

Moderate Alcohol Consumption, Estrogen Replacement Therapy, and Physical Activity Are Associated With Increased Insulin Sensitivity

Is abdominal adiposity the mediator?

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OBJECTIVE — To investigate 1) associations between environmental factors (alcohol consumption, hormone replacement therapy [HRT], and physical activity) and insulin resistance and secretion, independent of genetic influences; 2) the contribution of abdominal adiposity to these relationships; and 3) whether gene-environment interactions mediate these associations.

RESEARCH DESIGN AND METHODS — Reported effects of lifestyle factors on insulin resistance and secretion are inconsistent, possibly due to difficulty in dissecting environmental from genetic influences and to confounding by adiposity. We examined these relationships in 798 nondiabetic female twins. Insulin resistance and secretion were estimated by modified homeostasis model assessment (HOMA-R' and HOMA-β', respectively). Percent total body fat and percent central abdominal fat (CAF) were measured by dual-energy X-ray absorptiometry.

RESULTS — All categories of alcohol consumption were associated with lower insulin levels and HOMA-β' than abstinence. Only moderate alcohol consumers (11–20 units/week) had lower HOMA-R' than abstainers (-0.16 ± 0.09 vs. 0.14 ± 0.13 SD, $P = 0.048$). This difference was attenuated after controlling for percent CAF ($P = 0.57$), which was lower in moderate drinkers. Controlling for genetic and smoking effects in cotwin case-control analysis, monozygotic pairs discordant for alcohol consumption had greater within-pair differences in HOMA-R' than concordant pairs ($P = 0.02$). Postmenopausal women using estrogen-only HRT had lower HOMA-R' than non-HRT users (-0.33 ± 0.16 vs. 0.17 ± 0.08 SD, $P = 0.003$), even after controlling for percent CAF. Lower fasting glucose levels and insulin resistance and secretion indexes in physically active subjects were partly explained by lower abdominal adiposity.

CONCLUSIONS — Moderate alcohol consumption, estrogen replacement, and physical activity are associated with increased insulin sensitivity in female twins. The favorable effects of moderate alcohol consumption and physical activity on insulin sensitivity are partly mediated by lower abdominal adiposity.

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Abbreviations: CAF, central abdominal fat; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA, homeostasis model assessment; HRT, hormone replacement therapy; WHR, waist-to-hip ratio.

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

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Insulin resistance is a pivotal component of the metabolic syndrome, and its presence predicts type 2 diabetes and atherosclerotic cardiovascular disease (1). Recent evidence suggests that light to moderate alcohol consumers have a lower risk of developing type 2 diabetes (2,3) and coronary artery disease (4,5) than abstainers. Although increased levels of HDL cholesterol may contribute to this association (5), improvements in other metabolic syndrome parameters, such as insulin resistance and body fat distribution, may also contribute. In support of this hypothesis, the inverse association between alcohol intake and diabetes risk in the Nurses' Health Study was attenuated after adjustment for adiposity, albeit estimated by BMI (2). In addition, a recent report from the Insulin Resistance and Atherosclerosis Study demonstrated that the protective association between moderate alcohol consumption and atherosclerosis was attenuated by 25% after adjustment for insulin action and by 60% after further adjustment for other covariates, including BMI, waist-to-hip ratio (WHR), and lipids (6).

Although a number of studies suggest that moderate alcohol consumption is associated with improved insulin sensitivity, there is some variation in their findings, which are dependent, at least in part, on the method used to estimate insulin action. The "gold standard" technique, the hyperinsulinemic-euglycemic clamp (7), is impractical in large studies, and various surrogate indexes have been developed (8–16). Inconsistency may also relate to the inability of standard epidemiological studies to control for genetic factors, which explain up to 60% of the population variance in insulin resistance in women (17), and to examine for interactions between genetic risk and environmental exposure. Twin studies provide a unique model for examining relationships

between environmental factors and metabolic syndrome parameters, independent of genetic effects, and permit the detection of gene-environment interactions.

Although a recent randomized study found that use of hormone replacement therapy (HRT) reduced incident diabetes by 35% in postmenopausal women with coronary disease (18), the reported effect of HRT on possible mediators of this reduction, including insulin resistance, is inconsistent (19–25). In addition to variability in HRT regimens and doses, route of HRT administration, study populations, insulin resistance measures and the extent of covariate adjustment, confounding by genetic factors, and gene-environment interactions may also contribute to this inconsistency. Although the beneficial effect of other environmental factors, such as physical activity, on insulin resistance is well recognized (26,27), the degree to which genetic influences mediate this association, and the contribution of closely related metabolic phenotypes such as abdominal adiposity, the population variance of which is also largely heritable (28), remain controversial (27).

Although insulin resistance is the core component of the metabolic syndrome, progression to impaired glucose tolerance and type 2 diabetes depends on impaired insulin secretion. We have recently shown that the progression of glucose intolerance in normoglycemic first-degree relatives of individuals with type 2 diabetes is related to the rate of decline in insulin secretion and that this deterioration is predicted by central adiposity (29). Therefore, in a large cohort of healthy female twins, we 1) quantified the effect of alcohol consumption, HRT, and physical activity on validated measures of both insulin secretion and resistance independent of genetic and other environmental confounders; 2) examined the contribution of intermediary variables, such as directly measured abdominal obesity, to these relationships; and 3) investigated whether gene-environment interactions modulate these associations.

RESEARCH DESIGN AND METHODS

Cohort characteristics and lifestyle variables

Subjects were healthy female twins, recruited via the media through St. Thomas'

Twin Research and Genetic Epidemiology Unit, St. Thomas' Hospital, London, U.K. The study was approved by Research and Ethics Committees at St. Thomas' Hospital, London, U.K., and St. Vincent's Hospital, Sydney, Australia. All participants provided written informed consent. For the current analysis, 798 twins were studied (114 monozygotic and 285 dizygotic pairs). Twin pairs were excluded if either twin had a personal history of coronary disease, diabetes, or gestational diabetes or was taking antihypertensive or lipid-lowering medications. All subjects included in the study had fasting plasma glucose (FPG) levels <6.1 mmol/l. In 498 subjects who had undergone glucose tolerance tests, 2-h postload glucose levels were <7.8 mmol/l.

Zygoty was derived by questionnaire and confirmed with multiplex DNA fingerprinting (PE Applied Biosystems, Foster City, CA) if results were uncertain. Menopause was defined as amenorrhoea ≥ 12 months in subjects ≥ 40 years of age or previous hysterectomy and bilateral salpingo-oophorectomy. Subjects reported average weekly alcohol intake as never, social, or 1–5, 6–10, 11–15, 16–20, 21–40, or >40 units/week, and were grouped as abstainers, "light" (≤ 10 units/week), "moderate" (11–20 units/week), or "heavy" (>20 units/week) alcohol consumers for analysis (1 unit = 8 g of alcohol). Menopausal status, HRT use, and smoking were recorded using standardized questionnaires. Participation in and duration of leisure-time physical activity were ascertained by questionnaire, as described previously (30,31).

Body composition

BMI (kg/m^2) was calculated from weight and height, measured in light clothing (nearest 0.1 kg and 0.01 m, respectively). WHR was expressed as waist (narrowest circumference between lower rib margin and anterior superior iliac spines) divided by hip circumference (widest circumference between anterior superior iliac spines and greater trochanters). In 696 subjects, dual-energy X-ray absorptiometry (Lunar DPXL, Madison, WI) was used to measure total body fat and percent central abdominal fat (CAF). CAF was demarcated by a single observer as the adipose tissue contained in a window formed by the upper border of the second and lower border of the fourth lumbar vertebral bodies and inner rib margin as pre-

viously described (28,31,32). Percent CAF was defined as the amount of fat in this window expressed as a percentage of its total soft tissue content.

Biochemical analysis and glycemic indexes

Twins had venous blood drawn after a minimum 8-h overnight fast. FPG was assayed using a glucose-oxidase method and fasting plasma insulin (FPI) by radioimmunoassay (Immunodiagnosics Enzum test; Boehringer Mannheim, Mannheim, Germany). Estimates of insulin resistance (R) and insulin secretion (β) were based on a modified homeostasis model assessment (HOMA). As previously described (33), minor modifications were made to original HOMA indexes (34) to reduce misclassification errors arising from the confounding of one index by the variability of the other. Modified indexes (HOMA') were defined as follows: $\text{HOMA-R}' = (\ln\text{FPI} - c) \times \text{FPG}$, and $\text{HOMA-}\beta' = (\ln\text{FPI} - c)/\text{FPG}$, where c is the intercept on the $\ln\text{FPI}$ axis, derived from its regression with FPG using the diagonally weighted least squares method (33). Although we have previously shown that the original and modified HOMA indexes correlate similarly with direct measures of insulin resistance and secretion, in contrast to the strong relationship between the original HOMA-R and HOMA- β estimates ($r = 0.78$, $P < 0.0001$), which may lead to misclassification, the relationship between HOMA-R' and HOMA- β' is weak ($r = 0.13$) and not different from that between direct measures of insulin resistance (by hyperinsulinemic-euglycemic clamp) and insulin secretion (by frequently sampled intravenous glucose tolerance testing) (33). The ratio of within-subject variances for $\ln\text{FPI}$ and FPG required by this procedure was obtained from repeated measures ($n = 6$) in 24 female subjects (35). HOMA estimates are expressed in units of the SD of the sample.

Statistical analysis

Descriptive data are reported as means \pm SD or medians (interquartile range). When analyzing the effect of categorical variables, data are expressed as means \pm SE or means (95% CI). Nonnormally distributed data (FPI and duration of physical activity) was \ln -transformed in analyses and back-transformed for presentation. The effect of age on FPG,

Table 1—Anthropometry, body composition, and glycemic parameters in healthy female twins

Age (years)	44.6 ± 12.6
Weight (kg)	66.1 ± 12.3
BMI (kg/m ²)	25.1 ± 4.5
Waist (cm)	78 ± 10
WHR	0.8 ± 0.1
Total body fat (kg)	22.2 ± 8.4
Total body fat (%)	33.6 ± 7.3
Central abdominal fat (kg)	1.2 ± 0.7
Central abdominal fat (%)	28.6 ± 10.4
Fasting plasma glucose (mmol/l)	4.4 ± 0.4
Fasting plasma insulin (mIU/l)	5.8 (4.1–8.2)
HOMA-β' (SD)*	0 ± 1
HOMA-R' (SD)*	0 ± 1

Data are means ± SD or medians (interquartile range). *Mean = 0 and SD = 1 as a consequence of expressing the data in units of the SD of the sample.

HOMA-β', and HOMA-R' was accounted for by including age as a covariate in ANCOVAs. Because standard statistical techniques may underestimate SE and overestimate significance when dealing with paired data, the generalized estimating equation was applied to account for within-pair phenotypic correlations (36). $P < 0.05$ was considered significant. Data were analyzed by Statview 5 (SAS Institute, Cary, NC) and Stata Statistical Software, release 7.0 (StataCorp, College Station, TX).

As previously described (31), cotwin case-control (monozygotic twin) analysis was used to quantify the impact of environmental factors on glycemic parameters, independent of genetic influences. Because monozygotic twins share 100% of their genes, within-pair differences in phenotype must be due to environmental factors for which the twins are discordant. Discordance for alcohol consumption was assigned when intakes within a twin pair fell into different alcohol consumption categories. For HRT and physical activity, discordance was assigned when one twin of a pair used HRT or participated in regular leisure-time physical activity, respectively, and the other did not. Within-pair differences in glycemic parameters were compared between discordant and concordant pairs by ANOVA.

Using methods described previously (31), gene-environment interaction an-

Table 2—Age-adjusted glycemic parameters according to alcohol consumption category and HRT use (latter in postmenopausal women)

	n	FPG (mmol/l)	FPI (mIU/l)	HOMA-β' (SD)	HOMA-R' (SD)
Alcohol consumption category					
Abstainers	60	4.3 ± 0.0	7.0 (6.2–7.9)	0.26 ± 0.12	0.14 ± 0.13
1–10 units/week	574	4.4 ± 0.0	5.9 (5.7–6.2)*	0.00 ± 0.05*	0.02 ± 0.05
11–20 units/week	118	4.3 ± 0.0	5.6 (5.1–6.1)†	−0.04 ± 0.09*	−0.16 ± 0.09*
>20 units/week	41	4.4 ± 0.1	5.4 (4.5–6.4)*	−0.28 ± 0.14‡	0.03 ± 0.15
HRT use					
Users	103	4.4 ± 0.0	5.5 (5.0–6.0)	−0.07 ± 0.10	−0.08 ± 0.10
Nonusers	204	4.5 ± 0.0*	6.0 (5.6–6.5)	−0.17 ± 0.07	0.16 ± 0.08*

Data are means ± SE or means (95% CI). Compared with abstainers or HRT users: * $P < 0.05$; † $P < 0.01$; ‡ $P < 0.005$ (using the generalized estimating equation). For alcohol consumption, five subjects with unrecorded alcohol data were excluded, and for HRT use, three subjects with unrecorded hormone therapy status were excluded.

alysis was used to assess whether associations between environmental factors and glycemic indexes were dependent or independent of genetic risk. For analyses involving alcohol consumption, 420 twins (73 monozygotic and 137 dizygotic twin pairs) concordant for HRT and smoking were grouped into tertiles of age-adjusted glycemic parameters. Because glycemic parameters are known to be highly heritable (17), a randomly selected twin from each pair was assigned a genetic risk category for each glycemic parameter based on the glycemic tertile of her cotwin. This subgroup of randomly selected twins was also divided into tertiles of alcohol consumption. Highest and lowest genetic risk categories and alcohol consumption tertiles were entered into a two-factor ANOVA to assess interactive effects of genetic risk and alcohol intake on glycemic parameters. A gene-environment interaction was present if the relationship between alcohol consumption and glycemic parameters was significantly different in the highest and lowest genetic risk categories. The analysis was repeated for physical activity and HRT use (the latter in 178 postmenopausal twins concordant for smoking and alcohol consumption).

RESULTS— Mean age was 44.6 ± 12.6 years (range 18.4–72); 310 subjects (39%) were postmenopausal. Of the subjects, 24% were smokers (mean duration 24.4 ± 11.5 years) and 23% were ex-smokers (mean time since quitting 16.1 ± 9.4 years). FPG ($r = 0.24$, $P < 0.001$) and HOMA-R' ($r = 0.11$, $P = 0.01$) increased with age, and HOMA-β'

($r = -0.26$, $P < 0.001$) decreased with age. In contrast, FPI was weakly and non-significantly related to age ($r = -0.07$, $P = 0.08$). Cohort characteristics are shown in Table 1.

Alcohol consumption

Sixty subjects abstained from alcohol. The majority of the cohort ($n = 574$) was in the light drinker category (≤ 10 units/week). Of the subjects, 118 drank moderate amounts of alcohol (11–20 units/week). Only 41 subjects reported drinking >20 units/week (heavy drinkers). Smoking prevalence increased with increasing alcohol consumption category: abstainers, 20%; light drinkers, 21%; moderate drinkers, 31%; and heavy drinkers, 41% ($P = 0.003$). The prevalence of regular physical activity was unrelated to the alcohol consumption category (not shown).

FPG was similar across alcohol consumption categories (Table 2). However, compared with abstainers, FPI and HOMA-β' were lower in subjects who consumed alcohol, with evidence of a dose-dependent effect ($P = 0.03$ and $P < 0.001$ for trend, respectively) (Table 2 and Fig. 1). Results were similar after controlling for smoking (not shown). Differences in FPI between abstainers and heavy ($P = 0.04$), but not light ($P = 0.31$) or moderate ($P = 0.19$), alcohol consumers remained significant when percent CAF was included as a covariate. Differences in HOMA-β' were similar after controlling for percent CAF (not shown).

Moderate alcohol consumers (11–20 units/week) had lower HOMA-R' than

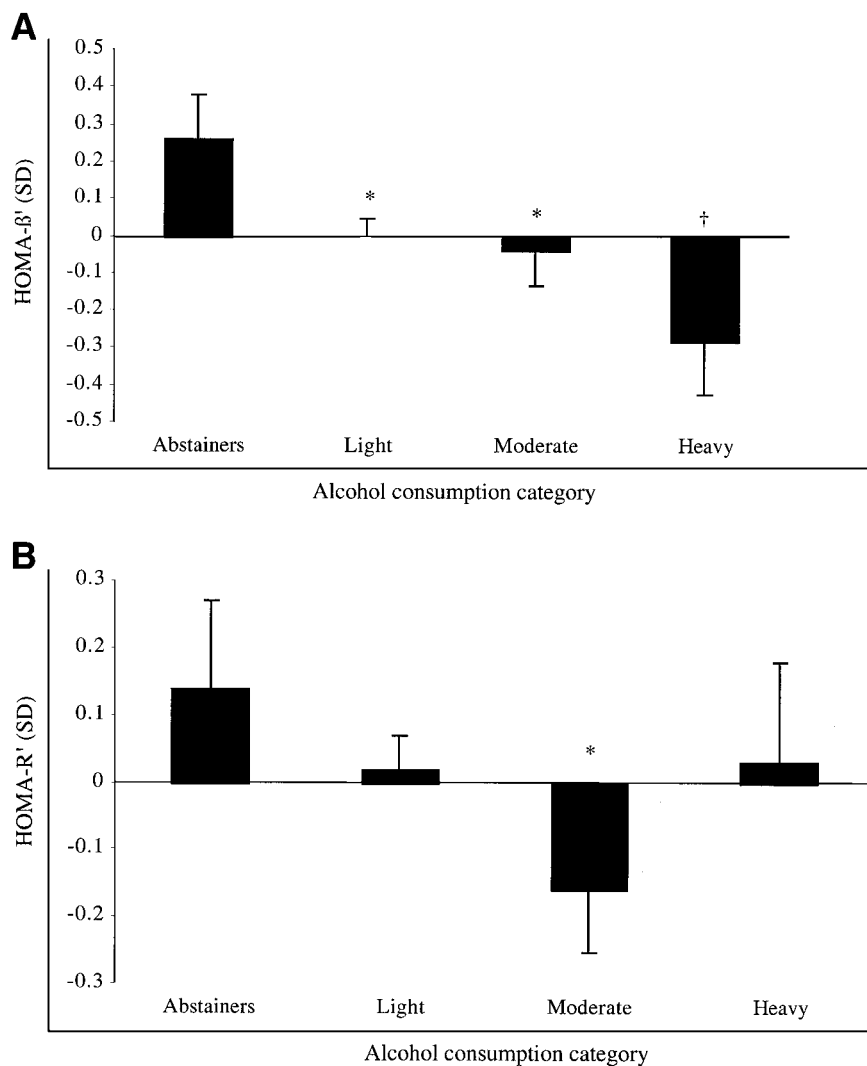


Figure 1—Relationship between alcohol consumption category and age-adjusted HOMA-β' and HOMA-R' in healthy female twins. Data are means ± SE. * $P < 0.05$, † $P < 0.005$ vs. abstainers (using the generalized estimating equation). Alcohol consumption categories: light, ≤ 10 units/week; moderate, 11–20 units/week; and heavy, > 20 units/week.

abstainers after adjusting for age ($P = 0.048$) (Table 2), resulting in a U-shaped relationship between alcohol consumption and HOMA-R' (Fig. 1). The difference between moderate drinkers and abstainers was similar after controlling for smoking and physical activity (not shown).

Compared with abstainers, subjects with moderate alcohol intakes had lower percent CAF (31.6 ± 1.5 vs. $26.4 \pm 0.9\%$, $P = 0.002$ and $P = 0.11$, before and after generalized estimating equation, respectively). Because abstainers had similar percent CAF to subjects drinking ≤ 10 or > 20 units/week (percent CAF: 28.8 ± 0.5 and $29.6 \pm 1.5\%$, respectively), the

association between alcohol intake and central adiposity was U-shaped, similar to that between alcohol intake and HOMA-R'. Adjusting for percent CAF attenuated the difference in HOMA-R' between moderate drinkers and abstainers ($P = 0.57$).

Cotwin case-control analysis was performed in 111 monozygotic twin pairs to examine if associations between alcohol consumption and metabolic indexes were independent of genetic influences. Discordance for alcohol intake ($n = 29$ pairs) was associated with greater within-pair differences in HOMA-R' than concordance (1.06 ± 0.13 vs. 0.66 ± 0.06 SD, $P = 0.001$). Within discordant twin pairs,

twins with higher alcohol intakes had lower HOMA-R' than twins with lower intakes (-0.02 ± 0.19 vs. 0.20 ± 0.18 SD). The difference between concordant and discordant pairs remained significant ($P = 0.02$) when pairs discordant for smoking ($n = 17$) were excluded. Although twin pairs discordant for alcohol consumption had greater within-pair differences in FPI than concordant pairs, this was not significant after excluding smoking-discordant pairs (not shown). Within-pair differences in HOMA-β' were similar in concordant and discordant twin pairs (not shown). No gene-environment interactions between alcohol intake and glycemic parameters were found.

Hormone replacement therapy

Of the postmenopausal subjects, 103 (34%) used HRT. HRT users were more likely to drink > 10 units/week than non-HRT users (25 vs. 14%, $P = 0.02$). The prevalence of smoking and leisure-time physical activity was similar in HRT users and nonusers. HRT users had lower FPG and HOMA-R' than postmenopausal nonusers (Table 2). Differences remained significant when alcohol consumption and percent CAF were included as covariates (FPG: $P = 0.02$; HOMA-R': $P = 0.04$).

To further investigate the influence of HRT on metabolic parameters, HRT users were categorized by type of hormone preparation. Of the subjects, 42 reported using combined estrogen and progestin, and 40 used estrogen alone (18 unrecorded; 3 using estrogen and progestin plus androgen excluded). FPG and HOMA-β' were similar in estrogen-only and estrogen plus progestin users (not shown). However, estrogen-only users were less insulin resistant than estrogen plus progestin users (HOMA-R': -0.33 ± 0.16 vs. 0.15 ± 0.15 SD, $P = 0.02$). Accordingly, only estrogen-only users were less insulin resistant than non-HRT users ($P = 0.003$), which remained significant when percent CAF was included as a covariate. Although estrogen-only users had lower FPI than estrogen plus progestin users ($P = 0.02$), this was attenuated when HOMA-R' was included as a covariate.

Of the 103 HRT users, 76 used oral preparations and 18 used transdermal preparations (in nine subjects, the route of administration was unknown). There were no significant differences between

oral and transdermal HRT users in glycemic parameters, although this may be limited by the small number of transdermal HRT users.

In cotwin case-control analysis, a comparison of within-pair differences in glycemic parameters between postmenopausal twin pairs concordant and discordant for HRT use did not reveal any significant differences (not shown). In gene-environment interaction analysis in postmenopausal twin pairs, all relationships were independent of genetic risk (no gene-environment interactions). Because a previous study found that HRT was associated with improved insulin sensitivity in subjects with high amounts of abdominal fat only (37), we also performed a gene-environment interaction analysis examining the association between HRT and HOMA-R' in subjects at low and high genetic risk of central adiposity. However, HRT was associated with lower HOMA-R' in both genetic risk groups, indicating that the effect of HRT on HOMA-R' was not modulated or abrogated by genetic risk or the degree of central abdominal adiposity.

Physical activity

The 331 subjects who participated in regular physical activity had lower FPI (5.5 [95% CI 5.2–5.9] vs. 6.2 [6.0–6.5] mIU/L, $P = 0.002$), HOMA- β' (-0.07 ± 0.06 vs. 0.06 ± 0.05 SD, $P = 0.04$), and HOMA-R' (-0.09 ± 0.06 vs. 0.07 ± 0.05 SD, $P = 0.02$) than nonparticipants. Among physically active subjects, duration of activity was unrelated to glycemic parameters. Differences in FPI, HOMA- β' , and HOMA-R' were attenuated ($P > 0.14$) when percent CAF, which was significantly lower in physically active subjects (26.9 ± 0.5 vs. $29.9 \pm 0.6\%$, $P < 0.001$), was included as a covariate. No significant differences in glycemic parameters were found between twin pairs concordant or discordant for physical activity (not shown). No interactions between physical activity and genetic risk of glycemic parameters were found.

CONCLUSIONS— A major finding of this study was that, even in healthy nondiabetic women, regular moderate alcohol consumption was associated with increased insulin sensitivity and lower abdominal adiposity than abstinence. In monozygotic twin pairs concordant for smoking, within-pair differences in

HOMA-R' were greater in pairs discordant for alcohol intake, indicating that alcohol-associated differences in insulin resistance were independent of genetic and other closely related environmental effects.

Early studies found that large amounts of alcohol acutely impair insulin sensitivity (38). In contrast, recent epidemiological studies associate regular moderate alcohol consumption with improved insulin sensitivity (8–16) and have found direct relationships between alcohol intake and insulin-mediated glucose uptake, measured by the hyperinsulinemic-euglycemic clamp (39). These observations are supported by some, but not all (40,41), intervention studies, which have found that insulin sensitivity improves after regular moderate alcohol consumption (42) and during acute alcohol ingestion (43). The findings of a recent study (44), which showed that a reduction in alcohol intake from 72 to 8 g/day did not improve insulin sensitivity, are consistent with our finding that heavy drinkers had similar HOMA-R' to light drinkers and nondrinkers. By studying twins, the current analysis extends previous reports by examining associations between alcohol intake and metabolic parameters after controlling for important genetic, environmental, and body composition confounders.

Although the biological mechanisms by which moderate alcohol consumption improves insulin sensitivity have not been fully elucidated, there is evidence to suggest that acetate, the main metabolite of alcohol oxidation, reduces fatty acid release from adipose tissue and inhibits uptake of circulating fatty acids by muscle (45). This reduction in systemic fatty acid availability would be expected to enhance glucose oxidation and insulin sensitivity by reducing substrate competition (43,46). Our study extends this evidence because we found that abdominal obesity, a major source of circulating fatty acids (46), was lower in moderate alcohol consumers than abstainers and that the latter partly accounted for the observation of lower insulin resistance in this group of moderate drinkers. Although a number of previous reports have suggested that enhanced insulin action associated with moderate alcohol consumption is independent of adiposity, these studies have mainly relied on BMI (10,11,14,42,47), a less accurate measure of adiposity. A ma-

ior strength of the current study was the direct measurement of abdominal fat, a stronger predictor of insulin sensitivity than total adiposity (32). Our results support a recent report that showed that adjusting for waist circumference, in addition to BMI, attenuated the U-shaped relationship between alcohol intake and insulin sensitivity (13), although this is in conflict with another report using WHR (9). Taken together, the current and previous studies suggest that alcohol improves insulin sensitivity both acutely, via direct effects on fatty acid uptake in muscle, and over the longer term, via reductions in central abdominal fat and, accordingly, fatty acid availability.

The finding that HRT users had lower FPG and were less insulin resistant than nonusers may explain, in part, the results of a recent study, which found that postmenopausal women with coronary heart disease randomized to HRT had a 35% lower risk of developing diabetes than placebo-treated women (18). We have previously shown that estrogen and progestin may have differential effects on glucose metabolism (19). The current study corroborates these observations because we found that estrogen-only HRT users were less insulin resistant than women who used a concomitant progestin. Although controversial (21,48), but consistent with our findings, a number of studies have found that physiological doses of oral estrogen replacement improve insulin sensitivity (19–22), whereas others suggest that higher doses (21–24), or the addition of a progestin (19–22,24,25), may negate or reverse this benefit. Some of these inconsistencies may also relate to differences in experimental design, study populations, duration of therapy, dose, type and route of hormone replacement, small sample sizes, incomplete adjustment for covariates and variability in the methods used to measure insulin resistance, and other phenotypic and lifestyle characteristics.

An interesting observation from this study was that lower abdominal adiposity partly explained the lower fasting glucose levels and insulin secretion and resistance indexes in physically active subjects. The finding of lower abdominal adiposity in physically active subjects is consistent with previous observations (26,31) and supports the suggestion that body fat distribution plays a major role in mediating

the beneficial metabolic changes associated with physical activity (27).

The strengths of the current examination relate to our use of a large cohort of pre- and postmenopausal women free of diabetes and heart disease, who were carefully phenotyped with accurate and validated indexes of insulin secretion, insulin resistance, and direct measures of body fat distribution. The finding that environmental factors had divergent effects on HOMA-R' and HOMA- β ' highlights the independence of these modified indexes compared with original HOMA estimates (33). Another significant strength is the use of the twin model to examine these relationships independent of genetic effects—a major confounding factor that cannot be controlled for in standard epidemiological studies. We were also able to examine for gene-environment interactions. Possible limitations should also be considered. The results of the current study may not be applicable to other populations or to men. Given the cross-sectional design of the study, causality cannot be inferred. Because alcohol intake was based on self-reported data, underreporting and reporting biases cannot be excluded. Because of the relatively small number of heavy drinkers in the current study, we cannot comment on the relationship between heavy alcohol consumption and metabolic parameters. Consideration should also be given to selection bias, particularly with regard to HRT use, because women who take HRT tend to have healthier lifestyles than those who do not (49).

In summary, in this large study of healthy pre- and postmenopausal female twins, using validated indexes of insulin secretion and sensitivity and direct measures of body fat, we examined relationships between common environmental factors and glucose metabolism. Moderate alcohol consumption, estrogen replacement therapy, and regular leisure-time physical activity were associated with lower insulin resistance. Although independent of genetic and smoking effects, the favorable relationship between alcohol intake and insulin resistance was partly mediated by abdominal obesity, which was lower in subjects with alcohol intakes in the moderate range. Lower abdominal adiposity also partly explained the favorable association between physical activity and glycemic parameters. Our findings clarify the relationship between

common environmental and metabolic factors and highlight the importance of body fat distribution in mediating these effects.

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