# Relationships Between Fasting Plasma Insulin, Anthropometrics, and Metabolic Parameters in a Very Old Healthy Population

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**Several studies have shown that insulin resistance and hyperinsulinemia are associated with many metabolic disorders predisposing to coronary heart disease (CHD). This syndrome has been termed syndrome X. However, it is not completely known whether these relationships are still present in the elderly, or whether other factors such as age, gender, and body fat distribution modulate them. Therefore, we investigated the relationship between fasting plasma insulin, total and regional adiposity, fasting plasma glucose and lipids, plasma plasminogen activator inhibitor-1 (PAI-1), fibrinogen, and coagulation factor VII in a sample of 100 healthy free-living octogenarians-nonagenarians (52 men and 48 women) who were disability-free according to the Katz index. By univariate analysis, fasting insulin correlated positively with all anthropometric measures except the waist to hip ratio (WHR) in women. There was a positive correlation between fasting insulin and fasting glucose ($r = .40$, $P < .01$), plasma triglycerides ([TGs] $r = .21$, $P < .05$), and PAI-1 levels ($r = .33$, $P < .01$), whereas a negative relation was found with high-density lipoprotein cholesterol (HDL-C) and apolipoprotein, A-I (apo A-I) levels ($r = -.22$ and $r = -.24$, respectively, $P < .05$). These relationships were weaker and less significant in women. In pooled data, stepwise multiple regression analysis showed an independent relationship of both the body mass index (BMI) and fasting insulin level with TGs ($R^2 = .14$), while gender and fasting insulin were the best predictors of HDL-C variance ($R^2 = .17$). Furthermore, fasting insulin was the only variable independently related to PAI-1 ($R^2 = .12$). Our findings support the existence of a metabolic syndrome even in very old age by showing that high insulin levels are related to various metabolic and hemostatic disorders.**


OVER THE LAST FEW DECADES, a progressive increase in life expectancy in Western nations has been observed.[1] Therefore, it is becoming more important to evaluate the risk factors for atherosclerosis since its clinical consequences, such as coronary heart disease (CHD) and stroke, are the leading causes of disability and death in the population over 65 years old.[2,3]

Several studies have highlighted the association between insulin levels and various metabolic disorders, including glucose intolerance and dyslipidemia (high triglyceride [TG] and low high-density lipoprotein cholesterol [HDL-C] levels).[4,5] These disorders tend to cluster in the same individuals, and Reaven[6,7] used the term "syndrome X" to refer to a complex plurimetabolic disease in which insulin resistance and hyperinsulinemia are the main underlying defects. Moreover, recent evidence demonstrates that plasminogen activator inhibitor-1 (PAI-1), coagulation factor VII, and fibrinogen levels are related to insulin resistance and hyperinsulinemia, as well as an increased risk of CHD.[8] However, these relationships were mostly confirmed in middle-aged individuals, and relatively little information in old populations is available.[9,10]

Recent studies have shown that aging is associated with an increase in total adiposity with a predominantly abdominal fat distribution.[11,12] Because abdominal adiposity is strongly related to insulin resistance and hyperinsulinemia, it could be argued that the metabolic abnormalities associated with syndrome X in aging are due to an increase in abdominal fat distribution.[13,14] On the other hand, aging is often characterized by a reduced level of physical activity, malnutrition, and chronic illness, which can impact the relationship between insulin levels and metabolic parameters.[15,16]

This study was undertaken to assess the relationship between fasting insulin, fat distribution, and several metabolic and hemostatic parameters typically associated with syndrome X in very old free-living healthy persons. This is the first known study to address these specific objectives in a healthy, self-sufficient population of both sexes aged over 80 years.

## SUBJECTS AND METHODS

The val Vibrata is a valley located in the province of Teramo in the northern Abruzzo region of central Italy. The val Vibrata Aging Project is an ongoing longitudinal observational study initiated in 1992; its aims are to measure metabolic and anthropometric parameters and plasma lipid peroxide levels in a sample of healthy free-living octogenarians-nonagenarians, and to evaluate the impact of these parameters on future disease, disability, and cause of death. Lipoprotein(a) levels and apolipoprotein (a) isoform distribution, as well as plasma lipid peroxides and antioxidant systems, in this sample were previously described in detail elsewhere.[17,18]

### *Subjects*

Fourteen thousand seven hundred eight inhabitants presenting to 10 family doctors (Associazione Medica Sabin) practicing in 11 different villages of the Vibrata valley were screened, and 711 octogenarians-nonagenarians were identified. From this sample, 466 free-living healthy subjects were distinguished, and 120 were randomly selected; of these, 100 agreed to participate in the study (58 men and 42 women aged 85.1 ± 0.3 and 86.4 ± 0.5 years [mean ± SE], respectively) and were enrolled. Inclusion criteria were as follows: (1) origin from the Vibrata valley or Abruzzo region; (2) no history of cardiovascular disease (angina, myocardial infarction, congestive heart failure class III-IV New York Heart Association, or peripheral vascular disease), severe respiratory disease requiring oxygen therapy (chronic bronchitis, emphysema, or asthma), neurological disease (transient ischemic attacks, stroke, Parkinson's disease, or dementia), neoplasia, diabetes mellitus, severe osteoarthritis, major depression, or alcoholism; (3) absence of major changes in blood cell count, clinical chemistry

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parameters, urinalysis, and neoplastic markers (carcinoembryonic antigen, alpha-fetoprotein, and prostate-specific antigen) suggestive of a major occult disease; and (4) no disability. Activities of daily living were evaluated by the Katz index, and only subjects in class A-B (independent in reference to feeding, continence, ambulating, using the toilet, dressing, and bathing, or in all but one of these functions) were enrolled.[19] The World Health Organization questionnaire was used to rule out CHD and peripheral vascular disease.[20] No subjects were being treated with β-blockers, and only four with diuretics. All information about the clinical status was verified with the help of the family doctors and available relatives. Diabetes mellitus was excluded by the following criteria: (1) no hystory of diabetes, (2) no current or previous treatment with insulin or oral hypoglycemic agents, and (3) fasting blood glucose not greater than 140 mg/dl.

### *Anthropometric Parameters*

All parameters were evaluated according to standardized methods by the same two physicians (G.Z. and R.F.) over the entire period of the study. The body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters. Waist circumference was measured between the lower rib and the iliac crest, at the end of a normal expiration; hip circumference was evaluated as the largest measurement in a horizontal plane around the buttocks. The waist to hip ratio (WHR) was then calculated.

### *Metabolic and Coagulation Parameters*

Blood samples were obtained early in the morning after a 12-hour overnight fast, kept at 4°C for 1 hour, and then centrifuged at 3,000 rpm for 10 minutes at 4°C; serum and plasma were kept at 4°C and assayed within 3 hours. For determination of insulin and coagulation parameters, samples of plasma were immediately frozen and stored at −20°C until assay.

Total cholesterol (TC), TG, and blood glucose were evaluated on a Shimadzu analyzer (model CL7000; Japan) by enzymatic methods.[21,22] HDL-C was determined after selective lipoprotein precipitation with polyanions.[23] Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation: LDL-C = [TC − (TG/5)] − HDL-C.[24] Apolipoprotein (apo) A-I and B levels were measured by rate-nephelometry (Kallestad analyzer, model QM300; Behring Institute, Scoppito, Italy). PAI-1 and factor VII activities were measured by a chromogenic substrate assay[25,26] using an automated device (Behring Chromo Time System). Fibrinogen concentrations were determined by radial immunodiffusion (NOR-Partigen plates; Behring Institute). The fasting plasma insulin level was measured by a radioimmunoassay kit (Insulina Myria; Techno Genetics, Recordati, Italy) with a cross-reactivity of proinsulin with insulin of 30%. For all methods used in the study, the coefficient of variation was 3% to 5%.

### *Statistical Analysis*

Data were stored and analyzed using the Systat package (Systat, Evanston, IL) for Windows. The fasting insulin level and plasma TG, PAI-1, and factor VII levels were logarithmically transformed before analysis (natural log), which resulted in a more normal distribution of these variables. Mean gender differences were compared by Student's unpaired *t* test or analysis of covariance when necessary. The prevalence of metabolic disorders among fasting insulin quartiles was evaluated by $\chi^2$ test. Pearson's product-moment correlation coefficients were calculated, and stepwise multiple linear regression analysis was performed to determine relationships between variables of interest (the accepted software defaults for α to enter and be removed and tolerance were .15, .15, and .01, respectively). All data are expressed as the mean ± SE, and statistical significance was accepted at *P* less than .05.

**Table 1. Anthropometric, Metabolic, and Coagulation Parameters in 100 Free-Living Healthy Octogenarians-Nonagenarians (mean ± SE)**

| Variable | Men | Women |
|---|---|---|
| No. of subjects | 58 | 42 |
| Age (yr) | 85 ± 0.3 | 86 ± 0.5* |
| BMI (kg/m²) | 25.3 ± 0.4 | 26.1 ± 0.8 |
| Waist circumference (cm) | 92.4 ± 1.3 | 93.4 ± 2.2 |
| Hip circumference (cm) | 94.5 ± 1.0 | 98 ± 2 |
| WHR | 0.97 ± 0.006 | 0.95 ± 0.01† |
| Fasting glucose (mg/dL) | 87.4 ± 1.7 | 89.9 ± 2.2 |
| Fasting insulin (mU/L) | 5.7 ± 0.4 | 6.4 ± 0.5 |
| TC (mg/dL) | 212 ± 4.7 | 228.7 ± 6.4 |
| TG (mg/dL) | 104 ± 9.8 | 116.3 ± 8.3 |
| LDL-C (mg/dL) | 139.9 ± 4 | 148.3 ± 5.6 |
| HDL-C (mg/dL) | 51.2 ± 1.2 | 56.5 ± 1.8 |
| Apo A-I (mg/dL) | 146.5 ± 4.1 | 156.7 ± 4.2† |
| Apo B (mg/dL) | 97.2 ± 3.8 | 96.9 ± 3.8 |
| LDL-C/apo B ratio | 1.5 ± 0.04 | 1.6 ± 0.06 |
| Fibrinogen (g/L) | 2.5 ± 0.07 | 2.7 ± 0.08 |
| Factor VII | 136.8 ± 13.04 | 125.2 ± 13.2 |
| PAI-I | 1.92 ± 0.13 | 1.98 ± 0.18 |

*$P < .05$ (unpaired *t* test).
†$P < .01$ (analysis of covariance by age).

## RESULTS

The anthropometric and metabolic characteristics of the sample are presented by gender in Table 1. The BMI was slightly higher in women compared with men, while the WHR was higher in the latter group. TC, LDL-C, HDL-C, and apo A-I were lower in men than in women, but only for apo A-I was the difference statistically significant. Fasting glucose and insulin were slightly higher in women, whereas no gender differences were detected for fibrinogen, coagulant factor VII, and PAI-1 levels.

Table 2 shows Pearson's simple correlations between fasting insulin and anthropometric, metabolic, and hemostatic param-

**Table 2. Pearson's Correlations Between Fasting Plasma Insulin and Anthropometric, Metabolic, and Coagulation Parameters in 100 Free-Living Healthy Octogenerians-Nonagenarians**

| Variable | Total | Men | Women |
|---|---|---|---|
| BMI | .49‡ | .56‡ | .45† |
| Waist circumference | .48‡ | .57‡ | .41† |
| Hip circumference | .46‡ | .55‡ | .38† |
| WHR | .23* | .32† | .21 |
| Fasting glucose | .40‡ | .48‡ | .28 |
| TC | −.18 | −.09 | −.34* |
| TG | .21* | .32* | .13 |
| LDL-C | −.25† | −.18 | −.37* |
| HDL-C | −.22* | −.31* | −.22 |
| LDL-C/apo B | −.22* | −.27* | −.21 |
| Apo A-I | −.24* | −.35* | −.15 |
| Apo B | .04 | .12 | −.06 |
| Fibrinogen | .16 | .18 | .10 |
| Factor VII | .04 | .09 | −.10 |
| PAI-I | .33‡ | .32* | .33* |

*$P < .05$.
†$P < .01$.
‡$P < .001$.

eters. Insulin was significantly correlated with all anthropometric measures in the overall population; the correlation of fasting plasma insulin with the BMI and WHR is shown in Fig 1. When the analysis was made by gender, the correlation between fasting insulin and the WHR in women was weaker and no longer significant. In the overall population, insulin was positively and significantly correlated with fasting glucose, plasma TG, and PAI-1 levels, and these relationships were generally stronger in men versus women. Interestingly, fasting insulin was inversely correlated with TC and LDL-C, especially in women; otherwise, it was inversely and significantly correlated with HDL-C and apo A-I only in men. The BMI was correlated with the waist circumference ($r = .91$, $P < .001$), fasting glucose ($r = .20$, $P < .05$), plasma TG ($r = .31$, $P < .01$), HDL-C ($r = -.19$, $P < .05$), and PAI-1 ($r = .28$, $P < .05$, data not shown).

To determine the relative contribution of age, gender, BMI, waist circumference, and fasting insulin to fasting glucose, TG, HDL-C, and PAI-1, we performed a stepwise linear multiple regression analysis of the pooled data (Table 3). The waist circumference was selected as a measure of abdominal obesity,

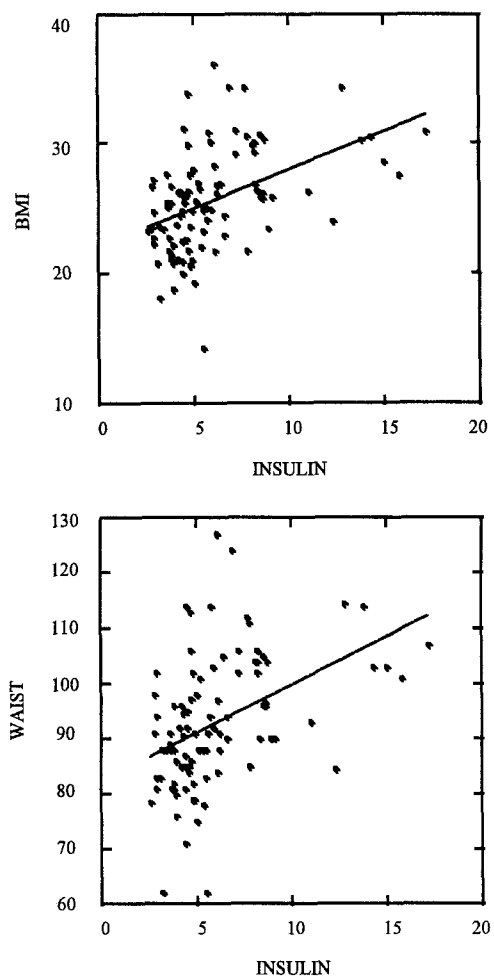


**Fig 1. Pearson's correlation between fasting plasma insulin, BMI, and waist circumference.**

**Table 3. Stepwise Multivariate Regression Analysis of the Effects of Age, Gender, BMI, Waist Circumference, and Fasting Plasma Insulin Levels on Metabolic Parameters in 100 Free-Living Healthy Octogenerians-Nonagenarians**

| Variable | Parameters | $R^2$ | $P$ |
|---|---|---|---|
| Fasting glucose | Insulin | .234 | |
| | Age | .260 | .0005 |
| TG | Insulin | .105 | |
| | BMI | .137 | .006 |
| HDL-C | Gender | .08 | |
| | Insulin | .17 | .001 |
| LDL-C/apo B ratio | Waist circumference | .126 | .0005 |
| PAI-I | Insulin | .12 | .001 |

due to its stronger correlation with fasting insulin than the WHR. Fasting insulin levels were independently related to all variables but the LDL-C/apo B ratio. Age, BMI, and gender were also independently associated with fasting glucose, TG, and HDL-C, respectively. Insulin was the only significant predictor of PAI-1 levels ($R^2 = .12$). The same results were obtained when plasma TG levels were taken into account (data not shown).

Figure 2 shows the prevalence of some metabolic risk factors in this population subdivided according to fasting insulin quartiles. Subjects in the highest quartile (n = 24) had a fasting insulin greater than 10 mU/mL. These individuals had a higher prevalence of high TG (>200 mg/dL, 16% *v* 5%), low HDL-C (<39 and 43 mg/dL in men and women, respectively, 17% *v* 1.4%), and high PAI-1 (>3.5 U/mL, 23% *v* 5%) compared with subjects in the lower three quartiles, but the difference was significant only for HDL-C ($P < .05$). On the contrary, the prevalence of high TC (> 240 mg/dL) was lower in the upper-quartile subjects (15% *v* 32%). About 50% of individuals in the highest insulin quartile had none of the metabolic disorders (high TG, low HDL-C, and high PAI-1), compared with 75% in the lower quartiles ($P < .05$).

## DISCUSSION

There is substantial evidence that the inability of insulin to stimulate glucose transport and the ensuing compensatory hyperinsulinemia are frequently associated with several metabolic alterations predisposing to CHD even in the absence of glucose intolerance.[6,7,27] Indeed, insulin resistance and hyperinsulinemia are often found in patients with dyslipidemia and hemostatic abnormalities.[7,8]

The results of our study extend these relationships to a very old healthy population whose selection was based on the absence of significant chronic illness and disability. Furthermore, because fasting insulin levels correlate inversely with insulin sensitivity, our results also provide evidence for a relationship between insulin resistance and several metabolic abnormalities in old age.[28] Similar conclusions were found by the Zuphten Elderly Study, a larger cross-sectional survey; however, this Dutch population sample was, on average, 10 years younger than ours and only men were studied.[9] We ruled out the presence of diabetes on the basis of the medical history and a single fasting plasma glucose determination. By not

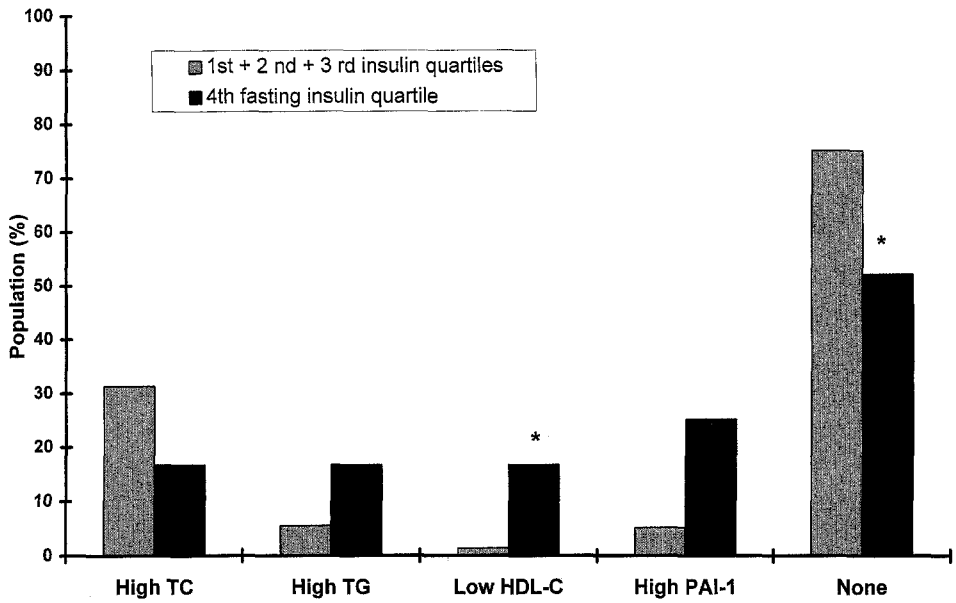


**Fig 2. Prevalence of metabolic risk factors for CHD among insulin quartiles in the entire sample. *$P < .05$.**

performing an oral glucose tolerance test (OGTT), it is theoretically possible that we underestimated the presence of glucose intolerance; however, whether current OGTT criteria are appropriate in older subjects is a still a debated issue because the clinical significance of postchallenge hyperglycemia in the elderly is not completely clear.[29] Fasting insulin was significantly related to fasting glucose independently of potential confounding variables such as age, gender, obesity, and abdominal fat distribution. High TG and low HDL-C levels are the most common lipoprotein disorders associated with insulin resistance and hyperinsulinemia.[6,30] We found a significant correlation between fasting insulin and TG only in men; moreover, by multivariate analysis, the BMI was a predictor of plasma TG even though fasting insulin was independently correlated. With the exception of the Zuphten Elderly Study,[9] these findings partially agree with data obtained in previous studies of old populations.[10] We further observed a negative correlation between fasting insulin and HDL-C only in men, whose HDL-C levels were lower than in women (difference not significant). Nevertheless, gender ($R^2 = .08$) and fasting insulin ($R^2 = .09$) were both predictors of HDL-C levels by multivariate analysis independently of age, overall and abdominal adiposity, and TG levels. Thus, it could be argued that besides insulin, other sex-related factors might play a role in influencing HDL-C levels in this very old population.

We found a significant positive relationship between fasting insulin and PAI-1 levels in both sexes independently of several confounding factors such as age, obesity, fat pattern, and TG level. The mechanisms linking hyperinsulinemia to increased PAI-1 levels are still unclear, but it was shown that insulin may directly stimulate hepatocyte PAI-1 synthesis in culture.[31] Since previous studies have shown an age-related increase in PAI-1 levels, the association between hyperinsulinemia and hypofibrinolysis might be very important to explain the high risk of thromboembolic disease in the elderly.[32,33]

Our results also confirm the positive relationship between insulin levels and the degree of both total and regional adiposity in a very old population. The highest correlation emerged when the analysis was presented by gender with waist circumference, a direct measure of trunk obesity. Cigolini et al[34] observed similar findings in a larger survey of premenopausal women from different European countries, particularly in the Italian sample. Recent studies indicate that older individuals tend to have a more central fat distribution, which is closely associated with insulin resistance, hyperinsulinemia, and several metabolic alterations such as dyslipidemia and glucose intolerance.[11,12] Hence, it has been advanced that an excess of highly metabolically active fat located within the abdomen might be a key factor in syndrome X.[13,14] Nonetheless, by multivariate analysis, we found that fasting insulin was still a significant predictor of many but not all metabolic risk factors for cardiovascular disease after adjustment for anthropometric measurements of fat patterning. Interestingly, a negative correlation between insulin levels and TC or LDL-C was found in our sample of very old individuals; these data confirm the unexplained previous findings of an association between high fasting insulin and low LDL-C levels.[35]

However, since our study is cross-sectional and consequently cannot elucidate any causality, it can only suggest the presence of associations. Moreover, since all of the above-mentioned factors were tightly related to each other, the finding of an independent effect for each of them does not rule out any interference by other correlated variables. On the other hand, insulin itself might be atherogenic, as suggested by some epidemiological studies showing an independent relationship between insulin levels and CHD.[36,37] However, the only prospective study in elderly men, aged 67 years, did not confirm this outcome.[38]

Finally, two limitations of the present study have to be considered. First, we could not include two important possible confounders, arterial hypertension and the level of physical activity, in our analysis because complete information was not

available. Second, the method we used for insulin determination had a 30% cross-reactivity with proinsulin, and this is a possible bias. Nevertheless, it must be emphasized that proinsulin levels usually correlate with insulin levels in both normal subjects and those with impaired glucose tolerance, and of consequence, the proinsulin to insulin ratio does not change.[39]

In conclusion, our results suggest that hyperinsulinemia is associated with several metabolic risk factors for CHD even in a very old healthy population. To gain a better understanding of the link between aging and atherosclerosis, these associations require further studies.

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