A Tangled Threesome: Adiponectin, Insulin Sensitivity, and Adiposity

Can Mendelian Randomization Sort Out Causality?

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The discovery nearly two decades ago that the adipocyte is more than a passive storehouse for lipids, that it is also an active secretory unit for peptides capable of system-wide regulation of energy metabolism, ushered in an exciting era of investigation into these newfound adipose-derived hormones (1). One intriguing object of these efforts has been the 244–amino acid protein adiponectin (2). Alone among major adipokines, adiponectin exhibits decreased rather than increased production with fat-mass expansion. Adiponectin is also the most abundantly secreted of all adipose-tissue peptides, and circulating levels correlate negatively with insulin resistance (IR), dyslipidemia, and inflammation (3). Such features have heightened interest in this molecule as a potential therapeutic target against the modern scourges of obesity and obesity-related disorders (2,3).

That adiponectin has insulin-sensitizing effects in mice is now well established (2). Available experimental data are most compelling for adiponectin's actions on the liver, where it promotes fatty acid oxidation and suppresses gluconeogenesis, and on adipocytes, where it inhibits lipolysis (3). In addition, adiponectin has direct anti-inflammatory properties (1), further enabling salutary fat-mass expansion, which is deemed pivotal to its insulin-sensitizing effects (3).

Consistent with data in mice, prospective epidemiological studies have shown higher circulating adiponectin to be associated with lower risk of diabetes (4,5). Yet such observational data, susceptible as it is to confounding and reverse causation, cannot determine causality (6). In fact, observations from naturally occurring disorders of insulin action or from exogenous administration of insulin in humans have been cited to support the proposition that the direction of the association may be the reverse of that supposed (7). According to this hypothesis, the inverse association between adiponectin and IR may in fact reflect suppression of adiponectin production by hyperinsulinemia acting through spared, as yet undefined, signaling pathways (7). Hence, the (bi)directionality of the adiponectin–IR association in the clinical setting has remained an open question.

Stepping into the breach, Gao et al. (8) report in this issue of the journal a Mendelian randomization (MR) analysis of adiponectin's relation to insulin sensitivity in a population-based cohort of older Swedish men. The authors chose as instrumental variables (IVs)—genetic stand-ins for circulating adiponectin levels that, based on the random allocation of alleles during gametogenesis, ought to be free from bias/confounding—several adiponectin-raising single nucleotide polymorphisms (SNPs) in *AdipoQ*. These IVs were used to generate age-adjusted estimates of adiponectin's effect on insulin sensitivity that proved to be significant for all variants and were moreover comparable in magnitude to the direct association observed for serum adiponectin. Such IV estimates of adiponectin's effects, however, were substantially attenuated after additional adjustment for body mass index and waist circumference, leading the authors to conclude that the association of adiponectin with higher insulin sensitivity is likely a causal relation mediated by reduced adiposity.

The study by Gao et al. is the first of its kind and counts as a clear strength use of the euglycemic insulin clamp, the gold standard for determination of insulin sensitivity (9). This technique may have permitted detection of a significant association between the IVs and outcome notwithstanding the study's relatively modest size (10).

But determining whether this study supports a causal role for adiponectin as relates to insulin sensitivity, with adiposity as a mediator, requires careful assessment of the extent to which the three fundamental assumptions underpinning IV analysis are satisfied (10). The first assumption requires that the genetic variants in *AdipoQ* be associated with the modifiable exposure of interest, circulating adiponectin. This is well supported in the current as in previous studies (11,12). According to the second assumption, the *AdipoQ* SNPs must not be associated with factors apt to confound the association between adiponectin and insulin sensitivity. The third assumption in turn stipulates that these SNPs must be related to insulin sensitivity only via their association with circulating adiponectin and not be otherwise affected by this outcome. It is in the case of assumptions 2 and 3 that the potential for violations exists.

In regard to assumption 2, the negative correlation observed between *AdipoQ* SNPs and adiposity is of particular concern. The current paradigm is that caloric excess leads to fat-mass expansion, with resulting adipose tissue stress and inflammation leading to reduced adiponectin production (13). This places adiposity primarily upstream of adiponectin, not downstream. Yet because adiposity in late adulthood should not influence the random allocation

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of *AdipoQ* alleles during meiosis, the authors posit that the inverse relation with adiposity, and the attenuation of the *AdipoQ*–insulin sensitivity association by measures of fat mass cannot reflect confounding but mediation (8). Consistent with this proposition, adiponectin infusion into the central nervous system of mice does lead to weight loss (14). But in a genetic model, overexpression of adiponectin led to enhanced fat-mass expansion and weight gain in mice, with protection against IR (15). Casting further doubt on this premise, no consistent associations between *AdipoQ* SNPs and reduced adiposity have emerged from human genetic association studies (11,16,17).

Given the paucity of independent support for the notion of reduced adiposity as a mediator of adiponectin's insulin-sensitizing role, the possibility of bias/confounding warrants special attention (Fig. 1). This could occur if genetic variants at nearby loci causing reduced adiposity were in linkage disequilibrium with the examined adiponectin-raising SNPs (10), but no such variants have been described. Another possibility is that of interaction between *AdipoQ* variants and adiposity in the former's relationship with insulin sensitivity (18), wherein the alleles lowering adiponectin levels do so as a result of, for instance, increased susceptibility to obesity-associated proinflammatory cytokines' suppression of adiponectin production (19). Indeed, the importance of accounting for gene-by-adiposity interactions in unmasking genetic determinants of IR has been recently demonstrated (20). In the current study, the IV estimates appeared more strongly associated with insulin sensitivity in participants with higher than lower adiposity, but there was insufficient power to address this question adequately. How such an interaction would account for the negative correlation between adiposity and *AdipoQ* is not immediately clear, however, since a survival advantage to individuals harboring the adiponectin-raising alleles would occur only in an obesogenic context.

Moreover, if the proposed hypothesis that hyperinsulinemia can suppress adiponectin production in the setting of IR (7) proves correct, then a pathway whereby outcome could affect genotype would be present—in violation of assumption 3 (Fig. 1). Such transcriptional regulation would mostly apply for the promoter SNP (rs17300539) (11), but if the latter's adiponectin-lowering allele acted through enhanced binding of an insulin-stimulated repressor, this could account for its association with lower insulin sensitivity. Again, however, this proposed mechanism fails readily to explain the negative correlation of the promoter variant—let alone the remaining SNPs—with fat-mass measures, since any health advantage of *AdipoQ* resistance to hyperinsulinemic suppression of transcription would not be predictably associated with lower adiposity.

Hence, a tangled web of association among the variables involved, wherein a bidirectional relation between adiponectin and insulin sensitivity could exist (7), and a different obesogenic milieu (11,20) might affect the impact of or upon *AdipoQ* variants, mandates caution in interpreting MR findings in this context. Although the current findings could be interpreted as supporting a causal role for adiponectin in insulin sensitivity, these will require replication in larger studies with greater power to assess effect modification by obesity. Ultimately, however, more confident causal inference from IV analysis for this question will require advances in understanding the molecular regulation of adiponectin and still-elusive pathways of insulin signaling. Pending development of suitable pharmacotherapies that specifically target adiponectin in humans, MR approaches may offer the best alternative to randomized trials for unraveling the chain of causality, but judging the soundness of the underlying assumptions—and the merit of the resulting conclusions—will require basic knowledge to more forward apace.

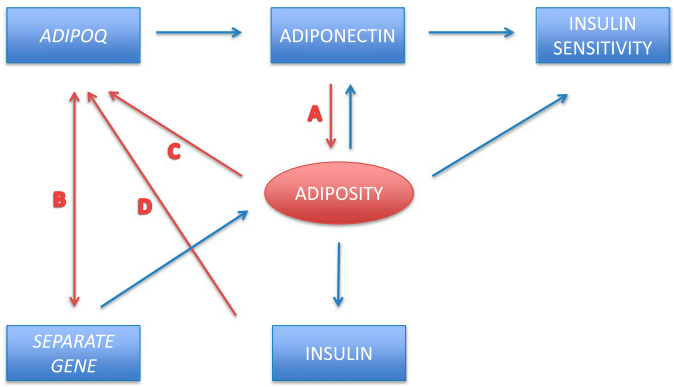

FIG. 1. Relations among *AdipoQ* variants, circulating adiponectin, adiposity, and insulin sensitivity/insulinemia with known or potential bearing on the interpretation of a corresponding MR analysis. Red arrows represent proposed causal associations, whereas blue arrows represent accepted causal associations. "A" denotes mediation of a causal adiponectin–insulin sensitivity association by adiposity, the most tenable interpretation of the MR findings reported by Gao et al. under a scenario in which all three of the fundamental assumptions of IV analysis are met. "B" denotes linkage disequilibrium between *AdipoQ* variants and those of a second gene regulating adiposity—and therefore insulin sensitivity, which would violate the assumption that *AdipoQ* variants are not susceptible to confounders that may influence the association between circulating adiponectin and outcome. Linkage disequilibrium with such a locus is not known to exist. "C" denotes gene × adiposity interaction as a potential violation of the assumption that *AdipoQ* is independent of environmental influences, which could bias the IV estimate of adiponectin's effect on insulin sensitivity; however, how such an interaction would explain the negative correlation between adiponectin-raising *AdipoQ* SNPs in the current study is unclear. "D" denotes hypothetical suppression of adiponectin production by hyperinsulinemia acting through signaling pathways separate from those directly affecting glucose transport, an instance of reverse causality that would bias the association between the IV estimate of effect of adiponectin on insulin sensitivity. Once again, this would not readily explain the negative correlation reported in the study by Gao et al. between adiponectin-raising *AdipoQ* variants and adiposity.

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