Evidence of a Causal Relationship Between Adiponectin Levels and Insulin Sensitivity

A Mendelian Randomization Study

He Gao,[1,2,3] Tove Fall,[1] Rob M. van Dam,[2,3] Allan Flyvbjerg,[4] Björn Zethelius,[5] Erik Ingelsson,[1] and Sara Hägg[1]

The adipocyte-secreted protein adiponectin is associated with insulin sensitivity in observational studies. We aimed to evaluate whether this relationship is causal using a Mendelian randomization approach. In a sample of Swedish men aged 71 years (n = 942) from the Uppsala Longitudinal Study of Adult Men (ULSAM), insulin sensitivity (*M/I* ratio) was measured by the euglycemic insulin clamp. We used three genetic variants in the *ADIPOQ* locus as instrumental variables (IVs) to estimate the potential causal effect of adiponectin on insulin sensitivity and compared these with results from conventional linear regression. The three *ADIPOQ* variants, rs17300539, rs3774261, and rs6444175, were strongly associated with serum adiponectin levels (all $P \leq 5.3 \times 10^{-9}$) and were also significantly associated with *M/I* ratio in the expected direction (all $P \leq 0.022$). IV analysis confirmed that genetically determined adiponectin increased insulin sensitivity (β = 0.47–0.81, all $P \leq 0.014$) comparable with observational estimates (β = 0.50, all $P_{\text{difference}} \geq 0.136$). Adjustment for BMI and waist circumference partly explained the association of both genetically determined and observed adiponectin levels with insulin sensitivity. The observed association between higher adiponectin levels and increased insulin sensitivity is likely to represent a causal relationship partly mediated by reduced adiposity. ***Diabetes*** **62:1338–1344, 2013**

Adiponectin is the most abundantly secreted protein by white adipose tissue. Unlike most other adipokines, it has a well-known inverse relationship with adiposity and risk of type 2 diabetes (1–3). Insulin resistance is a major link between excessive adiposity and the development of type 2 diabetes, and murine studies have demonstrated that adiponectin exerts an insulin-sensitizing effect (4–6). Although adiponectin is also inversely associated with insulin resistance in human populations (7–9), it remains unclear whether this represents a causal relationship (10).

Mendelian randomization is a method that uses genetic variants [instrumental variables (IVs)] as robust proxies for an environmentally modifiable exposure to assess and quantify potential causal relationships with health outcomes (11,12). Because genotypes that influence adiponectin levels are assigned at conception, they are unlikely to be related to confounders such as lifestyle, socioeconomic and environmental risk factors, or reverse causation. Given sufficient power and carefully selected genetic variants that have robust association with adiponectin levels, a Mendelian randomization study has the potential to help disentangle adiponectin as cause or consequence of insulin sensitivity. Thus far, there is to our knowledge no such existing study.

Genome-wide linkage scan and association studies have revealed a few loci that affect the circulating level of adiponectin (13). Among them, *ADIPOQ*, which encodes for the adiponectin protein, is by far the most established locus, with many studies demonstrating its robust and strong association with adiponectin levels (7,14–16). With no known pleiotropic effect, *ADIPOQ* variants presumably fulfill the key assumptions of a Mendelian randomization design (Fig. 1), and therefore they are good candidate instruments for a Mendelian randomization study with adiponectin as the exposure variable.

The aim of our study was to use single nucleotide polymorphisms (SNPs) in *ADIPOQ* that are reliably associated with serum adiponectin levels as IVs to elucidate the potential causal effect of adiponectin on insulin sensitivity measured by euglycemic insulin clamp in nondiabetic men in a population-based cohort.

RESEARCH DESIGN AND METHODS

Study sample. The Uppsala Longitudinal Study of Adult Men (ULSAM) was initiated between September 1970 and September 1973 with an invitation to all men aged 50 years living in Uppsala County, Sweden. The sample used for this study comes from the third investigation, during 1991–1995, when the subjects were aged ~71 years. A total of 1,221 men (73% of those invited, i.e., men still alive and residing in Uppsala County) participated, and the examination included a medical questionnaire, blood pressure and anthropometric measurements, collection of blood samples, a 75-g oral glucose tolerance test, and insulin sensitivity measurements. Detailed information can be found at the cohort website (http://www.pubcare.uu.se/ulsam/).

Of the 1,221 participants, 199 were excluded for the following reasons: unavailable clamp data (n = 61), unavailable measurement of adiponectin (n = 15), or presence of type 2 diabetes [defined as fasting glucose >7.0 mmol/L (>126 mg/dL) or use of antidiabetes medication (n = 123)]. Of the remaining 1,022 participants, genotypes were available for 943 participants, and after exclusion of 1 person with missing genotypes in >1 of the 16 *ADIPOQ* SNPs, 942 participants were eligible for the final analysis. The study was approved by the ethics committee of Uppsala University, and all participants provided written informed consent.

From the [1]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; the [2]Saw Swee Hock School of Public Health, National University of Singapore, Singapore; the [3]National University of Singapore Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore; the [4]Medical Research Laboratories, Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark; and the [5]Department of Public Health and Caring Sciences/Geriatrics, Uppsala University, Uppsala and Medical Products Agency, Uppsala, Sweden.

Corresponding author: Erik Ingelsson, erik.ingelsson@ki.se.

Received 13 July 2012 and accepted 27 October 2012.

DOI: 10.2337/db12-0935

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db12-0935/-/DC1.

E.I. and S.H. contributed equally to this study.

See accompanying commentary, p. 1007.

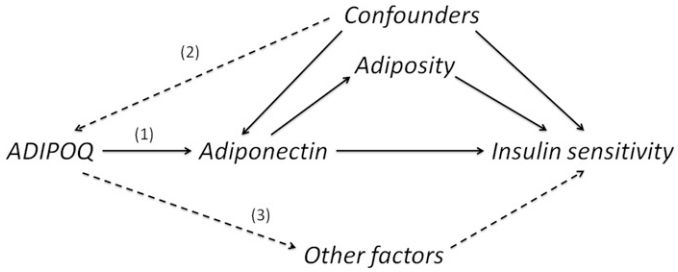

FIG. 1. Hypothetical model for the relationship between *ADIPOQ*, adiponectin, and insulin sensitivity. This paradigm illustrates the key relationships in the study of the influence of adiponectin on insulin sensitivity using a Mendelian randomization design. Solid arrows represent causal influences that are expected, and dotted arrows are causal influences that are assumed not to exist. Genetic variants from the *ADIPOQ* gene are used as IVs based on three main assumptions: *1*) The IVs are robustly associated with the exposure of interest, i.e., adiponectin levels; *2*) the IVs are independent of confounders for the association between adiponectin and insulin sensitivity in observational studies; and *3*) the IVs are independent of the outcome insulin sensitivity given adiponectin levels and the confounders. Moreover, we hypothesize that part of the effect of adiponectin on insulin sensitivity is mediated by adiposity, which is represented by BMI and waist circumference.

Adiponectin and insulin sensitivity measurements. Serum adiponectin was measured in plasma samples frozen at −70°C for 11 ± 2 years without previous thaw-freeze cycles and using a validated in-house time-resolved immunofluorometric assay with reagents from R&D Systems (Abingdon, U.K.). The intra- and interassay coefficient of variation averaged <5 and 10%, respectively, as previously described in detail (17).

In vivo sensitivity to insulin was determined by the euglycemic insulin clamp, according to the procedure described by DeFronzo et al. (1979) (18), but with a higher insulin infusion rate per body surface area to better suppress liver glucose output [56 instead of 40 mU · min^{-1} $(m^2)^{-1}$]. After a primary dose in the initial 10 min, continuous infusion of insulin lasted for 110 min and hepatic glucose production was assumed to be entirely suppressed. Glucose disposal (*M*) was calculated as the total amount of glucose infused during the last 60 min (the steady state) of the clamp divided by kilograms body weight and minutes. The insulin sensitivity index (*M/I* ratio) was derived by dividing *M* by the steady-state mean insulin concentration (*I*). *M/I* thus represents the amount of glucose metabolized per unit of plasma insulin and was given as 100 × mg · kg^{-1} · min^{-1} · mU^{-1} · L.

Genotypes and IVs. The ULSAM cohort has undergone prior genotyping on the Human CardioMetabo beadchip (19), which is designed to interrogate 200,000 markers of interest for cardiovascular and metabolic diseases. Of the 1,221 individuals who underwent genotyping, we removed those with a genotyping call rate <0.99 (n = 5), failing sex check (n = 1), close relatedness (n = 36), or large heterozygosity (n = 7). Quality control also ensured that all SNPs had good call rate (>0.99) and did not deviate from Hardy-Weinberg equilibrium ($P > 1 \times 10^{-6}$). In total, genotypes of 183,357 SNPs were available for 1,175 individuals, of whom 942 were eligible for the current study (see above).

We searched the genomic region ±50 kb of the *ADIPOQ* gene based on Human Genome build 36 and identified 16 SNPs that were present on the Metabochip. Their associations with serum adiponectin in the ULSAM cohort were examined by linear regression under an additive genetic model adjusting for age. Pairwise linkage disequilibriums (LDs) were then assessed with information from the HapMap2 release 22 CEU panel, and SNPs in moderate LD ($r^2 > 0.5$) were assigned to the same LD block. Of the SNPs that were significantly associated with adiponectin, we selected rs17300539 and rs3774261, which were the top associated SNPs in their respective LD block as IVs. Since rs6444175 only showed intermediate LD (r^2 = 0.506) with rs3774261, this variant was evaluated as an instrument in separate analyses (but not in the genotype score). We excluded rs864265 (P = 0.02) for further analyses, which was from another LD block, because its association with adiponectin has not been consistent in previous studies. Moreover, a Filipino study found the association between this SNP and adiponectin to be mediated by R221S, a rare variant affecting immunoassay binding affinity with artificially low adiponectin levels as a consequence (20).

To maximize the power of our study, we further generated an allele score from rs17300539 and rs3774261 (r^2 = 0.137) by adding up the number of effect alleles and dividing the sum by the total copies of alleles, resulting in a range from 0 to 1. Thus, we had three single SNPs and one allele score as IVs of serum adiponectin for subsequent Mendelian randomization analyses.

Statistical analysis. Adiponectin and insulin sensitivity index (*M/I* ratio) were natural log transformed to achieve normality. Values with a distance of >4 SD from the mean were truncated to ±4 SD, respectively, to avoid effects of outliers on the effect estimates (did not occur for insulin sensitivity or adiponectin; four, five, and three values were truncated for age, BMI, and waist, respectively).

The observed effects of adiponectin on *M/I* ratio, as well as effects of IVs on *M/I* ratio, were obtained by linear regression under additive genetic models with adjustment for age. In further analyses, BMI and waist residual, which is the BMI-adjusted waist circumference, were included as covariates into the model to evaluate effects independent of adiposity. To test the linearity of the relationship between adiponectin and insulin sensitivity, we examined the residual plots after the multivariate linear regression. Furthermore, cubic spline curves were plotted to visualize the degree of deviation from linearity.

IV analysis was then performed using a two-stage least squares approach with the STATA package ivreg2. In brief, the first stage was a conventional linear regression assessing the association between the SNPs and serum adiponectin. The predicted value of adiponectin from the model was saved and used as an independent variable in the second stage, where the dependent variable was insulin sensitivity index (*M/I* ratio). This β coefficient, or the IV estimate, reflects an unconfounded effect of genetically determined adiponectin level on insulin sensitivity. Endogeneity, or the difference between the IV estimate and the observed effect size, was examined by a Durbin-Wu-Hausman χ^2 test implemented in the package. The percentage of change in insulin sensitivity with x% increase in adiponectin estimated by each instrument was calculated as follows: $[(1 + x\%)^{\beta} - 1] \times 100\%$, where β is the corresponding IV estimate. To test the generalizability of our results, we performed secondary analyses including the 123 individuals with type 2 diabetes. In order to examine potential effect modification by adiposity, we conducted a BMI-stratified analysis by defining overweight using the median BMI level in our sample (BMI ≥25.7 kg/m^2).

Data were analyzed using Stata/IC (version 12.1; StataCorp, College Station, TX). Two-sided P values <0.05 were considered significant.

RESULTS

Characteristics of the study participants are summarized in Table 1.

Association between the instruments and serum adiponectin. Table 2 shows the 16 *ADIPOQ* SNPs available on the Metabochip and their associations with adiponectin levels in the ULSAM cohort. Based on LD structure, they were assigned to 11 different blocks. Excluding rs864265, seven SNPs belonging to two different LD blocks showed highly significant associations with serum adiponectin (all $P \leq 4.8 \times 10^{-7}$). The three SNPs chosen as IVs, namely, rs17300539, rs3774261, and rs6444175, were all strongly associated with adiponectin levels ($P = 1.1 \times 10^{-10}$, 1.3×10^{-9}, and 5.3×10^{-9}, respectively). As expected, the allele score generated from rs17300539 and rs3774261 was even more strongly associated with adiponectin level ($P = 1.2 \times 10^{-13}$) compared with each of the single *ADIPOQ* SNPs. The instruments explained between 3.5 and 6.0% (rs17300539, 4.3%; rs3774261, 3.8%; and rs6444175, 3.5%; all three SNPs combined: 6.0%) of the total variance in adiponectin levels.

Association between the instruments and insulin sensitivity. All of the IVs including the three *ADIPOQ* SNPs and the allele score were significantly associated with insulin sensitivity represented by the *M/I* ratio ($P \leq 0.022$ for all SNPs and $P = 8.0 \times 10^{-4}$ for the allele score) (Supplementary Table 1). Adjustment for adiposity represented by BMI and waist residual weakened these associations, but some of the instruments remained significantly associated with the *M/I* ratio (rs6444175, P = 0.011; allele score, P = 0.042).

IV analysis of the effect of adiponectin on insulin sensitivity. To examine the potential causal effect of adiponectin on insulin sensitivity in an IV analysis, we assessed the first-stage F statistic in which an empirical value >10 indicates sufficient strength of the genetic

TABLE 1
Characteristics of the study participants (n = 942)

Age (years)	71.0 (0.59)
BMI (kg/m^2)	26.0 (3.24)
Waist circumference (cm)	94.0 (9.17)
LDL (mmol/L)	3.92 (0.88)
HDL (mmol/L)	1.30 (0.35)
Total cholesterol (mmol/L)	5.84 (0.99)
Fasting glucose (mmol/L)	5.38 (0.56)
Adiponectin (mg/L)	9.75 (7.65–12.81)
Fasting insulin (mU/L)	11.00 (7.80–14.90)
Insulin sensitivity *M/I* ratio [mg/kg/min/(mU/L)]*	5.15 (3.48–6.84)
HOMA-IR	2.57 (1.82–3.62)
Free fatty acids (mmol/L)	0.48 (0.38–0.59)
Triglycerides (mmol/L)	1.23 (0.92–1.65)

Data are means (SD) or median (interquartile range). **M/I* ratio was defined as total glucose disposal per kilograms body weight per minute divided by mean insulin concentration under a steady state (last 60 min of the euglycemic insulin clamp).

variant as a proxy of the exposure. The large F statistics (42.6 for rs17300539, 37.6 for rs3774261, 34.7 for rs6444175, and 56.7 for the allele score in age-adjusted analyses) ensured that the SNPs chosen were strong instruments for adiponectin levels (Table 3).

The IV-estimated effect size of adiponectin levels on insulin sensitivity was highly significant and consistent for all instruments [β = 0.47 (95% CI 0.10–0.84) for rs17300539, 0.68 (0.28–1.08) for rs3774261, 0.81 (0.38–1.23) for rs6444175, and 0.60 (0.27–0.93) for the allele score] (Table 3). These estimates reflect the effect of genetically influenced adiponectin levels on insulin sensitivity and are assumed to be free from confounding. Thus, these results indicate that the relationship between adiponectin level and insulin sensitivity is potentially causal.

We then compared the IV analysis results with conventional multiple linear regression of insulin sensitivity on adiponectin levels. Endogeneity tests suggested that there was no statistical difference between the IV estimate and the observational estimate (Table 3). This was consistent for each of the individual SNP instruments and also the allele score, demonstrating that genetically determined adiponectin levels affect insulin sensitivity to the same degree as expected based on the observed association. In our secondary analyses including individuals with type 2 diabetes, the results were similar to those from our main analyses (Supplementary Table 2).

For some of the IVs, we still observed an association between the IV-estimated adiponectin and insulin sensitivity after adjustment for BMI and waist residual [β = 0.55 (95% CI 0.13–0.96) for rs6444175 and 0.34 (0.03–0.65) for the allele score] (Table 3), although the effect sizes were substantially attenuated. This suggests that at least part of the causal beneficial effect of adiponectin levels on insulin sensitivity was mediated by lower adiposity as measured by BMI and waist circumference. All the instruments except for rs17300539 were strongly associated with BMI in an age-adjusted model. However, none of them were associated with BMI after adjustment for adiponectin levels (Supplementary Table 3).

BMI-stratified analysis (Supplementary Table 4) suggested that the association between increased adiponectin levels and higher insulin sensitivity was stronger in the group with higher BMI, for which the observed causal relationship between adiponectin and insulin sensitivity also persisted. However, in the group with lower BMI, as indicated by the wide CIs of the IV estimates and nonsignificant heterogeneity P values for most of the instruments, we did not have enough power to robustly confirm or refute an association owing to limited sample size.

Estimated impact of adiponectin on insulin sensitivity. Depending on the IV used, the age-adjusted effect estimate

TABLE 2
ADIPOQ SNPs available on the Metabochip and their associations with serum adiponectin

SNP or allele score	C	Position	Alleles*	EAF	Call rate	HWE P	Association with adiponectin (age adjusted) β (95% CI)	P	LD group†
rs3917086	3	188007420	A/C	0.029	0.997	0.621	0.022 (−0.078 to 0.122)	0.668	1
rs864265	3	188036986	T/G	0.149	1	0.015	−0.055 (−0.101 to −0.009)	0.020	2
rs822387	3	188038731	C/T	0.072	1	0.660	0.162 (0.099–0.224)	4.8×10^{-7}	3
rs17300539‡	3	188042154	A/G	0.072	1	0.189	0.205 (0.144–0.267)	1.1×10^{-10}	3
rs16861209	3	188045808	A/C	0.067	0.968	0.088	0.214 (0.149–0.278)	1.5×10^{-10}	3
rs16861210	3	188049192	A/G	0.080	1	0.550	0.188 (0.128–0.248)	1.1×10^{-9}	3
rs16861194	3	188042119	G/A	0.087	1	0.855	−0.051 (−0.114 to 0.011)	0.106	4
rs822396	3	188049571	G/A	0.173	1	0.475	0.013 (−0.032 to 0.059)	0.562	5
rs3774261‡	3	188054253	A/G	0.375	0.999	0.950	0.109 (0.074–0.144)	1.3×10^{-9}	6
rs6773957	3	188056399	A/G	0.375	0.999	0.950	0.109 (0.074–0.144)	1.4×10^{-9}	6
rs6444175‡	3	188062438	A/G	0.291	1	0.438	0.113 (0.076–0.151)	5.3×10^{-9}	6
rs3774262	3	188054508	A/G	0.085	1	0.185	0.030 (−0.030 to 0.091)	0.327	7
rs9853541	3	188095161	A/G	0.378	1	0.121	0.002 (−0.035 to 0.038)	0.927	8
rs11708293	3	188096021	G/A	0.043	0.999	1.000	−0.043 (−0.129 to 0.042)	0.322	9
rs11716002	3	188096058	G/A	0.236	1	0.105	0.011 (−0.031 to 0.052)	0.616	10
rs17301514	3	188096103	A/G	0.134	0.999	1.000	0.008 (−0.043 to 0.060)	0.757	11
Allele score§							0.404 (0.299–0.509)	1.2×10^{-13}	

SNPs were first sorted based on physical position in the *ADIPOQ* gene and then aggregated by LD group. C, chromosome; EAF, effect allele frequency; HWE, Hardy-Weinberg equilibrium. *The first allele is the effect allele. †LD group was assigned based on pairwise $r^2 > 0.5$ in the HapMap2 CEU panel. ‡SNPs selected for IV analysis. §The allele score was created using genotypes for rs17300539 and rs3774261 (r^2 = 0.137).

TABLE 3
Comparison of IV estimated and observed association between adiponectin and insulin sensitivity

IV	First-stage *F* statistic	IV estimate β (95% CI)	IV estimate *P*	Observational estimate β (95% CI)	Observational estimate *P*	Endogeneity *P*†
Age adjusted				0.498 (0.420–0.576)	1.2×10^{-33}	
rs17300539	42.6	0.470 (0.097–0.842)	0.014			0.878
rs3774261	37.6	0.678 (0.278–1.077)	9.1×10^{-4}			0.364
rs6444175	34.7	0.805 (0.381–1.228)	2.1×10^{-4}			0.136
Allele score*	56.7	0.600 (0.274–0.926)	3.3×10^{-4}			0.528
Age, BMI, and waist residual adjusted				0.275 (0.206–0.344)	1.3×10^{-14}	
rs17300539	38.5	0.265 (−0.079 to 0.609)	0.131			0.955
rs3774261	29.3	0.387 (−0.007 to 0.781)	0.055			0.572
rs6444175	28.1	0.546 (0.133–0.960)	0.010			0.178
Allele score	46.7	0.340 (0.026–0.654)	0.034			0.677

Waist residual, BMI-adjusted waist circumference. †Endogeneity was assessed by Durbin-Wu-Hausman test and reflects whether the difference between the IV estimate and the observational estimate was statistically significant. *The allele score was created using genotypes for rs17300539 and rs3774261 (r^2 = 0.137).

for genetically determined adiponectin on insulin sensitivity varied from 0.470 to 0.805. Based on these point estimates, we estimated the relationship between percent change in adiponectin levels and corresponding percent change in insulin sensitivity (Supplementary Fig. 1). For example, we calculated that a 10% increase in serum adiponectin level would lead to an improvement of insulin sensitivity ranging between 4.6 and 8.0%. Similarly, excluding the mediation through obesity, the percent increase in insulin sensitivity associated with a 10% elevation in adiponectin level was estimated to be between 2.6 and 5.3%. However, owing to the large CIs for the IV estimates, these numbers should be interpreted with caution.

DISCUSSION

In a population-based cohort study of men aged 71 years assessed with gold-standard measures of insulin sensitivity, for the first time we provide evidence of a causal effect of increased adiponectin levels on improved insulin sensitivity in humans using a Mendelian randomization approach with *ADIPOQ* SNPs as instruments.

The *ADIPOQ* gene, which encodes for adiponectin, has been widely investigated for variants associated with adiponectin levels. The *ADIPOQ* SNPs available in our study belonged to the two previously identified LD blocks (21), and the ones selected as IVs have been repeatedly reported for their association with adiponectin in individuals of European descent (14,15,22–25) (Supplementary Table 5). This confirms that the SNPs we selected are robust instruments for adiponectin levels in European populations.

Studies of the relationship between *ADIPOQ* and insulin sensitivity in candidate gene studies have been less consistent (26). The 3q27 region in which the *ADIPOQ* gene is located was reported for its linkage signal with seven metabolic traits and was suggested for an association with obesity and insulin sensitivity (27). In a genome-wide linkage and association study of adiponectin, the two instrument SNPs in our study, rs6773957 and rs3774261, were identified as top hits, but neither was significantly associated with fasting insulin, fasting glucose, or other metabolic parameters (16). Richards et al. (25) also reported no significant associations for four other SNPs at the *ADIPOQ* locus with the homeostasis model assessment index of insulin resistance (HOMA-IR). Similarly, Warren et al. (24) did not find an association between seven independent SNPs at *ADIPOQ*, including rs3774261, and metabolic traits such as fasting glucose, fasting insulin, and HOMA-IR. In a recent large study, Dastani et al. (28) demonstrated that several individual SNPs that were significant in their genome-wide association studies of adiponectin levels, including a few *ADIPOQ* SNPs, were associated with type 2 diabetes and related traits. Furthermore, they found a multi-SNP genotypic risk score based on the identified hits to be strongly associated with metabolic traits related to insulin resistance. Therefore, even though *ADIPOQ* has been established as a major determinant of adiponectin levels, its associations with measures of insulin sensitivity have not been consistent, which may have been due to lack of statistical power to detect this indirect effect. Of note, prior studies have not assessed associations of *ADIPOQ* variants and insulin sensitivity measured with intravenous methods in a larger study sample.

The impact of *ADIPOQ* variants on adiposity has not been conclusive. The SNPs rs2241766 and rs1501299 have been associated with obesity in Swedish individuals (29). In the Finnish Diabetes Prevention Study, a few *ADIPOQ* SNPs were associated with baseline and 4-year follow-up measurements of body weight as well as BMI (30). However, no consistent effect on BMI was demonstrated for SNPs in the *ADIPOQ* locus in a previous meta-analysis of published candidate gene studies on *ADIPOQ* (31). Our observation that the *ADIPOQ* SNPs selected as IVs were not associated with measures of adiposity after adjustment for adiponectin levels indicates that the instruments do not have a pleiotropic effect that would otherwise violate the assumptions of Mendelian randomization. Moreover, it indicates that differences in adiposity for carriers of different *ADIPOQ* alleles could be mediated through adiponectin levels.

The role of adiposity in the relationship between adiponectin and insulin sensitivity is debatable. The degree of adiposity is associated with both adiponectin levels and insulin sensitivity (2,3,7) and thus could be a confounder for the adiponectin-insulin sensitivity relationship. If this is the case, adjustment for adiposity by including BMI and waist residuals into the regression model should not affect the association between *ADIPOQ* and insulin sensitivity level because under independent assortment *ADIPOQ* genotypes should not be associated with adiposity. This could occur if *ADIPOQ* SNPs cosegregate with other genetic determinants of adiposity, but no such gene variants

in LD with the *ADIPOQ* variants are known to date. In this study, what we observed were attenuated associations of the SNPs and insulin sensitivity with adjustment for adiposity (Supplementary Table 1), which does not support a role of adiposity as a confounder for the adiponectin–insulin sensitivity relationship. Instead, it implies that adiposity partly mediates the effect of adiponectin on insulin sensitivity, as illustrated in Fig. 1. This is supported by previous studies showing a positive correlation between adiponectin levels and clamp-measured insulin sensitivity independent of BMI (32) and that administration of recombinant adiponectin increases insulin sensitivity in both lipotrophic and obese mice (5). As proposed by Kadowaki et al. (33), obesity caused by environmental risk factors could interact with genetic factors leading to reduced adiponectin levels, which in turn plays a crucial causal role in the development of insulin resistance, type 2 diabetes, and metabolic disease. Our results suggest that this causal effect on insulin sensitivity is modulated partially through adiposity, which may work by antagonizing the secretion of proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-6 (34).

It is possible that at increased levels of adiposity, adiponectin plays a more important causal role in the regulation of insulin sensitivity compared with that in normal-weight individuals. There is a possibility that an interaction exists between adiposity and the *ADIPOQ* SNPs, wherein transcription factors that may be upregulated by, for example, inflammatory mediators, could have differential binding and thus different effects. In this case, the adiposity-adjusted IV estimates that represent causal effects not mediated by adiposity may be prone to less accuracy. Given the modest sample size when stratifying on median BMI, our study is not suitable for examining these issues, but further studies on the interrelationship between adiposity, adiponectin, and insulin sensitivity are warranted.

Cook and Semple (10) reviewed the evidence of causality in the relationship between adiponectin and insulin resistance and concluded that existing evidence indicated that adiponectin can improve insulin sensitivity in mice. For instance, intraperitoneal injection of adiponectin enhanced insulin action by suppressing hepatic glucose production and decreasing serum glucose levels (4). Further evidence came from studies on rhesus monkeys where the decrease of adiponectin levels was in parallel with the development of insulin resistance (35). However, hyperinsulinemia seemed to suppress adiponectin in both mice and humans suggesting that low adiponectin levels may also be a consequence of insulin resistance (10). Our results using a Mendelian randomization approach support the hypothesis that adiponectin levels are causally related to the degree of insulin sensitivity in humans.

Adiponectin has been suggested to be a stronger marker for insulin resistance and risk of type 2 diabetes than for cardiovascular disease (8,36). Hypoadiponectinemia was also more closely associated with the degree of insulin resistance and hyperinsulinemia than with adiposity and glycemia in several studies (3,37). In line with this, results from our study indicate that genetically determined adiponectin levels influence insulin sensitivity and that this effect is not fully mediated by adiposity, reflecting that there may be pathways between adiponectin and insulin sensitivity that do not act through adiposity. Together with findings that adiponectin shares genetic backgrounds with insulin resistance–related metabolic traits (7,28), emerging evidence is pointing toward an important role of adiponectin, independent of adiposity, in the development of insulin resistance and type 2 diabetes.

We acknowledge that our research question is novel and that there are no previous studies with which our results can be compared. Despite the fact that this is a single study with a relatively modest sample size, our results are unlikely to represent chance findings for the following reasons: First, the ULSAM cohort is the largest single-center population-based cohort in the world with insulin sensitivity measured by the euglycemic insulin clamp technique. Compared with indirect measurements and surrogate indices, this method directly measures whole-body glucose disposal at a given level of insulinemia under steady-state conditions and is therefore regarded as the gold standard for measuring insulin resistance (38). Second, *ADIPOQ* is the most established gene influencing adiponectin levels, and the SNPs chosen as IVs were robustly associated with adiponectin levels. Genetic variation in *ADIPOQ* affects adiponectin production and is not known to affect insulin sensitivity in other ways. Therefore, by focusing on *ADIPOQ*, concerns over potential pleiotropic effects of the instrument could largely be eliminated. Third, the study was undertaken in a homogeneous, age-standardized sample of men aged 71 years from Uppsala in Sweden, and therefore population stratification that could violate assumptions of Mendelian randomization is unlikely.

Limitations of our study include the relatively small sample size. However, this was partially compensated by the accurate euglycemic insulin clamp measurement of insulin resistance that increased statistical power. Further, as our study was based on elderly men of Northern European descent, the generalizability to women, other age-groups, and other ethnicities is unknown. It is of note that a recent large cohort study of elderly adults demonstrated a nonlinear relationship between adiponectin and risk of incident diabetes (39). In our study, the association between adiponectin and insulin sensitivity measured by the euglycemic clamp was deemed to be linear, except at very high levels of adiponectin, where there may be a slight deviation from linearity. It is plausible that the previously observed nonlinear relationship between adiponectin levels and new-onset diabetes characterized by leveled-off associations at high levels of adiponectin is a result of insufficient enhancement of β-cell function, despite increased insulin sensitivity. Potential deviation from linearity may affect the accuracy of the effect estimates in a Mendelian randomization study, but a linear model is still sound in this case to test the null hypothesis of no effect of exposure on outcome (11).

In summary, our results indicate that genetically determined adiponectin levels influence insulin sensitivity to the same degree as the observed epidemiological associations. This suggests that the association between adiponectin levels and insulin sensitivity is likely to represent a causal relationship. Further elucidation of this relationship could provide insights into the physiological roles of adiponectin in the etiology of insulin resistance and shed light on the therapeutic potential of adiponectin for the metabolic syndrome and type 2 diabetes.

ACKNOWLEDGMENTS

This project was supported by grants from the Swedish Research Council (2009-2298), the Swedish Heart Lung Foundation (20100401), the Swedish Foundation for

Strategic Research (ICA08-0047), and the Swedish Diabetes Foundation and Diabetes Wellness Network (2145/2011SW). H.G. is supported by a PhD scholarship from National University of Singapore. T.F. and S.H. are supported through funds from the European Community's Seventh Framework Programme (FP7/2007-2013) and ENGAGE Consortium (HEALTH-F4-2007-201413). Genotyping was performed by the SNP&SEQ Technology Platform in Uppsala (www.genotyping.se), which is supported by Uppsala University, Uppsala University Hospital, Science for Life Laboratory (Uppsala), and the Swedish Research Council (contract nos. 80576801 and 70374401).

No potential conflicts of interest relevant to this article were reported.

H.G. formulated the proposal, performed the data analysis, wrote the first draft of the manuscript, interpreted the results, and contributed to the writing and revision of the manuscript. T.F. interpreted the results and contributed to the writing and revision of the manuscript. R.M.v.D. conceived the study and contributed to the writing and revision of the manuscript. A.F. and B.Z. contributed to phenotyping and contributed to the writing and revision of the manuscript. E.I. conceived the study, jointly supervised the project progress, and contributed to the writing and revision of the manuscript. S.H. jointly supervised the project progress and contributed to the writing and revision of the manuscript. E.I. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in abstract/poster form at the American Heart Association Scientific Sessions 2012, Los Angeles, California, 3–7 November 2012, and at the 62nd American Society of Human Genetics Annual Meeting, San Francisco, California, 6–10 November 2012.

The authors thank Arvid Sjölander, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, for his valuable comments and suggestions on methodological issues. The authors are indebted to the participants of the ULSAM study.

REFERENCES

1. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2009;302:179–188
2. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595–1599
3. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930–1935
4. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947–953
5. Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941–946
6. Fruebis J, Tsao TS, Javorschi S, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 2001;98:2005–2010
7. Menzaghi C, Salvemini L, Paroni G, et al. Circulating high molecular weight adiponectin isoform is heritable and shares a common genetic background with insulin resistance in nondiabetic White Caucasians from Italy: evidence from a family-based study. J Intern Med 2010;267:287–294
8. Wannamethee SG, Tchernova J, Whincup P, et al. Associations of adiponectin with metabolic and vascular risk parameters in the British Regional Heart Study reveal stronger links to insulin resistance-related than to coronory heart disease risk-related parameters. Int J Obes (Lond) 2007;31:1089–1098
9. Stefan N, Vozarova B, Funahashi T, et al. Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. Diabetes 2002;51:1884–1888
10. Cook JR, Semple RK. Hypoadiponectinemia—cause or consequence of human "insulin resistance"? J Clin Endocrinol Metab 2010;95:1544–1554
11. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008;27:1133–1163
12. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1–22
13. Breitfeld J, Stumvoll M, Kovacs P. Genetics of adiponectin. Biochimie 2012;94:2157–2163
14. Wassel CL, Pankow JS, Jacobs DR Jr, Steffes MW, Li N, Schreiner PJ. Variants in the adiponectin gene and serum adiponectin: the Coronary Artery Development in Young Adults (CARDIA) Study. Obesity (Silver Spring) 2010;18:2333–2338
15. Heid IM, Henneman P, Hicks A, et al. Clear detection of ADIPOQ locus as the major gene for plasma adiponectin: results of genome-wide association analyses including 4659 European individuals. Atherosclerosis 2010;208:412–420
16. Ling H, Waterworth DM, Stirnadel HA, et al. Genome-wide linkage and association analyses to identify genes influencing adiponectin levels: the GEMS Study. Obesity (Silver Spring) 2009;17:737–744
17. Andersen KK, Frystyk J, Wolthers OD, Heuck C, Flyvbjerg A. Gender differences of oligomers and total adiponectin during puberty: a cross-sectional study of 859 Danish school children. J Clin Endocrinol Metab 2007;92:1857–1862
18. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223
19. Voight BF, Kang HM, Ding J, et al. The metabochip, a custom genotyping array for genetic studies of metabolic, cardiovascular, and anthropometric traits. PLoS Genet 2012;8:e1002793
20. Croteau-Chonka DC, Wu Y, Li Y, et al. Population-specific coding variant underlies genome-wide association with adiponectin level. Hum Mol Genet 2012;21:463–471
21. Heid IM, Wagner SA, Gohlke H, et al. Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. Diabetes 2006;55:375–384
22. Henneman P, Aulchenko YS, Frants RR, et al. Genetic architecture of plasma adiponectin overlaps with the genetics of metabolic syndrome-related traits. Diabetes Care 2010;33:908–913
23. Menzaghi C, De Cosmo S, Copetti M, et al. Relationship between ADIPOQ gene, circulating high molecular weight adiponectin and albuminuria in individuals with normal kidney function: evidence from a family-based study. Diabetologia 2011;54:812–818
24. Warren LL, Li L, Nelson MR, et al. Deep resequencing unveils genetic architecture of ADIPOQ and identifies a novel low-frequency variant strongly associated with adiponectin variation. Diabetes 2012;61:1297–1301
25. Richards JB, Waterworth D, O'Rahilly S, et al.; GIANT Consortium. A genome-wide association study reveals variants in ARL15 that influence adiponectin levels. PLoS Genet 2009;5:e1000768
26. Enns JE, Taylor CG, Zahradka P. Variations in adipokine genes AdipoQ, Lep, and LepR are associated with risk for obesity-related metabolic disease: the modulatory role of gene-nutrient interactions. J Obes 2011;2011:168659
27. Kissebah AH, Sonnenberg GE, Myklebust J, et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci USA 2000;97:14478–14483
28. Dastani Z, Hivert MF, Timpson N, et al.; DIAGRAM+ Consortium; MAGIC Consortium; GLGC Investigators; MuTHER Consortium; DIAGRAM Consortium; GIANT Consortium; Global B Pgen Consortium; Procardis Consortium; MAGIC investigators; GLGC Consortium. Novel loci for adiponectin levels and their influence on type 2 diabetes and metabolic traits: a multi-ethnic meta-analysis of 45,891 individuals. PLoS Genet 2012;8:e1002607
29. Ukkola O, Ravussin E, Jacobson P, Sjöström L, Bouchard C. Mutations in the adiponectin gene in lean and obese subjects from the Swedish obese subjects cohort. Metabolism 2003;52:881–884
30. Siitonen N, Pulkkinen L, Lindström J, et al. Association of ADIPOQ gene variants with body weight, type 2 diabetes and serum adiponectin concentrations: the Finnish Diabetes Prevention Study. BMC Med Genet 2011;12:5

31. Menzaghi C, Trischitta V, Doria A. Genetic influences of adiponectin on insulin resistance, type 2 diabetes, and cardiovascular disease. Diabetes 2007;56:1198–1209
32. Tschritter O, Fritsche A, Thamer C, et al. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 2003;52:239–243
33. Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J Clin Invest 2006;116:1784–1792
34. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev 2005;6:13–21
35. Hotta K, Funahashi T, Bodkin NL, et al. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 2001;50:1126–1133
36. Lawlor DA, Davey Smith G, Ebrahim S, Thompson C, Sattar N. Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. J Clin Endocrinol Metab 2005;90:5677–5683
37. Abbasi F, Chu JW, Lamendola C, et al. Discrimination between obesity and insulin resistance in the relationship with adiponectin. Diabetes 2004;53:585–590
38. Kim JK. Hyperinsulinemic-euglycemic clamp to assess insulin sensitivity in vivo. Methods Mol Biol 2009;560:221–238
39. Kizer JR, Arnold AM, Benkeser D, et al. Total and high-molecular-weight adiponectin and risk of incident diabetes in older people. Diabetes Care 2012;35:415–423