension incidence, taking into account other abnormalities of the insulin-resistance syndrome.

Methods In all, 2968 non-hypertensive and non-diabetic Caucasian men were followed for 3 years. Hypertension incidence was defined as systolic blood pressure (SBP) ≥160 mmHg or diastolic blood pressure (DBP) ≥95 mmHg or drug treatment for hypertension.

Results Free fatty acid elevation was a highly significant risk factor for hypertension when controlled for age, family history of hypertension, alcohol consumption, body mass index, iliac circumference and weight change. Further controlling for SBP, heart rate and fasting insulin and glucose did not decrease its predictive power (hazard rate ratio [RR] = 1.58, 95% confidence interval [CI] : 1.30–1.91 comparing the 90th to the 10th percentiles at fasting; RR = 1.54, 95% CI : 1.33–1.79 at 2 h). In a forward stepwise model controlled for age, family history of hypertension, alcohol consumption and SBP, the selected variables explaining the occurrence of hypertension were, in order, weight change, 2-h free fatty acids, iliac circumference and fasting free fatty acids, whereas body mass index, heart rate, insulin, glucose and other lipids did not enter into the model.

Conclusions Free fatty acids elevation, when controlled for all known risk factors and other abnormalities of the insulin-resistance syndrome, is a risk factor for hypertension. These results highlight the possible benefits of treatment using free fatty acid oxidation inhibitors.

Keywords Free fatty acids, obesity, essential hypertension, insulin, lipids, men

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The insulin-resistance syndrome is an association of hypertension with insulin-resistance, hyperinsulinaemia, glucose intolerance, increase of very-low-density-lipoprotein triglycerides and decrease of high-density-lipoprotein cholesterol,[1] and insulin-resistance is thought to be the underlying abnormality.[2] However, the role of abdominal fat distribution and free fatty acids has been emphasized recently.[3,4] The glucose-free fatty acid cycle was first proposed by Randle who suggested that an increased availability of free fatty acids resulted in increased fat oxidation in muscle with a secondary decrease in the muscle glucose uptake.[5] A high free fatty acid level, which is related to abdominal fat, diabetes and insulin resistance,[6] may also stimulate gluconeogenesis, reduce the action of insulin to suppress hepatic glucose production,[7] worsen the glucose tolerance,[8] and have a direct toxic effect on beta cells.[9] In fact, free fatty acids have been found to be a risk factor for diabetes in two epidemiological studies.[10,11] Thus, a high free fatty acid level may be considered as one of the components of the insulin-resistance syndrome.

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The link between insulin-resistance and hypertension has not been consistent in prospective epidemiological studies when adjusted for obesity and abdominal fat distribution.[12–19] In the Paris Prospective Study, obesity and abdominal fat distribution were strongly predictive of hypertension, and, when controlled for both factors, hyperinsulinaemia was a significant predictive factor for hypertension only in men who gained weight over the 3 years of follow-up.[20] An underlying abnormality, related to obesity or glucose tolerance (e.g. free fatty acid elevation), may be responsible for this relationship. However, it is also possible that free fatty acids have a direct effect on blood pressure.

No previous large prospective study has examined free fatty acids and their role in hypertension. Therefore, we studied lipid (total cholesterol, triglyceride, free fatty acid) abnormalities as factors predictive for hypertension in 2968 active, middle-aged Caucasian men from the Paris Prospective Study, who were followed for 3 years, taking into consideration the possible confounding effects of obesity, abdominal fat distribution, glucose and insulin levels.

Materials and Methods

Research design

The Paris Prospective Study included, between 1967 and 1972, 7991 Paris policemen. They were aged 43–54 years at the time of the first screening, which consisted of a questionnaire about health and habits and anthropometric measurements including height (to the nearest cm) with the subject wearing no shoes, and iliac circumference (at the iliac crest level, to the nearest mm). At the second examination, the baseline for this analysis, a blood sample was taken between 8.30 and 9 am, with subjects sitted and rested, at fasting and following a 75 g oral glucose tolerance test. Fasting and 2-h plasma glucose were measured by an automatic enzymatic procedure[21] and insulin by a radioimmunologic method.[22] Smoking and exercise were prohibited before and during the test. Fasting and 2-h plasma free fatty acid levels were assayed using a colorimetric method[23] after immediate centrifugation at +4°C. Total cholesterol (colorimetric method[24]) and triglyceride (fluorometric method[25]) levels were measured at fasting. At this examination, and at the subsequent three examinations, weight was measured to the nearest 0.5 kg with the subjects wearing light clothing, and blood pressures were taken once by the same trained personnel following a standard protocol. Subjects were seated for 15 min and the first and fourth Korotkoff sounds were measured on the right arm to the nearest 10 mmHg with a standard mercury sphygmomanometer. The heart rate was recorded at the same time. Hypertension was defined according to WHO criteria[26] but at only one of the three examinations after baseline: diastolic blood pressure (DBP) $\geq$95 mmHg and/or systolic blood pressure (SBP) $\geq$160 mmHg and/or current use of antihypertensive medications. As blood pressures were measured to the nearest 10 mmHg, our definition of hypertension included men with SBP $\geq$155 mmHg. Body mass index was calculated as weight (kg) divided by the square of height (m^2). A family history of hypertension included hypertension in any parent, brother or sister. Excessive alcohol consumption was defined by a clinical examination typical of cirrhosis or clinically diagnosed alcoholism or by a mean corpuscular volume >98 μ^3, a marker linearly related with alcohol intake.[27]

From the 7070 people born in France who attended the second (baseline) examination and had blood pressure measurements, fasting and 2-h free fatty acid measurements were measured in 5492 unselected men. At baseline, there were 1447 hypertensive and 92 diabetic men, 745 men who had missing values for total cholesterol, triglyceride, fasting or 2-h insulin, glucose levels, body mass index, iliac circumference, alcohol consumption or family history of hypertension, and 240 men who did not attend at least one of the three follow-up examinations, and who were successively excluded. The present analysis included 2968 men.

Statistical analysis

Insulin and triglyceride levels were log-transformed. Analysis of covariance and χ^2 tests were used for comparing means and prevalence rates between subgroups. The relationship between baseline variables and hypertension incidence was assessed by Cox's proportional hazards regression analysis.[28] Adjustment was made for factors known to be predictive of hypertension: age, body mass index, central adiposity (iliac circumference), body weight change, family history of hypertension, excessive alcohol consumption, baseline SBP and heart rate, a marker of stress[29] and a measure of sympathetic nervous system activity, and for the possible confounding factors, fasting insulin and glucose. The curvilinearity of and the interactions between variables were evaluated by a likelihood ratio test:[30] body weight change showed a curved relationship with the incidence of hypertension, and both linear and squared terms were used in the models. The hazard rate ratios (RR) and 95% confidence intervals (CI) of developing hypertension were calculated comparing the 90th and 10th percentiles. The validity of the proportional hazards assumption for the covariates was verified as suggested by Kalbfleish and Prentice.[31] The follow-up time was restricted to 3 years so that the free fatty acid levels respected the proportionality assumption of the model. Because age and baseline SBP violated the proportionality assumption, strata were used for the baseline hazard function: SBP at baseline (100–120 mmHg; 130 mmHg; 140 mmHg; 150 mmHg) and two age groups divided by the median (49.5 years). In order to check the validity of the time-dependent analysis, a logistic regression was performed, including the entire follow-up time. Similar results were found (data not shown).

All statistical analyses used the SAS statistical package. Statistical significance was defined as $P < 0.05$.

Results

The mean length of follow-up of the 2968 men was 2.7 years (range: 0.3–3). Table 1 shows the characteristics of the subjects at baseline. Rates of excessive alcohol consumption and family history of hypertension were 31% and 45%, respectively.

The incidence rates of hypertension increased significantly with fasting and 2-h free fatty acid levels ($P < 0.001$) (Figure 1). In proportional hazards models, a positive family history of hypertension (RR = 1.27, 95% CI : 1.07–1.50), but neither an excessive alcohol consumption (RR = 1.01, 95% CI : 0.84–1.21) nor age (Table 1), was a predictive factor for hypertension. A weight increase and high body mass index, iliac circumference, baseline blood pressures, heart rate, glucose, insulin, total cholesterol, triglyceride and free fatty acid levels were all univariate

Table 1 Baseline characteristics (median, 10th and 90th percentiles), hazards rate ratios and 95% confidence intervals for hypertension incidence from proportional hazards models: 90th compared with 10th percentile. 2968 subjects participating in the Paris Prospective Study and followed for 3 years (539 incident cases)

	10th percentile	Median	90th percentile	Hazard rate ratio [95% confidence interval]
Age (years)[a]	46.0	49.5	51.4	0.81 [0.64–1.03]
Body mass index (kg/m^2)	21.8	25.3	29.1	2.29 [1.88–2.79]
Iliac circumference (mm)	790	900	1010	2.50 [2.02–3.09]
Weight change (kg/m^2/year)[b]	–0.41	+0.09	+0.54	1.29 [1.10–1.52]
Systolic blood pressure (mmHg)[a]	120	130	150	4.27 [3.34–5.46]
Diastolic blood pressure (mmHg)	70	80	90	2.39 [1.93–2.96]
Heart rate (min^{-1})	56	64	75	1.48 [1.23–1.77]
Fasting insulin (pM)	22	72	136	1.63 [1.27–2.09]
2-hour insulin (pM)	86	237	603	1.45 [1.17–1.81]
Fasting glucose (mM)	4.88	5.55	6.11	1.74 [1.43–2.11]
2-hour glucose (mM)	3.77	5 33	7.44	1.44 [1.18–1.75]
Total cholesterol (mM)	4.27	5.44	6.86	1.23 [1.00–1.51]
Triglyceride (mM)	0.66	1.11	2.21	1.43 [1.17–1.73]
Fasting free fatty acids (mM)	0.16	0.27	0.44	1.75 [1.47–2.09]
2-hour free fatty acids (mM)	0.07	0.11	0.18	1.47 [1.28–1.69]

[a] The proportionality assumption of the proportional hazards model was not respected.

[b] 2873 subjects had no missing values for weight change (524 incident cases).

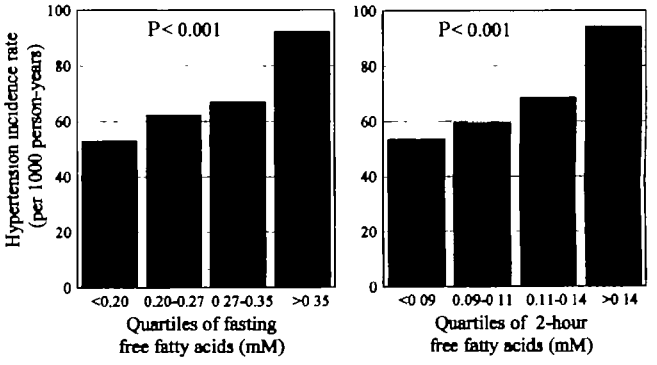

Figure 1 Incidence rates of hypertension by quartiles of fasting and 2-hour free fatty acids. 2968 subjects participating in the Paris Prospective Study and followed for 3 years (539 incident cases)

predictive factors for hypertension. Further analyses were controlled for age, family history of hypertension and excessive alcohol consumption because of their known predictive value for hypertension in other populations. High cholesterol and triglyceride levels were no longer significant risk factors when further controlled for body mass index, iliac circumference and weight change (Table 2). High fasting and 2-h free fatty acid levels were still highly significant risk factors when additionally controlled for SBP and heart rate. Further controlling for fasting insulin and glucose, which were not significant predictors in these models, did not decrease the predictive power of free fatty acids for hypertension. Similar results were found when the models were controlled for 2-h insulin and glucose (data not shown). In a forward stepwise model controlled for age, family history of hypertension, excessive alcohol consumption and SBP, the variables selected to explain hypertension incidence were, in order, weight change, 2-h free fatty acids, iliac circumference and fasting free fatty acids, whereas body mass index, heart rate, insulin, glucose and other lipid levels did not enter into the model (Table 3).

Table 2 Hazards rate ratios and 95% confidence intervals for hypertension incidence from proportional hazards models: 90th compared with 10th percentile. 2968 subjects participating in the Paris Prospective Study and followed for 3 years (539 incident cases)

	Hazard rate ratio [95% confidence interval]			
	Models 1[a]	Models 2[b]	Models 3[c]	Models 4[d]
Total cholesterol	1.22 [0.99–1.50]	1.07 [0.86–1 32]	0.97 [0.78–1.20]	0.95 [0.77–1.18]
Triglyceride	1.43 [1.18–1.74]	1.14 [0.92–1.41]	1.02 [0.83–1.26]	1.01 [0.82–1.25]
Fasting free fatty acids	1.79 [1.50–2.14]	1.73 [1.44–2.08]	1.58 [1.31–1.90]	1.58 [1.30–1.91]
2-hour free fatty acids	1.47 [1.28–1.69]	1.55 [1.34–1.79]	1.53 [1.32–1.78]	1.54 [1.33–1.79]

[a] Controlled for age, family history of hypertension and excessive alcohol consumption.

[b] Controlled for variables in model 1 and body mass index, abdominal fat distribution and weight change 2873 subjects and 524 incident cases.

[c] Controlled for variables in model 2 and systolic blood pressure and heart rate. 2873 subjects and 524 incident cases

[d] Controlled for variables in model 3 and fasting insulin and glucose. 2873 subjects and 524 incident cases.

Table 3 Hazards rate ratios and 95% confidence intervals for hypertension incidence from a final forward stepwise proportional hazard model controlled for age, family history of hypertension, excessive alcohol consumption and systolic blood pressure[a] 90th compared with 10th percentile. 2873 subjects participating in the Paris Prospective Study and followed for 3 years (524 incident cases)

	Hazard rate ratio [95% confidence interval]	P^b
1. Weight change	1.35 [1.14–1.61]	<0.0001
2. 2-hour free fatty acids	1.40 [1.18–1.66]	<0.0001
3. Iliac circumference	1.85 [1.48–2.31]	<0.0001
4. Fasting free fatty acids	1.35 [1.11–1.66]	0.0034

[a] Variables that did not enter into the model ($P > 0.05$). body mass index, heart rate, fasting and 2-h glucose and insulin, total cholesterol and triglyceride levels.

[b] P-value from a likelihood ratio test.

Discussion

In the present population of middle-aged Caucasian men, high fasting and 2-h free fatty acid levels were risk factors for hypertension over 3 years of follow-up after controlling for possible confounding factors. No effect of triglyceride or cholesterol levels was found.

First, high free fatty acids may have a direct role on hypertension. No other epidemiological study has examined the role of free fatty acids on hypertension incidence but clinical studies have suggested such a direct effect and gave possible explanations for a causal relationship.[32–41] Free fatty acids may increase the neurovascular tone by enhancing α_1-adrenoreceptor sensitivity and raising sympathetic drive, and may inhibit endothelium-dependent vasodilation, as recently reviewed.[42] Different mechanisms of signal transduction for free fatty acids to increase blood pressure have been proposed in the same review. Free fatty acids may activate protein kinase C[42], and may affect the Na^+, K^+ and Ca^{2+} currents and the composition of membrane phospholipids and the membrane fluidity.[43] Free fatty acids may also inhibit the Na^+ K^+ ATPase pump, thereby leading to high intracellular sodium and calcium, and increasing vascular muscle tone and blood pressure.[44,45] They may also regulate aldosterone secretion[46] and may inhibit endothelial nitric oxide synthase.[47] Moreover, free fatty acids may play a role in cardiovascular diseases.[48] A Randle cycle has been demonstrated recently in the human heart and long-term adverse effects on myocardial function may occur.[8,49] Free fatty acids are the preferred substrate for the heart,[50] but may be deleterious during ischaemia when glucose oxidation becomes more important due to its lower cost of oxygen per ATP generated.[51,52] However, haemodynamic modifications, related with free fatty acids, have not been well established in humans and must be further studied.

Other factors may confound the relationship between free fatty acids and hypertension. First, catecholamines activate adipose tissue hydrolysis, and free fatty acid concentration increases during stress and exercise; both are responsible for an increase in blood pressure.[53] However, the subjects were seated and rested, and smoking and exercise were prohibited before and between the fasting and the 2-h draw. To take into account a remaining effect of catecholamines, and because the subjects were policemen who may be particulary exposed to stress, adjustment was performed for baseline heart rate, which is a reflection of stress, a measure of sympathetic nervous system activity and a risk factor for hypertension in our study, that may be related to free fatty acid level.[42] We also adjusted for baseline SBP, a strong predictive factor for hypertension. However, high free fatty acid levels remained significant risk factors for hypertension when controlled for baseline SBP and heart rate. These results may also suggest that the major mechanism by which free fatty acids lead to hypertension may be independent of their effect on the sympathetic drive.

Free fatty acids may also be related to hypertension through their association with other factors included in the insulin-resistance syndrome, such as obesity and insulin and glucose levels. Free fatty acid portal flux is mainly related to the enlarged fat mass.[6] In the present analysis, weight increase and high iliac circumference were highly predictive factors for hypertension in the forward stepwise model. However, in a cross-sectional analysis in the same population,[54] as well as in another population,[55] free fatty acid level was elevated in hypertensive subjects at any body mass index, but especially in the lean hypertensive subjects in the Paris population. In the present prospective analysis, elevated fasting and 2-h free fatty acid concentrations, when controlled for body mass index, iliac circumference and weight change, were both strong risk factors for hypertension. Analyses performed among obese and non-obese subjects (defined by the medians of body mass index and iliac circumference) gave similar results (data not shown). Thus, obesity does not explain the relationship between free fatty acids and hypertension. Insulin, which is a free fatty acid regulator[56] and a marker of insulin-resistance in non-diabetic subjects, may be another confounder, although the link between insulin and hypertension has not been consistent in prospective epidemiological studies when adjusted for obesity and abdominal fat distribution.[12–19] Fasting insulin and fasting and 2-h glucose have been found to be predictive factors for hypertension in the Paris Prospective Study but mainly in men who gained weight or had a positive family history of hypertension.[20] However, free fatty acids were strong risk factors for hypertension when the models were further controlled for these two variables in the present analysis. Moreover, insulin and glucose levels did not enter into the forward stepwise model, when free fatty acids did. Thus, free fatty acids may be a risk factor for hypertension, independent of the insulin-resistance syndrome abnormalities.

Another important confounding factor may be alcohol consumption, because of its high level in this French population,[57] but it was imprecisely ascertained in our study. Alcohol consumption is related to an increase in blood pressure[58] and in triglycerides,[59,60] and a possible increase in free fatty acids.[60] However, it is unlikely that alcohol by itself would explain the predictive power of free fatty acids on hypertension, because hypertriglyceridaemia was not predictive of hypertension in this cohort. A limitation of the present study is the definition of hypertension: the use of the fourth Korotkoff sound and of a single measure of blood pressure. The lack of accuracy in the measurement of blood pressure (to the nearest 10 mmHg) was only partially accounted for by the methodology we used (Cox models and stratification by class of blood pressure at baseline to respect the proportionality assumption). The 3-year incidence

rates of hypertension were higher than in other studies,[16–19,61] but this is also probably due to the age and social characteristics of the population studied. Misclassifications and overestimation of hypertension incidence would have resulted in decreasing the likelihood of detecting risk factors. Moreover, similar results were found when we used a higher cutoff point for SBP to define hypertension (SBP ⩾165 mmHg, 3303 men and 642 cases of incident hypertension). Neither the 95 men with missing information for weight during the 3 years of follow-up, nor the 13 subjects who died during the 3 years of follow-up before the occurrence of hypertension, differed from other subjects on blood pressure and free fatty acid levels (data not shown). However, the 354 men who had a following examination but were lost to follow-up before the occurrence of hypertension and the third year had lower baseline DBP, but higher fasting and 2-h free fatty acid levels than subjects who were followed, when adjusted for age ($P \leqslant 0.04$, data not shown). Including these 354 men lost to follow-up in the analysis as if they were followed for 3 years without developing hypertension, the free fatty acid predicting power on hypertension did not decrease (data not shown) but these results were not adjusted for weight change. The 240 men who were not selected in the previous analysis because they had no examination after baseline had similar blood pressures but a higher age-adjusted fasting free fatty acid level ($P = 0.03$, data not shown) than subjects who were followed. Including these 240 men in the analyses did not modify the results either (data not shown). Therefore, biases in the subject selection are not likely to have influenced our results.

In conclusion, elevated fasting and 2-h free fatty acid concentrations were strong predictive factors for hypertension among active middle-aged Caucasian men. This effect on hypertension was independent of all known risk factors and of the effect of other abnormalities of the insulin-resistance syndrome. The present results suggest also that the major mechanism by which free fatty acids lead to hypertension may not be by raising sympathetic drive. Because free fatty acid elevation has also been found to be an independent risk factor for mortality[62] and for diabetes[11] in the same population, it may be the underlying abnormality of the risk factor cluster called the insulin-resistance syndrome, which is responsible for a high rate of cardiovascular disease. These results highlight the metabolic and haemodynamic implications of free fatty acid elevation and the possible benefits of treatment using free fatty acid oxidation inhibitors,[48] and must be further investigated.

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