

# Association of Serum Proinsulin With Hormone Replacement Therapy in Nondiabetic Older Women

## The Rancho Bernardo Study

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**OBJECTIVE** — One putative benefit of hormone replacement therapy (HRT) is a reduced risk of diabetes or reduced fasting glucose level. We report here the association of HRT with proinsulin, insulin, and fasting and postchallenge glucose levels in older adults.

**RESEARCH DESIGN AND METHODS** — Current HRT use was validated and cross-sectionally compared with diabetes-related variables in 785 women without diabetes by history or glucose tolerance test.

**RESULTS** — Median age was 72 years (range 50–97); median value of fasting plasma glucose, postchallenge plasma glucose, and proinsulin was 5.08 mmol/l, 6.93 mmol/l, and 9.3 pmol/l, respectively. In age-adjusted comparisons, current HRT use was associated with significantly lower fasting plasma glucose and higher postchallenge plasma glucose compared with never/previous HRT use, as well as with lower LDL and higher HDL cholesterol and higher triglycerides. Fasting and postchallenge intact insulin did not differ by HRT group, but proinsulin was significantly lower in current HRT users than in previous and never HRT users. The significant association between proinsulin and HRT status persisted after adjustment for age, waist-to-hip ratio, pulse pressure, LDL-to-HDL cholesterol ratio, triglycerides, fasting and postchallenge glucose, and intact insulin.

**CONCLUSIONS** — Reduced fasting and increased 2-h glucose replicate findings in a randomized clinical trial. The proinsulin effect has not been previously reported. Decreased fasting glucose and proinsulin levels in current HRT use suggest a potential antidiabetes effect of HRT. Increased postchallenge glucose in HRT, however, suggests insulin resistance and would be expected to increase the risk of heart disease.

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In 2002, our group reported that proinsulin was more strongly and consistently associated with coronary heart disease (CHD) than insulin (1). Many other studies have shown that serum proinsulin is a strong predictor of CHD (2–7), diabetes (8–10), and the metabolic syndrome (11). In one clinical trial (12),

human proinsulin increased the risk of cardiovascular events several-fold in comparison with intact human insulin, which the authors attributed to thromboembolic mechanisms. In vitro, proinsulin stimulates production of plasminogen activator-1 (PAI-1; 13,14), a putative link between atherothrombosis and elevated

proinsulin concentrations preceding the onset of CHD (15).

Hormone replacement therapy (HRT) has not been shown to prevent cardiovascular disease in clinical trials (16) despite multiple biologically plausible mechanisms for cardiac protection (17), but clinical trials have shown a reduced risk of diabetes (18–21) or reduced fasting glucose levels (22,23).

To our knowledge, no report has been published on a possible association between proinsulin and HRT. In the present study, we investigate the cross-sectional association of proinsulin, intact insulin, and other traditional cardiovascular risk factors with HRT in community-dwelling older women without diabetes. Women with diabetes were excluded because there is evidence that women with diabetes are less likely to be prescribed HRT.

### RESEARCH DESIGN AND METHODS

From 1992 to 1996, adults aged 50–97 years from a southern California residential community attended a Rancho Bernardo Study clinic visit. The 1992–1996 study population ( $n = 1,781$ ) included ~75% of surviving Rancho Bernardo participants who were still local community-dwelling residents. All were Caucasian and most were middle to upper middle class. Informed consent was obtained, and the study was approved by the institutional review board of the University of California, San Diego.

Personal history of hypertension, diabetes, current cigarette smoking, daily alcohol consumption, daily fat intake, and regular exercise (three or more times per week) were determined by standardized questionnaire and interview. Blood pressure and lipid-lowering medications and estrogen use (current/previous/never) were determined at the 1992–1996 clinic visit using standard questionnaires. HRT use, type, and dose were validated by a specially trained nurse who examined pills and prescriptions brought to the clinic for that purpose.

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**Abbreviations:** CHD, coronary heart disease; DEXA, dual-energy X-ray absorptiometry; HERS, Heart and Estrogen/Progestin Replacement Study; HOMA-R, R value of homeostasis model assessment; HRT, hormone replacement therapy; PAI-1, plasminogen activator-1; PEPI, Postmenopausal Estrogen/Progestin Interventions; WHI, Women's Health Initiative.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Height and weight were measured in participants wearing light-weight clothing without shoes. BMI was calculated as weight in kilograms divided by the square of height in meters. Waist and hip girth were measured in centimeters over single-thickness clothing with the participant standing in an erect position with feet together. Percentage of total body fat and truncal fat was determined by dual-energy X-ray absorptiometry (DEXA; model QDR-2000; Hologic, Waltham, MA). Lipids were measured in a lipid research clinic laboratory certified by the Centers for Disease Control and Prevention. Total cholesterol and triglycerides were measured by enzymatic methods with an ABA-200 biochromatic analyzer (Abbott), HDL cholesterol was measured by precipitation according to the lipid research clinic protocol (24), and LDL cholesterol was calculated using the formula by Friedewald et al. (25). A morning 75-g oral glucose tolerance test was performed after a minimum 8-h overnight fast. Fasting and 2-h postchallenge plasma glucose levels were measured with a glucose oxidase assay.

Participants with type 1 or type 2 diabetes, based on history or hyperglycemia defined by the 1999 World Health Organization criteria (26) (fasting plasma glucose  $\geq 7.0$  mmol/l or 2-h plasma glucose in 75-g oral glucose tolerance test  $\geq 11.1$  mmol/l), were excluded. The present study includes all 785 women without diabetes who had measurements of insulin and proinsulin. Fasting and postchallenge insulin levels were measured in the research laboratory of S. Edwin Fineberg (Indiana University) using a double-antibody kit for human intact insulin-specific radioimmunoassay in Linco research (27). This assay cross-reacts with both human proinsulin and des-64,65 proinsulin  $< 0.2\%$ . des-64,65 proinsulin cross-reacts 76%, but des-64,65 proinsulin is an extremely minor component of insulin-like materials, and the sensitivity was 2  $\mu\text{U/ml}$ . Two levels of control external standards were used. Intra-assay and interassay coefficients of variations for control level 1 were 5.5 and 15%, respectively, and for control level 2, 3.7 and 5.1%, respectively.

Proinsulin was measured with radioimmunoassay based on a method by Bowsher et al. (28). Human insulin does not cross-react with proinsulin in this assay. Sensitivity was 2 pmol/l and intra-assay and interassay coefficients of variation of insulin were from 5 to 16%. The estimate

of insulin resistance by the *R* value of homeostasis model assessment (HOMA-R) score was calculated with the following formula: fasting serum insulin ( $\mu\text{U/ml}$ )  $\times$  fasting plasma glucose (mmol/l)/22.5 (29).

The metabolic syndrome was defined as described in the National Cholesterol Education Program Adult Treatment Panel III report (30) as three or more of the following characteristics for women: 1) waist circumference  $> 88$  cm, 2) hypertriglyceridemia  $\geq 1.7$  mmol/l, 3) HDL cholesterol  $< 1.29$  mmol/l, 4) blood pressure  $\geq 130$  mmHg systolic or  $\geq 85$  mmHg diastolic or current use of antihypertensive medication, and 5) fasting glucose  $\geq 6.1$  mmol/l. The presence of CHD was based on a positive answer to the prolonged severe chest pain ( $> 30$  min) question on the Rose Questionnaire (31), diagnosis by a physician, or major electrocardiogram abnormalities by Minnesota code criteria (Minnesota codes 1.1 and 1.2) (32). A standard 12-lead resting electrocardiogram was performed before the oral glucose tolerance test after the subjects had been supine for at least 5 min. Medical history of heart disease was validated in 85% of the 30% sample whose hospital records were requested.

### Statistical methods

Data were analyzed with SPSS Version 12.0. Because BMI, waist circumference, trunk fat amount by DEXA, systolic and diastolic blood pressure, pulse pressure, alcohol intake, LDL-to-HDL cholesterol ratio, triglycerides, fasting glucose, fasting insulin, fasting proinsulin, postchallenge glucose and insulin, and proinsulin-to-insulin ratio showed at least slightly skewed distributions, all analyses were performed using log-transformed data. All probability values were based on logarithmic data. Clinical and laboratory variables were compared by HRT treatment status (never, previous, and current) using a Student's *t* test for continuous variables and a  $\chi^2$  test for categorical variables. ANCOVA was used to assess the independent association of proinsulin with HRT use, age, waist-to-hip ratio, pulse pressure, LDL-to-HDL cholesterol ratio, triglycerides, fasting and postchallenge plasma glucose, and insulin as covariates. Age-adjusted partial correlation analyses were used to determine the relation of proinsulin with HRT status, the presence of CHD, and other cardiovascular risk factors. Multiple linear regression analyses were used to assess

the independent association of proinsulin with HRT status, the presence of CHD, and other cardiovascular risk factors. All probability values were two tailed, and statistical significance was defined as  $P < 0.05$ .

**RESULTS**— Participants in the present study were Caucasian, older (aged  $70.9 \pm 11.6$  years), not obese (BMI  $24.5 \pm 4.0$  kg/m<sup>2</sup>), and reported a healthy lifestyle: 69.6% women exercised more than three times per week, and only 7.9% were current smokers. Their average age at menopause was  $47.4 \pm 6.4$  years; 33% ( $n = 260$ ) had never used HRT, 23% ( $n = 180$ ) had used HRT previously but had quit ( $16.7 \pm 12.2$  years ago), and 44% ( $n = 345$ ) were current HRT users. There was no difference in HRT start age between previous and current HRT users ( $50.7 \pm 9.2$  vs.  $51.2 \pm 9.5$  years). Duration of HRT use in the current HRT category was  $16.0 \pm 11.0$  years, and it was  $7.5 \pm 7.6$  years in previous HRT users. Age at menopause in never HRT was significantly older than age in previous HRT and current HRT, respectively ( $48.9 \pm 5.0$  vs.  $47.4 \pm 6.0$  and  $46.3 \pm 7.2$  years). Seventy-eight women reported an oophorectomy (4.3% in never HRT, 7.9% in previous HRT, and 16.6% in current HRT).

In age-adjusted comparisons, there was no difference in daily fat intake or smoking among the three groups (total fat intake:  $26.2 \pm 1.0$  in never HRT,  $27.9 \pm 1.2$  in previous HRT, and  $27.1 \pm 0.8$  g/day in current HRT; saturated fat intake:  $17.7 \pm 0.6$  in never HRT,  $19.2 \pm 0.8$  in previous HRT, and  $18.5 \pm 0.5$  g/day in current HRT; and past or current smoking: 47.3% in never HRT, 53.5% in previous HRT, and 54.8% in current HRT). Exercise and alcohol consumption did not differ (exercise three or more times per week: 66.2% in never HRT, 71.7% in previous HRT, and 71.8% in current HRT; alcohol intake:  $44.0 \pm 4.0$  in never HRT,  $48.3 \pm 4.9$  in previous HRT, and  $49.5 \pm 3.5$  g/week in current HRT). There was no difference in numbers of women using antihypertensive medications or lipid-lowering medications among never HRT, previous HRT, and current HRT (antihypertensive medications: 23.4, 26.8, 27.4%, respectively; antilipid medications: 10.4, 14.1, 9.7%, respectively).

Table 1 shows age and age-adjusted characteristics by HRT use status. Women with previous HRT were the oldest among three groups, and those with current HRT

**Table 1—Age and age-adjusted characteristics for nondiabetic older women (n = 785) according to estrogen replacement therapy: the Rancho Bernardo Study, 1992–1996**

	Never HRT	Previous HRT	Current HRT	P
n	260	180	345	
Age (years)	71.9 ± 0.8	75.5 ± 0.7	67.9 ± 0.6	<0.001
BMI (kg/m <sup>2</sup> )*	24.6 ± 0.2	24.7 ± 0.3	24.2 ± 0.2	NS
Waist circumference (cm)*	79.5 ± 0.7	79.9 ± 0.8	78.4 ± 0.6	NS
Waist-to-hip ratio	0.85 ± 0.01	0.86 ± 0.01	0.85 ± 0.01	NS
Trunk fat by DEXA (g)*	9,173 ± 278	9,617 ± 329	9,134 ± 236	NS
Systolic blood pressure (mmHg)*	136.3 ± 1.3	135.4 ± 1.5	137.8 ± 1.1	NS
Diastolic blood pressure (mmHg)*	75.0 ± 0.6	74.9 ± 0.7	74.4 ± 0.5	NS
LDL cholesterol (mmol/l)	3.47 ± 0.05	3.48 ± 0.06	3.07 ± 0.05	<0.001
HDL cholesterol (mmol/l)	1.60 ± 0.03	1.58 ± 0.03	1.82 ± 0.02	<0.001
LDL-to-HDL cholesterol ratio*	2.32 ± 0.05	2.35 ± 0.06	1.83 ± 0.05	<0.001
Triglyceride (mmol/l)*	1.28 ± 0.04	1.32 ± 0.05	1.50 ± 0.04	<0.001
Fasting glucose (mmol/l)*	5.21 ± 0.03	5.19 ± 0.03	5.04 ± 0.03	<0.001
Postchallenge glucose (mmol/l)*	6.96 ± 0.12 (n = 209)	6.74 ± 0.15 (n = 138)	7.31 ± 0.10 (n = 309)	0.006
Fasting insulin (pmol/l)*	74.0 ± 3.4	72.3 ± 3.4	65.4 ± 3.4	NS
Postchallenge insulin (pmol/l)*	482.2 ± 22.4 (n = 209)	483.9 ± 29.3 (n = 138)	451.2 ± 18.9 (n = 309)	NS
Fasting proinsulin (pmol/l)*	11.7 ± 0.4	11.3 ± 0.5	9.5 ± 0.4	0.001
Proinsulin-to-insulin ratio*	0.16 ± 0.01	0.16 ± 0.02	0.15 ± 0.01	NS
HOMA-R*	2.22 ± 0.10	2.16 ± 0.13	1.90 ± 0.09	NS
Metabolic syndrome ATP III (%)	10.8	11.3	10.9	NS
CHD (%)	24.8	26.5	20.6	NS

Data are means ± SEM or percent. CHD was defined by Rose Questionnaire, electrocardiogram, and physician's reports. \*Log transformed. ATP III, Adult Treatment Panel; NS, not significant.

were the youngest (each  $P < 0.01$ , previous HRT vs. never HRT vs. current HRT in ANOVA). Neither obesity parameters nor blood pressure differed by HRT status. However, LDL cholesterol was significantly lower and HDL cholesterol and triglycerides significantly higher in women with current HRT than in never or previous HRT users ( $P < 0.001$ ). Fasting plasma glucose was lower in current HRT women than in women with previous or never HRT ( $P < 0.001$ ). Postchallenge glucose in current HRT was higher than those in previous and never HRT ( $P = 0.004$  vs. previous HRT,  $P = 0.023$  vs. never HRT). After controlling for both age and BMI, these significant differences

among never, previous, and current HRT remained statistically significant (fasting glucose  $5.21 \pm 0.03$ ,  $5.18 \pm 0.03$ , and  $5.05 \pm 0.03$  mmol/l, respectively,  $P < 0.001$ ; postchallenge glucose  $6.95 \pm 0.12$ ,  $6.72 \pm 0.15$ , and  $7.32 \pm 0.10$  mmol/l, respectively,  $P = 0.002$ ).

Fasting and postchallenge insulin did not differ by HRT group, but proinsulin was significantly lower in current HRT users than in previous and never HRT users ( $P = 0.013$  vs. previous HRT;  $P < 0.001$  vs. never HRT). Although comparison of HOMA-R among three groups showed no statistical significance ( $P = 0.051$ ), HOMA-R was significantly lower in current HRT users than in previous and

never HRT users (each  $P < 0.05$ ). Also, in a comparison between combined never and previous HRT users ( $n = 440$ ) vs. current HRT users ( $n = 345$ ), HOMA-R was significantly lower in current HRT users ( $1.90 \pm 0.09$  in current HRT vs.  $2.19 \pm 0.08$  in combined previous and never HRT,  $P = 0.016$ ). After controlling for BMI in addition to age, this significance was maintained ( $1.92 \pm 0.09$  vs.  $2.17 \pm 0.08$ ,  $P = 0.040$ ). There was no significant difference between never and previous HRT users for any variables shown in Table 1.

In age-adjusted correlation analyses, shown in Table 2, current HRT, CHD, body size and fat distribution, HDL cho-

**Table 2—Age-adjusted correlation coefficient between fasting serum proinsulin and other variables for nondiabetic older women (n = 785): the Rancho Bernardo Study, 1992–1996**

Variable	r	P	Variable	r	P
Age	0.128	<0.001	Systolic blood pressure*	0.058	NS
Previous HRT	0.041	NS	Diastolic blood pressure*	0.050	NS
Current HRT	-0.130	<0.001	Pulse pressure*	0.036	NS
CHD	0.130	<0.001	LDL cholesterol	0.053	NS
BMI*	0.327	<0.001	HDL cholesterol	-0.216	<0.001
Waist girth*	0.306	<0.001	Triglycerides*	0.249	<0.001
Waist-to-hip ratio	0.087	<0.015	Fasting glucose*	0.245	<0.001
Trunk fat by DEXA (g)*	0.305	<0.001	Fasting insulin*	0.284	<0.001

\*Log transformed.

**Table 3—Serum fasting proinsulin (pmol/l) according to HRT for nondiabetic older women: the Rancho Bernardo Study, 1992–1996**

	Never HRT	Previous HRT	Current HRT	P for trend
Model 1	11.51 ± 0.42*	11.15 ± 0.52†	9.70 ± 0.38	0.005
Model 2	11.59 ± 0.48*	11.15 ± 0.61†	9.75 ± 0.41	0.005

Data are means ± SEM. Model 1: controlling for age, waist-to-hip ratio, pulse pressure, LDL-to-HDL cholesterol ratio, triglycerides, fasting glucose, and fasting insulin. Model 2: controlling for variables in model 1 plus postchallenge glucose and postchallenge insulin. \* $P < 0.01$ ; † $P < 0.05$  vs. current HRT (aged 70.8 years, waist-to-hip ratio = 0.85, pulse pressure = 61.9 mmHg, LDL-to-HDL cholesterol ratio = 2.11, triglycerides = 1.38 mmol/l, fasting glucose = 5.13 mmol/l, and fasting insulin = 68.9 pmol/l).

lesterol, triglycerides, fasting plasma glucose, and insulin were significantly associated with proinsulin levels, but blood pressure parameters and LDL cholesterol were not. In multiple linear regression analysis, BMI (standardized  $\beta = 0.178$ ,  $P < 0.001$ ), fasting insulin (standardized  $\beta = 0.167$ ,  $P < 0.001$ ), fasting glucose (standardized  $\beta = 0.153$ ,  $P < 0.001$ ), and triglycerides (standardized  $\beta = 0.115$ ,  $P = 0.001$ ) were significantly and independently associated with proinsulin, respectively. In the same model, age (standardized  $\beta = 0.063$ ) and LDL-to-HDL cholesterol ratio (standardized  $\beta = 0.035$ ) showed no significant association with proinsulin.

Given the strong association between proinsulin and current HRT use, we tried to determine whether this association was independent of all measured cardiovascular risk factors and glucose homeostasis parameters. In this model, proinsulin was significantly decreased in current HRT users compared with previous or never HRT users independent of age, waist-to-hip ratio, pulse pressure, LDL-to-HDL cholesterol ratio, and triglycerides. Significant differences persisted in the model adding fasting, postchallenge glucose, and insulin as covariates (Table 3).

Fifty percent of current hormone users used a combined estrogen and progesterone therapy. The estrogen-only women were older than women using combined therapy ( $69.7 \pm 10.7$  vs.  $66.1 \pm 9.9$  years). In an age-adjusted comparison between estrogen-only and combined-therapy women, estrogen-only women showed larger trunk fat by DEXA (means ± SEM,  $9,876 \pm 319$  vs.  $8,684 \pm 319$  g,  $P = 0.008$ ) and higher triglycerides ( $1.66 \pm 0.05$  vs.  $1.34 \pm 0.05$  mmol/l,  $P < 0.001$ ). In these age-adjusted analyses, there was no difference in fat intake, smoking habit, BMI, waist circumference, blood pressure, LDL cholesterol, HDL cholesterol, and fasting and postchallenge glucose and insulin. Higher proinsulin

value in estrogen-only women compared with combined-therapy women ( $9.97 \pm 0.47$  vs.  $9.05 \pm 0.47$  pmol/l,  $P = 0.014$ ) was observed after controlling for age but was no longer significant after controlling for age and triglycerides ( $9.85 \pm 0.48$  vs.  $9.17 \pm 0.47$  pmol/l,  $P = 0.322$ ).

**CONCLUSIONS**— In this study, current HRT use was associated with lower fasting plasma glucose and higher postchallenge plasma glucose compared with never and previous HRT use, as well as with lower LDL and higher HDL cholesterol and higher triglycerides. These results are all comparable with the results in the Postmenopausal Estrogen/Progestin Interventions (PEPI) clinical trial (33). In addition, lower proinsulin was closely associated with current HRT, independent of age, obesity, lipid profile, glucose, and insulin levels. To our knowledge, this is the first report showing an association between proinsulin and HRT.

Many observational studies (34–38) have reported that postmenopausal women taking HRT have lower fasting glucose or glycosylated hemoglobin levels than those not taking hormones. Some randomized, controlled trials (22,39,40) of HRT versus placebo in women without diabetes have reported decreased fasting glucose or insulin levels but others (41–43) have not.

The PEPI study was the first large, randomized, placebo-controlled trial to evaluate the effect of postmenopausal hormone therapy on glucose metabolism (33). In a trial of four hormone groups compared with placebo in 875 younger postmenopausal women (45–64 years of age) who were followed for 3 years, hormone therapy caused a significant decrease in fasting plasma glucose levels and an increase in 2-h postchallenge glucose levels. No significant change in fasting or 2-h insulin was observed. The Heart and Estrogen/Progestin Replacement Study (HERS) reported a 35% lower risk for di-

abetes in women randomly assigned to HRT than in those assigned to placebo (19). The Women's Health Initiative (WHI) clinical trial also reported that estrogen plus progestin lowered fasting glucose, fasting insulin, and HOMA-R after 1 year (18). Postchallenge glucose was not measured in HERS or WHI. In this study, we observed an association of HRT with decreased fasting glucose and increased postchallenge glucose similar to the results in PEPI, although Rancho Bernardo participants were older than PEPI women and were not obese compared with HERS and WHI women.

The precise mechanism for the decreased fasting glucose and increased postprandial glucose is not clear. This apparently paradoxical combination of findings may be caused by the ability of estrogen to diminish glucagon action and secretion and to increase glucocorticoid activity (44). It is possible that an HRT-related change of body composition may affect insulin sensitivity, but the change in body fat and its distribution in trials have been modest (45). In the present study, there was no significant difference in diverse obesity parameters including BMI, waist circumference, and truncal fat by HRT status. Therefore, it is less likely that HRT-related change of body composition altered insulin sensitivity. Similarly, both WHI and HERS reported that the lower fasting glucose with HRT was independent of obesity. Some studies of women taking estrogen reported improved insulin sensitivity (46–48) and better rates of glucose disposal (49), but most studies (42,50–54) have not replicated this finding.

In a previous study (55) of Rancho Bernardo women who were not using HRT, endogenous levels of bioavailable estradiol were positively associated with increasing 8-year follow-up levels of fasting insulin and HOMA-R. Comparable with WHI study results, in the present study insulin resistance measured by HOMA-R was significantly decreased in current HRT users compared with nonusers. Proinsulin and insulin concentrations were measured in the fasting state and could thus reflect hepatic insulin resistance to a greater extent than insulin-mediated glucose uptake in muscles. Many studies reported that both oral and transdermal estrogen therapies suppress hepatic glucose production (23,56). It is possible that the association between HRT and glucose metabolism is indirect through alterations of levels of growth hormone or catecholamines (56–59).



In this study, proinsulin was associated with HRT, independent of glucose and insulin and other established cardiovascular risk factors. One explanation for this finding was that proinsulin reflects insulin resistance more than intact insulin. Hyperproinsulinemia may be a sign of a pancreatic  $\beta$ -cell defect, augmented by an increased demand placed on the  $\beta$ -cell by hyperglycemia (60). It has also been suggested that hyperproinsulinemia is the result of secretion of immature proinsulin-rich granules from  $\beta$ -cells in response to an increased demand for insulin (61). Insulin has a considerably shorter half-life than proinsulin (62). Oscillations of insulin secretion (63) might contribute to more variation in insulin levels than observed for proinsulin, such that proinsulin could reflect insulin resistance and pre-diabetes more accurately than insulin.

Proinsulin is present in low concentrations except in diabetic subjects (64). The activity of proinsulin, either in vitro or in vivo, is only ~10% of the biological activity of insulin, so low concentrations of proinsulin are unlikely to have a significant biological, insulin-like effect. However, several reports suggest the possibility of direct proinsulin action on atherosclerosis: Proinsulin has been shown to increase PAI-1 in in vitro models (65). Adjustment for PAI-1 levels markedly attenuated the association between proinsulin and carotid wall thickness in the Insulin Resistance Atherosclerosis Study (66), suggesting a role for PAI-1 in mediating this association. Increased proinsulin levels have been associated with the sympathovagal balance of the automatic nervous function in both people with type 2 diabetes and in control subjects (66). Recently, in an 11-year follow-up of the Hoorn Study (67), fasting proinsulin was associated with all-cause and CHD mortality, independent of glucose tolerance status and insulin resistance and largely independent of other CHD risk factors. Given these findings, proinsulin may play a role in the relationship between insulin resistance and CVD through actions on a putative proinsulin receptor (68) or through other biological actions that are not yet known.

The present study has some limitations. First, these findings are limited to older Caucasian women. Second, the cross-sectional study design precludes assumption of causality.

In summary, decreased fasting plasma glucose and proinsulin were each significantly associated with current HRT

use. However, HRT was also associated with increased postchallenge glucose, which is a stronger cardiovascular risk factor than fasting glucose (69). Further study of proinsulin as a potential biological link between insulin resistance and CHD is needed.

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