Euglycemic Clamp Insulin Sensitivity and Longitudinal Systolic Blood Pressure: Role of Sex

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Abstract—Insulin resistance may be an independent risk factor for the development of hypertension, but change in blood pressure (BP) over time has not been adequately studied in healthy individuals fully characterized for insulin sensitivity. In the Relationship between Insulin Sensitivity and Cardiovascular disease (RISC) study, we measured insulin sensitivity (M/I) using the euglycemic clamp technique in 1073 healthy European adults (587 women, 486 men) aged 30 to 60 years followed up 3 years later. Systolic BP (SBP) at baseline was higher in insulin-resistant women (ie, those in the low sex-specific M/I tertile) compared with those in the intermediate (P<0.001) or high tertiles (P=0.06; mean±SD: 117±13, 111±12, 114±12 mm Hg, respectively). It did not differ across M/I tertiles in men. After adjustment for age, body mass index, baseline SBP, and other covariates, low insulin sensitivity (M/I) predicted a longitudinal rise in SBP in women but not in men; M/I was not associated with change in diastolic BP. SBP rose over time in both sexes and within all M/I tertiles (P<0.05), except in women with high insulin sensitivity. Therefore, in women (but not in men), low insulin sensitivity was associated with higher SBP at 3 years, and high insulin sensitivity was associated with a lower rise in SBP over time. (***Hypertension.*** **2013;62:404-409.)** ● **Online Data Supplement**

Key Words: blood pressure ■ hypertension ■ insulin ■ insulin resistance ■ sex

Hyperinsulinemia[1] and underlying insulin resistance are associated with essential hypertension,[2] but their exact role in pathophysiology remains unclear. In the Framingham Offspring study,[3] an insulin sensitivity index estimated from fasting and postload insulin and glucose levels[4] independently predicted blood pressure (BP) tracking during 4 years in nonobese middle-aged men and women.[5,6]

The euglycemic clamp technique, requiring intravenous insulin infusion with real-time glucose monitoring, is the acknowledged gold standard for the assessment of insulin sensitivity.[7] However, because it is invasive and labor-intensive, it has not been applied in adequately sized cohorts at risk of hypertension. Longitudinal studies have instead used fasting insulin concentrations, homeostasis model assessment (HOMA; calculated from fasting insulin and glucose concentrations), and insulin sensitivity index (calculated from fasting and postload insulin and glucose levels).[4,8] Most,[9–22] but not all,[23–27] have reported a relationship between insulin resistance and future BP rise or the development of hypertension.

Although these surrogate measures correlate with clamp-measured insulin sensitivity,[28] they depend on insulin immunoassays and do not account for body mass or body composition. Some previous studies in adults have used more direct physiological methods but have been either small[29] or cross-sectional.[30,31]

Having previously published cross-sectional euglycemic clamp data from the Relationship between Insulin Sensitivity and Cardiovascular disease (RISC) cohort (n=1384),[32] we now report the longitudinal relationship between baseline insulin sensitivity and BP during 3 years.

Methods

The RISC study is a large euglycemic clamp–based prospective study of the association between insulin resistance and atherosclerotic disease.[33] The RISC investigators and recruiting centers are listed in full in the online-only Data Supplement. The protocol was approved by Institutional Review Committees at all study centers, and the participants gave informed consent. All clinical assessments were performed according to the principles of the Declaration of Helsinki.

Participants were clinically healthy individuals aged 30 to 60 years recruited from 18 centers in 13 European countries. Exclusion criteria included presence of (or treatment for) cardiovascular disease, diabetes mellitus, dyslipidemia, or any other chronic disease. Of the 1563 participants screened, 1384 healthy individuals had a

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euglycemic clamp at baseline; of these, 1073 (587 women and 486 men) had complete data at 3-year follow-up after exclusion of those who had developed diabetes mellitus and symptomatic cardiovascular disease (n=21).

BP and Physical Examination

BP was measured sitting after 5-minute rest by trained study nurses (OMRON 705CP, Hamburg, Germany) in triplicate, according to a standard protocol: the median of these readings was used in this analysis. Median systolic BP (SBP) ≥140 mm Hg and median diastolic BP (DBP) ≥90 mm Hg at follow-up were taken to indicate hypertension.[34,35] Participants who had been identified and commenced on treatment in routine care (n=46) were classified as hypertensive. Height was measured by stadiometer. Body weight, percentage of body fat, and lean body mass (lbm) were evaluated by bioimpedance (TBF-300, Tanita International Division, United Kingdom).

Insulin Sensitivity

Insulin sensitivity was measured by a standard hyperinsulinemic euglycemic clamp technique as previously described, after central training of site staff.[33] Target plasma glucose concentration was between 4.5 and 5.5 mmol/L; insulin was infused at a rate of 240 $pmol \cdot min^{-1} \cdot m^{-2}$. Plasma glucose was measured at 5- to 10-minute intervals to ensure it remained within 0.8 mmol/L (±15%) of target glucose concentration. The steady-state period (for calculation of insulin sensitivity) was between 80 and 120 minutes. The glucose infusion rate (M value) was expressed in $mg \cdot min^{-1} \cdot lbm(kg)^{-1}$. Insulin sensitivity was expressed as M/I ($\mu mol \cdot min^{-1} \cdot kglbm^{-1} \cdot nmol/L^{-1}$), with the M value divided by steady-state insulin.

Biological Samples

Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were assayed by enzymatic colorimetric assay (Roche), and low-density lipoprotein cholesterol was calculated by the Friedewald formula. A 75-g oral glucose tolerance test was used to exclude participants with diabetes mellitus at inclusion (or developing diabetes mellitus during follow-up), with glucose assayed centrally by the glucose oxidase method (Cobas Integra, Roche).

Other Measures

Physical activity was measured during 1 week by Actigraph accelerometer (version AM7164-2.2, CSA Inc, Florida) expressed as average number of counts per minute.[36] Women were classified as postmenopausal if their last menstrual period was >12 months before baseline measurements.

Follow-Up

After annual telephone follow-up calls, participants attended a repeat visit at 3 years for medical history, concomitant medication, BP, anthropometry, blood examinations, and oral glucose tolerance test.

Statistical Analysis

Variables not following a Gaussian distribution were log-transformed. Because the sex*log M/I interaction term for SBP at Year 3 was significant in pooled regression with all of the data (P=0.003), subsequent analysis was performed separately by sex. Baseline measurements are shown in Table 1, with univariate Pearson correlation coefficients between change in systolic and DBP and other covariates in Table 2. ANOVA was used to examine change in BP across sex-specific tertiles of baseline insulin sensitivity with Tukey post hoc testing (corrected for multiple comparison) to examine changes between tertiles. Change in BP between baseline and follow-up was compared within each tertile of insulin sensitivity using paired t tests. Multiple linear regression analysis was used to determine whether insulin sensitivity predicted systolic and DBP at 3 years with covariates, including age, recruitment center (using indicator variables), baseline BP, body mass index (BMI), change in BMI, blood glucose, lipid profile, and lifestyle factors. The method of Cui et al[37] was used to estimate numeric BP values for individuals who had been commenced on antihypertensive medication between baseline and follow-up.[38] The relationship between log M/I and SBP at Year 3 adjusted for baseline SBP was used in preference to the unadjusted relationship with change in SBP, given that baseline BP and change in BP are usually highly correlated.[3] The relationship was curvilinear when adjusted for study center and age only, but this was no longer the case when baseline SBP was added to the model. β-coefficients for insulin sensitivity are shown along with R^2 for the coefficient of determination for the model. Binary logistic regression was used to assess prediction of hypertension defined according to ESH/JNCVII (European Society of Hypertension/Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure) as ≥140/≥90 mm Hg[34,35] (or by its treatment). SPSS version 18 was used in all analyses.

Results

At 3 years, BP had reached a diagnostic threshold for hypertension in 11.6% of all participants (n=125; 75 men, 50 women). A further 4.3% (n=46; 23 men, 23 women) had been commenced on antihypertensive treatment in routine care, that is, 16.0% (n=171; 98 men, 73 women) of all participants had developed incident hypertension.

Insulin concentrations achieved at steady state (mean±SD) were 416±111 pmol/L in men and 406±112 pmol/L in women. Men had lower insulin sensitivity (M/I) than women (Table 1); statistically significant differences were also noted between the sexes for all measurements, except smoking status and physical activity.

In univariate analyses (Table 2), change in both systolic and DBP correlated in the expected manner with baseline values in both sexes. Change in both systolic and DBP from baseline was correlated with insulin sensitivity (log M/I) in women but not in men. Weight correlated with change in diastolic, but not SBP, in both sexes. Other correlations were of borderline statistical significance.

When baseline SBP was expressed according to sex-specific tertiles of insulin sensitivity (M/I; mean±SD; Figure; for cut off values see figure legend), there were no differences between tertiles among men (123±11 [low]; 121±11 [intermediate]; 123±10 mm Hg [high]). However, women with low baseline M/I tended to have higher baseline SBP: 117±13 mm Hg (low), 111±12 mm Hg (intermediate), 114±12 mm Hg (high; low versus intermediate, 6.0 [95% confidence interval {CI}, 3.0–9.0] mm Hg, P<0.001; low versus high 3.0 [95% CI, −0.1 to 5.9] mm Hg, P=0.06).

During 3 years of follow-up, SBP increased from baseline in all tertiles for men (P<0.05; Figure). In women, SBP rose in those with low and intermediate M/I (P<0.05), but no change was observed in the high insulin sensitivity tertile (114±12 versus 114±14 mm Hg; P=0.791; 0.2 [95% CI, −1.8 to 1.4]). Comparing unadjusted 3-year SBP between insulin sensitivity tertiles within each sex (Tukey post hoc testing), no statistically significant differences were observed in men: (127±13 [low], 126±13 [intermediate], 126±14 mm Hg [high]). However, in women, 3-year SBP in the low M/I tertile (121±16 mm Hg) was significantly higher than in the intermediate (116±16 mm Hg; P=0.001; 5.6 [95% CI, 1.93–9.26]) and high (114±14 mm Hg) M/I tertiles (P<0.001; 7.1 [95% CI, 3.47–10.79]). Test for trend for 3-year SBP across the tertiles was significant in women (P<0.001) but not in men

Table 1. Baseline Characteristics of Men and Women in the RISC Study

Characteristics	Men (n=486)	Women (n=587)	*P* Value
Age, y	43.8±8.5	44.9±8.0	0.04
Systolic BP, mm Hg	126±14	117±16	<0.001
Diastolic BP, mm Hg	77±7	73±8	<0.001
Waist, cm	93±10	81±11	<0.001
Weight, kg	83.4±12.3	67.5±12.0	<0.001
BMI, kg/m^2	26.2±3.4	24.8±4.2	<0.001
Fat free mass, kg	64.8±7.0	44.8±4.3	<0.001
Fat mass, kg	18.6±7.6	22.7±9.0	<0.001
Glucose, mmol/L	5.2±0.5	5.0±0.5	<0.001
Total cholesterol, mmol/L	4.9±0.9	4.8±0.9	0.030
LDL cholesterol, mmol/L	3.1±0.8	2.8±0.8	<0.001
HDL cholesterol, mmol/L	1.3±0.3	1.6±0.4	<0.001
Triglycerides, mmol/L*	1.12 (1.07–1.17)	0.86 (0.83–0.89)	<0.001
Clamp insulin sensitivity, M/I*	112 (107–117)	141 (135–148)	<0.001
Smoker, %	26	26	0.910
Alcohol, g/wk*	81 (76–89)	47 (43–50)	<0.001
Physical activity, counts/min*	339 (316–355)	324 (316–339)	0.426
Menopause, Y/N	…	153/ 434	…
Creatinine, μmol/L	75±12.4	59±12.0	<0.001
eGFR, mL/min per 1.73 m^2	110±29.5	107±36.5	0.171

Data shown are as mean±SD, or geometric means and confidence intervals. BMI indicates body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M/I, glucose metabolised/insulin; and RISC, Relationship between Insulin Sensitivity and Cardiovascular disease.

*Log-transformed for analysis.

(*P*=0.307). When these analyses were repeated, excluding individuals on antihypertensive treatment, similar results were obtained (data not shown).

When key covariates (including recruitment center, age, baseline SBP, BMI, change in BMI, lipid profile, smoking status, and fasting glucose) were included in multiple regression analyses (Table 3), low insulin sensitivity (log M/I) significantly and independently predicted SBP at 3 years in women (β=–0.214; *P*<0.001) but not in men.

M/I was not a predictor of the development of hypertension, in either sex, after adjustment for baseline SBP, age, and BMI (data not shown), although the trend test across the M/I tertiles was significant for the development of hypertension in women (*P*<0.01) but not in men (*P*=0.260). There was no relationship between insulin sensitivity and SBP in either sex when HOMA was substituted for Log M/I as an independent variable (data not shown). When regression analyses were repeated substituting waist for BMI, results were very similar (data not shown). Insulin sensitivity did not predict DBP longitudinally (data not shown).

Discussion

In this analysis of 1073 healthy European adults, low insulin sensitivity measured using a robust euglycemic clamp technique predicted rise in SBP at 3 years in women but not in men. SBP was higher at baseline in women with low insulin sensitivity than that in those with intermediate or high insulin sensitivity. It increased during 3 years in all groups studied, except in women with high baseline insulin sensitivity. Insulin sensitivity predicted change in SBP independently of key covariates (including age, baseline BP, BMI, and change in BMI)[3] in women only. These findings offer new insights into the relationships between metabolic factors and BP by sex, clarifying and extending cross-sectional data we have previously reported from the RISC cohort.[32]

The most comprehensive previous study on this topic was the Framingham Offspring study in which insulin sensitivity was assessed in 1933 healthy adults using an insulin sensitivity index based on fasting and postload insulin and glucose levels.[3] In this report, insulin sensitivity (expressed categorically in sex-specific quartiles and stratified by age) was independently associated with BP over time in younger, leaner individuals of both sexes but not in those who were older, overweight, or obese.

A unique feature of the present analysis is that it is based on data from a large number of individuals undergoing a standard euglycemic clamp. Insulin sensitivity was directly derived from the glucose infusion rate during steady-state euglycemia adjusted only for centrally measured insulin concentrations, and lean body mass was measured using a standard device.

Only 2 previous investigations into the longitudinal relationship between insulin sensitivity and BP in adults have incorporated direct measures of insulin sensitivity,[20,29] and only 1 investigation used the euglycemic clamp. One of these studies was relatively small (n=54) and in men only: no effect was demonstrated.[29] The other used a modified frequently sampled intravenous glucose tolerance test in a triethnic population (n=840) and reported a modest protective association of insulin sensitivity on the risk of hypertension.[20]

Other longitudinal studies reporting a relationship between insulin sensitivity and BP[10–22] have been based on surrogate

Table 2. Pearson Correlations (*r*) Between Baseline Characteristics and Change (Δ) in Systolic and Diastolic BP During Three Years

	ΔSBP		ΔDBP	
	Male	Female	Male	Female
Characteristics	*r*	*r*	*r*	*r*
Age, y	0.162‡	0.027	0.074	−0.068
Baseline SBP, mm Hg	−0.293‡	−0.273‡	−0.162‡	−0.163‡
Baseline DBP, mm Hg	−0.293‡	−0.196‡	−0.340‡	−0.339‡
Waist, cm	0.056	0.055	0.067	0.008
Weight, kg	0.077	0.073	0.113*	0.134†
BMI, kg/m^2	0.034	0.081*	0.086	0.100*
Fat free mass, kg	0.092	0.050	0.079	0.162‡
Fat mass, kg	0.040*	0.074	0.110*	0.103*
Glucose, mmol/L	−0.008	0.016	−0.005	−0.037
Total cholesterol, mmol/L	0.029	0.076	−0.041	−0.012
LDL cholesterol, mmol/L	0.061	0.080	−0.044	−0.017
HDL cholesterol, mmol/L	−0.153†	−0.009	−0.100*	−0.007
TG, mmol/L	0.075	0.016	0.058	0.006
Insulin sensitivity, log M/I	−0.054	−0.132†	−0.045	−0.110†
Smoker, %	0.019	−0.004	−0.010	−0.022
Alcohol, g/wk	0.021	0.044	0.012	0.057
Physical activity, counts/min worn	−0.014	0.000	−0.024	−0.103*
Creatinine, μmol/L	−0.041	−0.031	0.052	0.080
eGFR, mL/min per 1.73 m^2	0.055	0.030	−0.035	−0.067

BMI indicates body mass index; BP, blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M/I, glucose metabolised/insulin; SBP, systolic blood pressure; and TG, triglyceride.

*P<0.05.

†P<0.01.

‡P<0.001.

measures of insulin resistance, including either fasting insulin[10–15,17,21,22] or fasting insulin and glucose concentrations (HOMA).[18,19] They have been performed in a variety of populations (Scandinavian[11,15,22]; Mexican-American[16,17]; Japanese individuals[13,14]), with some including only men[10,11,19,25,29] but none only women. Some studies of reasonable size and duration have reported no relationship between insulin sensitivity and BP[9,23,24,26,27] after adjustment

Table 3. Standardized β Coefficients for Predicting Systolic BP at Three-Year Follow-Up From Log M/I as Independent Variable (With Various Adjustment Factors)

		Men (n=486)			Women (n=587)		
Model	Adjustment Factors	R^2	β	*P* Value	R^2	β	*P* Value
1	…	0.005	−0.069	0.134	0.046	−0.214	<0.001
2	Center and age	0.147	−0.049	0.296	0.250	−0.194	<0.001
3	Model 2 + baseline systolic BP	0.365	−0.034	0.398	0.466	−0.121	<0.001
4	Model 3 + BMI	0.368	−0.007	0.871	0.474	−0.081	0.027
5	Model 4 + %change BMI	0.385	−0.003	0.946	0.481	−0.078	0.031
6	Model 5 + baseline eGFR	0.386	−0.004	0.935	0.481	−0.077	0.035
7	Model 6 + glucose, Chol, LDL, HDL, log TG	0.397	0.030	0.539	0.486	0.073	0.053
8	Model 7 + baseline HRT & OCP use	0.397	0.030	0.539	0.488	−0.079	0.038
9	Model 8 + smoking	0.398	0.031	0.523	0.488	0.079	0.040
10	Model 9 + phys. activity	0.396	0.026	0.683	0.482	−0.086	0.088
11	Model 10 + log alcohol	0.396	0.026	0.693	0.483	−0.088	0.126

BMI indicates body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HRT, hormone replacement therapy; LDL, low-density lipoprotein; M/I, glucose metabolised/insulin; OCP, oral contraceptive pill; and TG, triglyceride.

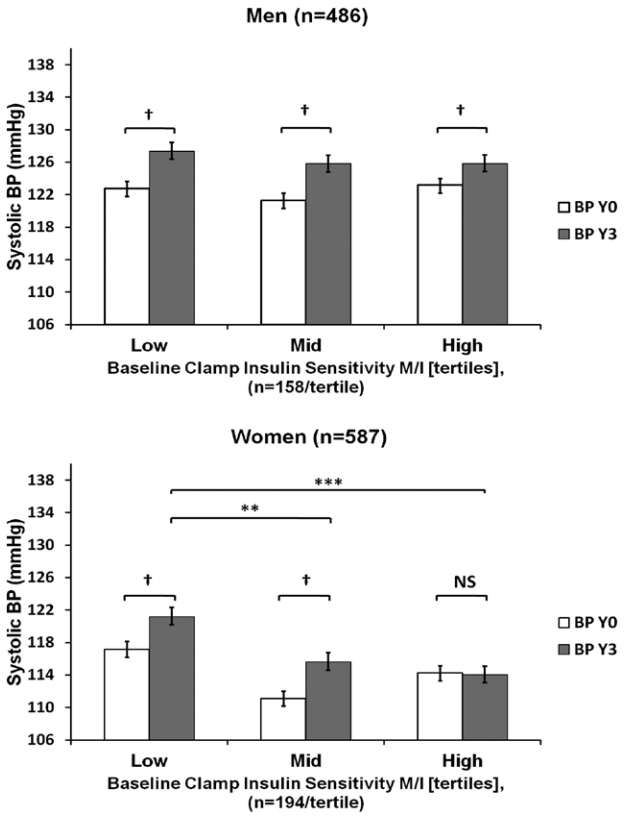

Figure. Unadjusted systolic blood pressure (BP; mean±SEM) at baseline and 3-year follow-up in men (**top**) and women (**bottom**) by tertiles of baseline insulin sensitivity (M/I). Men tertile ranges: low, 16.2 to 90.8; intermediate, 90.8 to 137; high, 137 to 454 $\mu mol \cdot min^{-1} \cdot kgffm^{-1} \cdot nmol/L^{-1}$. Women tertile ranges: low, 21.4 to 120.2; intermediate, 120.2 to 173.8; high, 173.8 to 977.2 $\mu mol \cdot min^{-1} \cdot kgffm^{-1} \cdot nmol/L^{-1}$. BPY0 indicates baseline systolic BP; and BPY3, systolic BP at 3 years (mean±SEM). Comparison between tertiles by ANOVA with Tukey post hoc testing with 95% confidence intervals for the difference in means between tertiles. †$P<0.05$. Differences in means between baseline and 3-year follow-up SBP by paired sample *t* tests. **$P<0.01$; ***$P<0.001$; NS, not significant.

for baseline BP and weight/adiposity, although it is difficult to interpret from some studies whether data were examined separately by sex.

Validation studies of HOMA against clamp insulin sensitivity report Pearson *r* values of between 0.5 and 0.6 but are based on small numbers of participants pooled for sex.[4,28] On average, women have a lower percentage of lean body mass compared with men. Therefore, in the presence of intact β-cell function, a given absolute value of fasting insulin (or HOMA) in women reflects a greater level of tissue insulin resistance than in men. Women in the Framingham Offspring cohort had (on average) lower BMI and lower fasting insulin than men, but a similar insulin sensitivity index. If BP tracking over time is related to tissue rather than whole-body insulin sensitivity, euglycemic clamp data are likely to be more precise by sex than indices based on fasting insulin. Differences in the relationships among insulin sensitivity and BP according to sex may reflect higher fat mass as a percentage of body weight in women, particularly with aging.[39]

Strengths of the present analysis are the following: use of a gold standard assessment of insulin sensitivity with a standard protocol among participating centers; central training of study personnel with focus on quality control; and accurate measurement of BP using the same standard and validated device in all centers. We acknowledge that the low proportion of cases that developed incident hypertension (16%) limited the power of our analysis to address a more clinically relevant outcome measure. Moreover, given the invasive nature of assessments, we were unable to use a truly population-based sampling method. Because only European centers were included, with very few participants representing ethnic minorities, it cannot be assumed that our findings can be generalized to other populations.

Perspectives

These prospective data from the Europe-wide RISC cohort of healthy adults indicate that low insulin sensitivity measured using the euglycemic clamp technique is an independent predictor of longitudinal change in BP over time in women but not in men. Women with high insulin sensitivity may be protected against rise in SBP over time. The physiological basis for the sex difference we report in the RISC cohort and its implications for the role of insulin resistance in the pathophysiology of hypertension remain uncertain. Further insights may be gained by further follow-up of the cohort.

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Disclosures

None.

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Novelty and Significance

What Is New?

- The RISC study is the largest longitudinal study of the relationship between euglycemic clamp insulin resistance and BP in healthy men and women.

What Is Relevant?

- Women with high insulin sensitivity may be protected against rise in systolic BP over time.

Summary

Low euglycemic clamp-measured insulin sensitivity independently predicted systolic BP at 3 years in women but not in men.

ONLINE SUPPLEMENT

For:

EUGLYCEMIC CLAMP INSULIN SENSITIVITY AND LONGITUDINAL SYSTOLIC BLOOD PRESSURE: ROLE OF GENDER

John R Petrie, MD, PhD; Muhammad Omar Malik, MD; Beverley Balkau, PhD; Colin G Perry, MD, PhD; Kurt Højlund, MD; Zoltan Pataky, MD; John Nolan, MD; Ele Ferrannini, MD, PhD; Andrea Natali, MD, PhD

for the RISC Investigators

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Short title: Insulin sensitivity, gender and blood pressure

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