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Insulin Resistance, Insulin Response, and Obesity as Indicators of Metabolic Risk

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Context: Insulin resistance (IR) and obesity, especially abdominal obesity, are regarded as central pathophysiological features of a cluster of cardiovascular risk factors (CVRFs), but their relative roles remain undefined. Moreover, the differential impact of IR *viz*. insulin response has not been evaluated.

Objective: The objective of this study was to dissect out the impact of obesity, abdominal obesity, and IR/insulin response on CVRF.

Design: This was a cross-sectional study.

Setting: The study was conducted at 21 research centers in Europe.

Subjects: The study included a cohort of 1308 nondiabetic subjects [718 women and 590 men, age 30–60 yr, body mass index (BMI) 17–44 $kg \cdot m^{-2}$].

Main Outcome Measures: We measured IR (by a standardized euglycemic insulin clamp), waist girth, insulin response to an oral glucose tolerance test, and major CVRF, and analyzed their associations by multivariate models and factor analysis.

Results: BMI was positively related to all CVRFs. Waist circumference was related to higher blood pressure and serum triglycerides and lower high-density lipoprotein-cholesterol, IR to reduced glucose tolerance, higher free fatty acids, triglyceride and low-density lipoprotein-cholesterol, and lower high-density lipoprotein-cholesterol, and insulin response to higher heart rate, blood pressure and fasting glucose, and the same dyslipidemic profile as IR ($P \leq 0.05$ for all). By factor analysis, three main factors (related to IR, age, and fatness, respectively) appeared to underlie this pattern of associations. Each of BMI, waist girth, IR, and insulin response was independently associated with total CVRF load (all $P < 0.001$).

Conclusions: When IR, fat mass and distribution, and insulin response are measured simultaneously in a large cohort, no one factor stands out as the sole driving force of the CVRF cluster, each being associated with one or more physiological pathways according to known cause-effect relationships. **(*J Clin Endocrinol Metab* 92: 2885–2892, 2007)**

A LARGE BODY of evidence has accrued to show that many cardiovascular risk factors (CVRFs) cluster, *i.e.* they tend to occur together in the same individual more often than by chance (1). Among such factors, glucose intolerance, hypertension, and dyslipidemia are those that have a high prevalence in populations of diverse ethnicity (2). Although overt diabetes, clinical hypertension, and high serum low-density lipoprotein (LDL)-cholesterol levels are established CVRF dating back to the Framingham study (3), lesser degrees of glucose intolerance, borderline-high blood pressure and non-LDL-cholesterol dyslipidemia, *i.e.* high serum triglycerides and low high-density lipoprotein (HDL)-cholesterol, have emerged as additional abnormalities with atherogenic potential (4). Because insulin resistance (IR) is a common feature of disordered carbohydrate and lipid metabolism and blood pressure homeostasis, Reaven (5) postulated that IR (with the attendant hyperinsulinemia) is the *primum movens* in CVRF clustering, *i.e.* the primary pathophysiological event driving the other components of the cluster. Additional subclinical abnormalities, such as hyperuricemia (6), abundance of small, dense LDL particles (7), elevated prothrombotic factors (8), and microalbuminuria (9), have also been related to the presence of IR and incorporated into a cardiometabolic syndrome.

In more recent years, much basic and clinical research has indicated a pathogenic role for ectopic fat accumulation, in particular in abdominal visceral depots. A relative excess of fat within the abdomen (10), liver (11), and chest (12), as opposed to subcutaneous tissues, has been linked with glucose intolerance, dyslipidemia, and hypertension (13), as well as IR (14). Although itself more insulin sensitive (15) and more metabolically active than subcutaneous fat (16), intra-

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Abbreviations: BMI, Body mass index; CVD, cardiovascular disease; CVRF, cardiovascular risk factor; FFA, free fatty acid; HDL, high-density lipoprotein; IFG, impaired fasting glycemia; IGT, impaired glucose tolerance; IR, insulin resistance; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; RISC, Relationship between Insulin Sensitivity and Cardiovascular Disease; WHR, waist-to-hip ratio.

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abdominal fat has been imputed of releasing excess toxic cytokines (17), proinflammatory molecules (18), and vasoactive hormones, as well as driving excess free fatty acids (FFAs) and cortisol directly to the liver (19). Thus, a large waist girth, as a clinical surrogate measure of intraabdominal fat accumulation, has replaced IR (rather, its surrogate measures) in algorithms and constructs devised to assess risk of incident diabetes and cardiovascular disease (CVD) (13, 20). However, whether IR (or the attendant hyperinsulinemia) and abdominal obesity cosegregate consistently in healthy humans and identify the same clinical phenotype has not been determined. Moreover, although IR is generally accompanied by hyperinsulinemia, the differential impact of these two metabolic traits is seldom evaluated. Therefore, we set forth to measure IR by the gold standard technique (*i.e.* the euglycemic hyperinsulinemic clamp) in a large cohort of nondiabetic subjects, and to dissect out the relative impact of obesity, abdominal obesity, and IR/hyperinsulinemia on multiple indicators of metabolic risk.

Subjects and Methods

Study subjects

RISC (Relationship between Insulin Sensitivity and Cardiovascular Disease) is a prospective, observational, cohort study whose rationale and methodology have been published (21). In brief, participants were recruited from the local population at 19 centers in 14 countries in Europe, according to the following inclusion criteria: either sex, age between 30 and 60 yr, and clinically healthy, stratified by sex and age according to 10-yr age groups. Initial exclusion criteria were: treatment for obesity, hypertension, lipid disorders or diabetes, pregnancy, cardiovascular or chronic lung disease, weight change of 5 kg or more in last 6 months, cancer (in last 5 yr), and renal failure. Exclusion criteria after screening were: arterial blood pressure 140/90 mm Hg or higher; fasting plasma glucose 7.0 mmol/liter or greater; 2-h plasma glucose [on a 75-g oral glucose tolerance test (OGTT)] 11.0 mmol/liter or greater; total serum cholesterol 7.8 mmol/liter or greater; serum triglycerides 4.6 mmol/liter or greater; and electrocardiogram abnormalities. Baseline examinations began in June 2002 and were completed in November 2004. The present analysis is based on the 1308 subjects (718 women and 590 men, mean age 44 yr, BMI 26 $kg \cdot m^{-2}$, range 18–44) who satisfied all criteria and whose clamp study (see later) passed the quality control check.

Lifestyle and medical history

Information was collected on personal and family medical history of CVD, stroke, hypertension, and diabetes in first-degree relatives, as well as information on smoking and alcohol habits and physical activity. A modified version of the Rose questionnaire and the Edinburgh claudication questionnaire were used for exclusion (21).

Physical examinations

Height was measured on a clinic stadiometer, and body weight, percent body fat, and fat-free mass were evaluated by the TANITA bioimpedance balance (Tanita International Division, Tanita, UK). Waist, hip, and thigh circumferences were measured by tape according to a standardized written protocol; the waist-to-hip ratio (WHR) was also calculated. Sitting blood pressure and heart rate were measured (OMRON 705 cp; OMRON Healthcare Europe, Hoofddorp, The Netherlands) three times over 10 min; the median value was used in statistical analyses.

OGTT

Fasting blood samples were taken before, and 30, 60, 90, and 120 min into the OGTT, together with samples for central analysis of routine blood chemistry. Blood collected during the studies was separated into plasma and serum, aliquoted, and stored at −20 C for glucose and −80 C for lipids. Serum aliquots were also stored at −80 C for insulin determination. Samples were transported on dry ice at prearranged intervals to central laboratories. Serum insulin was measured by a specific time-resolved fluoroimmunoassay (AutoDELFIA Insulin kit; Wallac Oy, Turku, Finland), with the following assay characteristics: detection limit more than 3 pmol/liter, intraassay variation 1.7%, and interassay variation 3.5% (22).

Insulin clamp

On a separate day within 1 month of the OGTT, a euglycemic hyperinsulinemic clamp was performed in all subjects. Exogenous insulin was administered as a primed-continuous infusion at a rate of 240 $pmol \cdot min^{-1} \cdot m^{-2}$ simultaneously with a variable 20% dextrose infusion adjusted every 5–10 min to maintain plasma glucose level within 0.8 mmol/liter (±15%) of the target glucose level (4.5–5.5 mmol/liter). Additional blood samples were obtained at 20-min intervals for insulin and FFA determination. The clamp procedure was standardized across centers with the use of a demonstration video and *ad hoc* operating instructions; the raw data from each clamp study were immediately transferred to the coordinating center where they underwent quality control scrutiny according to preset criteria.

Local Ethics Committee approval was obtained by each recruiting center. Volunteers were given detailed written information on the study and signed a consent form.

Data analysis

Fat mass was obtained as the difference between body weight and fat-free mass. LDL-cholesterol concentration was calculated by the Friedewald formula. Glucose tolerance was categorized into normal, impaired fasting glycemia (IFG), and impaired glucose tolerance (IGT) according to American Diabetes Association criteria (23). Insulin sensitivity was expressed as the ratio of the M value (24), averaged over the final 40 min of the 2-h clamp and normalized by the fat-free mass, to the mean plasma insulin concentration measured during the same interval (M/I, in units of $\mu mol \cdot min^{-1} \cdot kg_{ffm}{}^{-1} \cdot mM^{-1}$). The average insulin concentration during the OGTT is hereinafter referred to as insulin response. Average glucose and insulin concentrations were calculated by dividing the respective areas under time-concentration curve (calculated by the trapezium rule) by 120 (min of OGTT duration).

Statistical analysis

Data are reported as mean ± SD. Variables (alcohol intake, BMI, waist and hip circumference, M/I, fasting, and mean OGTT insulin levels and serum triglycerides and HDL-cholesterol) with a skewed distribution (by Shapiro-Wilk W test) are given as median and (interquartile range), and were logarithmically transformed for use in statistical testing. Groups were compared by the Mann-Whitney *U* test, proportions by χ^2 analysis. Association between two variables was tested by the Spearman rank correlation ρ value. General linear models were used to test the simultaneous dependence of continuous variables on multiple parameters; results are presented as the standardized regression coefficient. When using nominal variables, the Bonferroni-Dunn test was used to compare any two levels of nominal variables. Exploratory factor analysis was performed using orthotran/varimax transformation; the number of principal factors was decided on the basis of an eigenvalue (amount of variance in relation to total variance) greater than one. To reduce colinearity, mean blood pressure was used instead of systolic/diastolic blood pressure, and WHR instead of waist and hip circumference. Results are given as orthogonal score weights on principal components. Statistical analyses were performed using JMP version 3.1 (SAS Institute Inc., Cary, NC).

Results

Clinical and metabolic data are given in Tables 1 and 2 separately for men and women. In the whole data set, the distribution of insulin sensitivity (as the M to I ratio) was significantly ($P < 0.0001$) different from a normal distribu-

TABLE 1. Clinical phenotype

	Men	Women	*P* value[a]
n	590	718	
Age (yr)	43 ± 9	44 ± 8	0.001
Postmenopausal women (%)		22	
Familial diabetes (%)	26	28	ns
Never smokers (%)	46	48	ns
Impaired glucose tolerance (%)[b]	11.9	11.5	ns
Alcohol intake (g/wk)[c]	89 (120)	42 (54)	<0.0001
BMI (kg/m^2)	26.0 (4.4)	24.0 (5.3)	<0.0001
Fat mass (%)	22 ± 7	32 ± 8	<0.0001
Waist girth (cm)	93 (14)	79 (16)	<0.0001
Hip girth (cm)	101 (11)	98 (12)	<0.0001
WHR (cm/cm)	0.902 (0.081)	0.802 (0.098)	<0.0001
Heart rate (bpm)	66 ± 10	70 ± 10	<0.0001
Systolic blood pressure (mm Hg)	122 ± 11	113 ± 12	<0.0001
Diastolic blood pressure (mm Hg)	76 ± 8	73 ± 8	<0.0001
Mean blood pressure (mm Hg)	92 ± 8	86 ± 9	<0.0001

Unless stated, entries are mean ± SD or median (interquartile range). bpm, Beats per minute; ns, not significant
[a] Mann-Whitney *U* or χ^2 test.
[b] IGT + IFG.
[c] Data for subjects with an alcohol intake greater than 0.

tion, with a median of 129 and an interquartile range of 86 μmol·min^{-1}·kg$_{ffm}^{-1}$·nM^{-1}. Insulin sensitivity was reciprocally and nonlinearly related to both BMI and waist girth [power functions (r = 0.42 and 0.44, respectively) providing better fits than linear functions (r = 0.38 and 0.39)]; both relationships were significantly ($P < 0.0001$ for the interaction between sex and BMI or waist) steeper in men than women and displayed considerable dispersion in either gender. In multivariate regression, M/I was independently related to both BMI and waist girth, with standardized regression coefficients of −0.29 and −0.19, respectively ($P < 0.0001$ for both).

Despite the close association of IR with insulin response (ρ values of −0.63 in men and −0.50 in women), only 60% of the individuals in the bottom sex-specific quartile of M/I [72 (29) μmol·min^{-1}·kg$_{ffm}^{-1}$·nM^{-1}] were in the top quartile of mean insulin levels [345 (245) pmol/liter], indicating that the two characters, IR and insulin response, were partly dissociated in the population. Likewise, only 75% of subjects in the top sex-specific BMI quartile [30.1 (3.5) kg·m^{-2}] were in the top sex-specific quartile of waist girth [102 (12) cm], again indicating partial dissociation of these two traits in the population. Consequently, IR, a high BMI, a large waist, and a high insulin response identified only partially overlapping subgroups of individuals. Thus, when using the top quartile of each of IR, large waist, and insulin response, the overlap between each two traits ranged from 45–60% and amounted to only 11–15% of the total cohort, with only 109 subjects (8% of all subjects) presenting all three features (Fig. 1). Replacing waist with BMI yielded an almost identical pattern of overlap.

To analyze the independent association of BMI, waist girth, IR, and insulin response with CVRFs, we set up general linear models in which each CVRF was a dependent variable, and BMI, waist girth, M/I, and insulin response were independent variables; center and age were covariates, and hip circumference was added to all models to provide a finer assessment of the impact of body fat distribution. In separate analyses (data not shown), the general pattern of associations was found to be similar in women and men; therefore, subsequent analyses were run on pooled men and women data using sex as a covariate. The results are presented as standardized regression coefficients in Table 3. Gender and age were significant independent correlates of virtually all CVRFs. BMI was associated with higher levels of all CVRFs, except HDL-cholesterol levels. After controlling for sex, age, BMI, and hip circumference, waist girth was independently associated with higher diastolic blood pressure, fasting glucose concentrations (stronger in women), steady-state FFA, triglycerides, and LDL-cholesterol, and with lower HDL-cholesterol levels. On the other hand, IR was independently associated with all CVRFs except heart rate, blood pressure values, and fasting glucose levels. Insulin response was as-

TABLE 2. Metabolic phenotype

	Men	Women	*P* value
M/I (μmol·min^{-1}·kg$_{ffm}^{-1}$·nM^{-1})	112 (70)	144 (82)	<0.0001
Fasting glucose (mmol/liter)	5.22 ± 0.52	4.94 ± 0.56	<0.0001
2-h glucose (mmol/liter)	5.65 ± 1.41	5.76 ± 1.49	ns
Fasting insulin (pmol/liter)	32 (25)	29 (21)	<0.001
Average insulin response (pmol/liter)	206 (168)	195 (148)	ns
Fasting FFA (mmol/liter)	0.47 ± 0.22	0.59 ± 0.22	<0.0001
Steady-state FFA (mmol/liter)	0.06 ± 0.10	0.05 ± 0.12	<0.0001
Serum triglycerides (mmol/liter)	1.08 (0.75)	0.83 (0.47)	<0.0001
LDL-cholesterol (mmol/liter)	3.08 ± 0.77	2.77 ± 0.80	<0.0001
HDL-cholesterol (mmol/liter)	1.20 (0.37)	1.54 (0.45)	<0.0001

Entries and symbols are the same as those in Table 1.

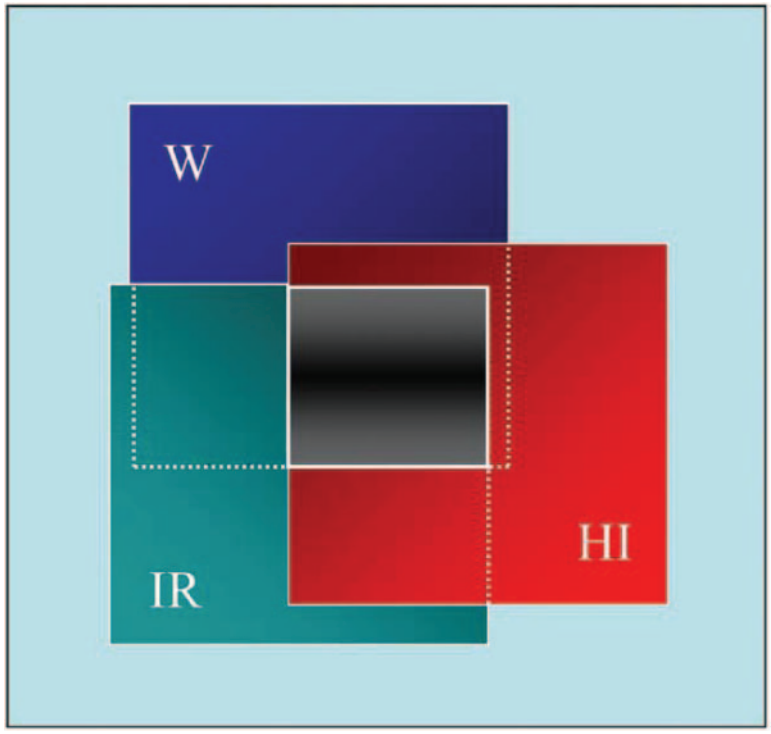

FIG. 1. Pattern of overlap of a large waist circumference (W) (top quartile), IR (top quartile), and insulin response (HI) (top quartile) in the study cohort (n = 1308). Each *square* is proportional to the corresponding number of subjects.

sociated with all parameters except fasting FFA and LDL-cholesterol levels. Of note, when both hip and waist girths were statistically significant predictors, their relationships had an opposite sign. Neither smoking habits nor alcohol consumption was a significant factor in these analyses.

Given the large number of interrelationships among CVRF and metabolic traits, we attempted to identify principal hidden components that might characterize clinical phenotypes with the use of exploratory factor analysis. The results (Table 4) showed that 13 variables could be described as clustering around a minimum of three main factors. Factor 1 had the highest score weight on IR, and clustered with higher insulin response, heart rate, triglycerides and FFA, and lower HDL-cholesterol levels. Factor 2 had the highest score weight on female sex, and clustered with higher fat mass and lower WHR, and higher HDL-cholesterol. Factor 3 had the highest score weight on age, blood pressure, WHR, LDL-cholesterol, triglycerides, and mean glucose (Fig. 2).

Finally, to explore further the independent contribution of BMI, waist circumference, IR, and insulin response to CVRFs, we constructed a CVRF score by adding up the (standardized) values of age, heart rate, mean blood pressure, mean glucose level, LDL-cholesterol, and triglycerides (and subtracting HDL-cholesterol level) in each subject and then regressing such CVRF score against BMI, WHR, IR, and insulin response (as the respective sex-specific quartiles). The results (Fig. 3) clearly show the graded increase in CVRF load with each metabolic variable in both men and women. By multivariate analysis, all four metabolic traits were simultaneously related to CVRF load after controlling for sex and center ($P < 0.0001$ for all).

Discussion

Our study demonstrates that IR, obesity, central fat accumulation, and insulin response each makes an independent

TABLE 3. Multivariate analysis of BMI, waist girth, IR, and insulin response

	Male sex	Age	BMI	Hip	Waist	IR	Insulin
Heart rate	−0.230	−0.082	ns	ns	ns	ns	0.177
Systolic BP	0.294	0.146	0.165	ns	ns	ns	0.070
Diastolic BP	0.137	0.115	0.127	ns	0.149	ns	0.069
Fasting glucose	0.182	0.137	0.142	ns	0.115	ns	0.163
2-h glucose	−0.153	0.095	0.122	−0.117	ns	0.268	0.100
Fasting FFA	−0.325	0.054	ns	ns	ns	0.224	ns
Steady-state FFA	0.088	ns	0.091	−0.090	0.138	0.178	0.206
Triglycerides	0.120	ns	0.115	−0.099	0.211	0.187	0.148
LDL-cholesterol	0.132	0.215	0.122	−0.081	0.108	0.086	ns
HDL-cholesterol	−0.297	0.110	−0.117	ns	−0.170	−0.133	−0.111

Entries are standardized regression coefficients; their sign indicates a positive or negative independent association. For each dependent variable in the leftmost column, the model is: y = intercept + center + sex + age + ln(BMI) + ln(hip) + ln(waist) + ln(M/I) + ln(insulin). BP, Blood pressure; Insulin, insulin response; ns, not significant.

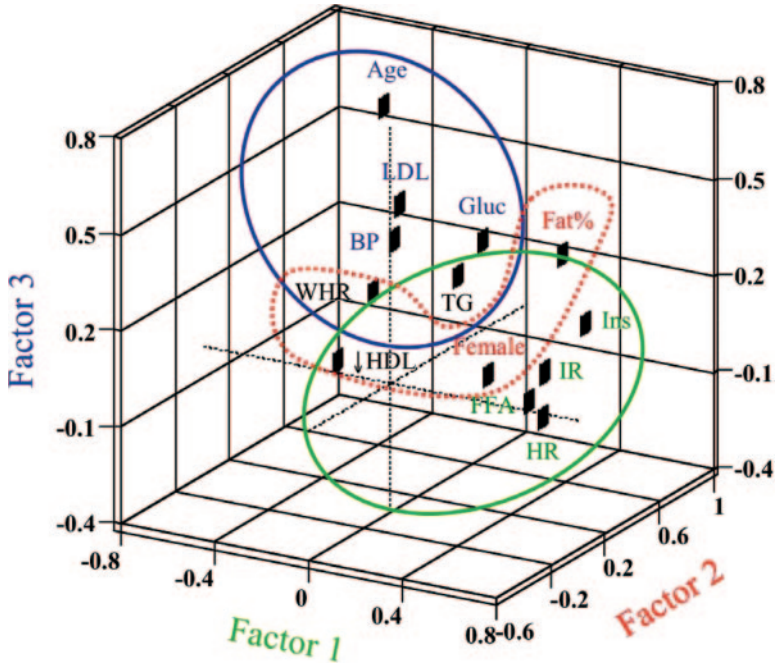

FIG. 2. Three-dimensional plot of the loadings of three main factors on the measured CVRFs (from Table 4). The *color contours* include subsets of variables with the highest loading on each factor. Labels in *black* identify variables shared by more than one factor. Note that HDL is a negative loading on factor 1 but a positive loading on factor 2; conversely, WHR is positive on factor 1 and negative on factor 2. BP, Blood pressure; Gluc, mean post-OGTT plasma glucose concentration; HR, heart rate; Ins, mean post-OGTT plasma insulin concentration; IR, insulin resistance [−ln (M/I)]; TG, triglyceride.

but partial contribution to the metabolic risk cluster that goes under the name of IR syndrome or metabolic syndrome. This conclusion requires preliminary qualification.

First, the cohort we recruited is representative of the healthy (or healthier) segment of a population of predominantly European descent. Although findings may vary in strictly population-based cohorts and/or in different ethnic groups, the general pattern of our results recapitulates individual findings reported in previous studies measuring either fasting insulin levels or waist circumference in relation to one or the other component of the cluster examined here (10–13, 17, 18). Second, the wide, skewed distribution of insulin sensitivity even in essentially healthy individuals confirms the findings of a previous data-pooling project using the clamp technique to measure insulin sensitivity (25). Likewise, the reciprocal association between insulin sensitivity and waist circumference reproduces previous observations (14). Of note is that the best fit of our data was a nonlinear function, whereby there appears to be little further decline in insulin sensitivity once waist girth exceeds 90–95 cm, with detectable differences between men and women emerging only in the lower waist girth range. The same was true of total adiposity, as the BMI. A corollary to these observations is that some transformation (*e.g.* logarithmic) is appropriate when using these measures in multivariate statistical analyses. Third, while assessing the relationship between either waist circumference or IR and risk factors, it is mandatory to account for sex, age, and overall adiposity (as the BMI or fat mass), each of which exerts a very significant influence on several parameters. For example, serum HDL-cholesterol concentrations are highly sex specific and sensitive to obesity; blood pressure levels are sex specific and increase with both age and obesity (Table 3). Finally, we used the gold standard technique to measure *in vivo* insulin sensitivity but a clinical measure, waist circumference, to index abdominal obesity. Although waist girth is well correlated with intraabdominal fat mass as measured by computerized axial tomography or magnetic resonance imaging (26), the amount of subcutaneous fat confounds this correlation. Therefore, we introduced both BMI and hip circumference

TABLE 4. Factor analysis of the RISC cohort

	Factor 1	Factor 2	Factor 3
Sex (1 = men, 2 = women)	−0.085	**0.885**	−0.207
Age (yr)	−0.194	0.306	**0.760**
Fat mass (% of body weight)	0.313	**0.742**	0.260
WHR (cm/cm)	0.236	**−0.518**	**0.448**
Heart rate (bpm)	**0.483**	0.310	−0.116
Mean blood pressure (mm Hg)	0.133	−0.185	**0.512**
IR [−ln (M/I)]	**0.742**	−0.124	0.172
FFA [ln (μmol/liter)]	**0.682**	−0.150	0.078
HDL-cholesterol (mmol/liter)	**−0.510**	**0.508**	−0.130
LDL-cholesterol (mmol/liter)	0.121	−0.128	**0.607**
Triglycerides [ln (mmol/liter)]	**0.443**	−0.259	**0.461**
Glucose [ln (mmol/liter)]	0.386	0.023	**0.496**
Insulin [ln (pmol/liter)]	**0.751**	0.151	0.255

Entries are orthogonal loadings. Values greater than 0.4 are in *bold*. bpm, Beats per minute.

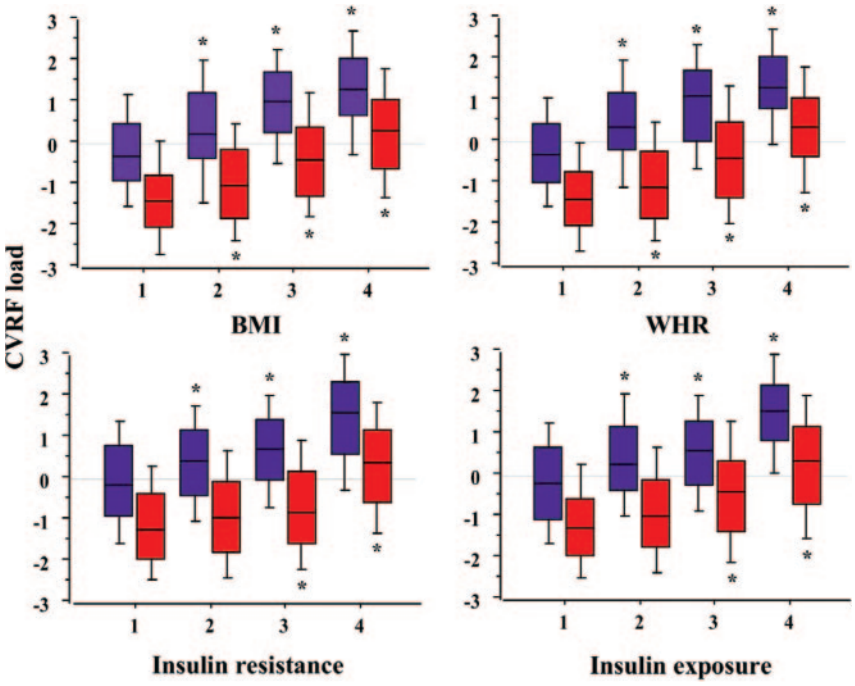

FIG. 3. Relation of BMI, WHR, IR, and insulin response (all as their sex-specific quartiles) to the CVRF score (sum of all measured CVRF, standardized) in men (*purple boxes*) and women (*red boxes*). *, $P \leq 0.01$ for the difference from the lowest quartile.

into our multivariate statistical models to derive the cleanest possible estimate of the impact of selective fat accumulation in the abdomen.

IR, a large waist (or BMI), and a high insulin response identified partially overlapping groups of individuals in our cohort (Fig. 1). This obviously means that each of obesity, IR, abdominal obesity, and insulin response can be found in isolation despite their strong tendency to cluster. Although this was not surprising for waist girth (or BMI) and IR given their loose correlation, the fact that IR can be dissociated from hyperinsulinemia/insulin response has been less well appreciated. The plasma insulin response to oral glucose is related to the degree of IR but mostly depends on the concomitant plasma glucose excursions as well as the set point of the β-cell (*i.e.* primary insulin hypersecretion) (27). Although fasting plasma insulin is used as a surrogate for IR, the biologically relevant hormone concentration to which tissues are chronically exposed is much closer to fed than fasting levels. Therefore, IR by the clamp and fed insulin levels reflect different phenomena, and may carry different pathogenic potential.

The results of our multivariate analysis of CVRFs (Table 3) recall specific pathophysiological pathways. IR was very clearly and uniquely associated with glucose tolerance, in full confirmation of the lesson learned from the Pima Indian studies (28). Reduced insulin-mediated glucose clearance is consistently found in patients with type 2 diabetes, IGT, and IFG, in whom it quantitatively contributes to glucose intolerance (29), and predicts deterioration of glucose tolerance in nondiabetic subjects (30). Also clear was the independent association of IR with dyslipidemia. Here, the underlying pathophysiological mechanism is IR of lipolysis, which commonly coexists with IR of skeletal muscle (5, 29) and results in higher circulating FFA levels. In turn, increased hepatic FFA uptake and enhanced triglyceride export in very low-density lipoprotein-cholesterol particles trigger the changes in the delipidation pathway, leading to decreased HDL-cholesterol concentrations. Changes in the catabolic rate of both apolipoprotein A and B have been described in nondiabetic insulin-resistant subjects (31). Of interest is that a large waist and a high insulin response both made a contribution to dyslipidemia independent of IR. With regard to hyperinsulinemia, studies in isolated livers perfused with high FFA levels have shown that *in vivo* hyperinsulinemia stimulates triglyceride synthesis and incorporation into very low-density lipoprotein (32). In addition, hyperinsulinemia down-regulates insulin action in the liver as well as peripheral tissues (33), thereby worsening any preexisting IR. With regard to fat distribution, highly lipolytic intraabdominal fat depots drain their FFA flux directly into the liver, thereby providing a substrate surplus for triglyceride synthesis (34). On the other hand, in the process of fat storage, the availability of an efficient subcutaneous reservoir of adipose tissue is essential to remove circulating triglycerides; in the presence of IR (and, possibly, an altered milieu of stress hormones) (35), excess circulating triglycerides may deposit ectopically in intraabdominal fat depots and liver (11). A reflection of this model of triglyceride traffic between the bloodstream, liver, and fat depots is found in our data, *i.e.* the opposite relation of hip circumference (more representative of subcutaneous fat mass) and waist circumference (more representative of intraabdominal obesity) to serum triglyceride concentrations even after accounting for sex and total adiposity (Table 3).

The association of a higher resting heart rate with hyperinsulinemia may reflect the ability of insulin to stimulate the sympathetic nervous system (36). The independent association of waist circumference with blood pressure, especially diastolic, could then reflect the release of angiotensinogen and other vasoactive substances from visceral fat (37). It must be recalled that the observed associations with blood pressure in the present cohort may have been weakened by the

exclusion of subjects with even mildly elevated blood pressure levels.

It is important to stress that the pattern of interrelationships emerging from our study is compatible with the pathophysiological mechanisms discussed previously but does not prove any of them. To extract more information from the data, we used exploratory factor analysis. The results suggested that our cohort could be seen as a mixture of three virtual phenotypes: the insulin-resistant subject, with a low level of physical fitness (=higher heart rate), hyperinsulinemia, and dyslipidemia (increased triglycerides and FFA and low HDL-cholesterol); the older subject, with increased blood pressure, LDL-cholesterol and triglyceride levels, and reduced glucose tolerance; and the subject, predominantly female, with abundant body fat but a low WHR, in whom IR is not prominent, and the metabolic profile is essentially normal. Obviously, the subclusters of variables are not separate but intersect each other (Fig. 2). Factor analysis was adopted by Meigs *et al.* (1), who, using data from 2458 nondiabetic subjects of the Framingham Offspring Study, also extracted three factors from 10 measured variables, with a somewhat different composition from ours, possibly because IR was not measured directly but inferred from hyperinsulinemia. On the other hand, Hanley *et al.* (38) measured insulin sensitivity (as the Si from an ivGTT-minimal model) in a tri-ethnic group of 1087 nondiabetic subjects; their factor analysis identified two main factors, one interpreted as "metabolic," the other as "blood pressure." More recently, the same group used confirmatory factor analysis to suggest that a single factor may underlie the metabolic syndrome (39). However, no previous study has used both direct measures of IR and insulin response together. More in general, it is difficult to compare detailed results of studies using different measures of insulin sensitivity and somewhat different sets of variables. In addition, the value of factor analysis of complex networks of physiological factors is in generating, rather than testing, hypotheses (40). Our factor analysis is nevertheless coherent with the standard multivariate approach (Table 3), and is further supported by the observation that each main trait examined, BMI, WHR, IR, and insulin exposure, made a graded contribution to global CVD risk (as expressed by the CVRF score) independently of the others (Fig. 3).

In conclusion, when IR, body fat mass and distribution, and insulin response are measured simultaneously in a standardized fashion in a large cohort (and adequate account is taken of relevant confounders), the emerging picture is one in which no one factor stands out as the sole driving force of the cluster, each exerting a statistical influence on one or more pathways according to established cause-effect relationships. How these associations will carry over to which clinical condition demands longitudinal studies with incident diabetes, dyslipidemia, or hypertension (and direct measures of cardiovascular damage) as endpoints.

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Further information on the RISC Study and participating centers can be found at www.egir.org.

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