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Failure of Homeostatic Model Assessment of Insulin Resistance to Detect Marked Diet-Induced Insulin Resistance in Dogs

METABOLISM

Accurate quantification of insulin resistance is essential for determining efficacy of treatments to reduce diabetes risk. Gold-standard methods to assess resistance are available (e.g., hyperinsulinemic clamp or minimal model), but surrogate indices based solely on fasting values have attractive simplicity. One such surrogate, the homeostatic model assessment of insulin resistance (HOMA-IR), is widely applied despite known inaccuracies in characterizing resistance across groups. Of greater significance is whether HOMA-IR can detect changes in insulin sensitivity induced by an intervention. We tested the ability of HOMA-IR to detect high-fat diet–induced insulin resistance in 36 healthy canines using clamp and minimal model analysis of the intravenous glucose tolerance test (IVGTT) to document progression of resistance. The influence of pancreatic function on HOMA-IR accuracy was assessed using the acute insulin response during the IVGTT (AIR_G). Diet-induced resistance was confirmed by both clamp and minimal model ($P < 0.0001$), and measures were correlated with each other ($P = 0.001$). In striking contrast, HOMA-IR ([fasting insulin (μU/mL) × fasting glucose (mmol)]/22.5) did not detect reduced sensitivity induced by fat feeding ($P = 0.22$). In fact, 13 of 36 animals showed an artifactual decrease in HOMA-IR (i.e., increased sensitivity). The ability of HOMA-IR to detect diet-induced resistance was particularly limited under conditions when insulin secretory function (AIR_G) is less than robust. In conclusion, HOMA-IR is of limited utility for detecting diet-induced deterioration of insulin sensitivity quantified by glucose clamp or minimal model. Caution should be exercised when using HOMA-IR to detect insulin resistance when pancreatic function is compromised. It is necessary to use other accurate indices to detect longitudinal changes in insulin resistance with any confidence.

Insulin sensitivity can be accurately quantified from the hyperinsulinemic glucose clamp (1) or intravenous glucose tolerance test (IVGTT) (2), while indices derived from the oral glucose tolerance test (e.g., Refs. 3,4) may be confounded by the influence of glucose absorption (5). The use of surrogate indices based solely on fasting measurements are often considered cost-effective in both small- and large-scale clinical and genetic studies (e.g., Refs. 6,7). One widely used surrogate is the homeostatic model assessment of insulin resistance (HOMA-IR) (8). HOMA-IR ([fasting insulin × fasting glucose]/22.5) is based on computer estimation of fasting values expected for a given degree of insulin resistance and pancreatic β-cell function and was derived from hyperglycemic clamp data obtained from normal healthy subjects (9). A value of 1.0 is considered normal, with higher indices reflecting a greater degree of insulin resistance. Subjects undergoing severe caloric restriction appear to be extremely insulin-sensitive, as reflected in HOMA-IR as low as 0.29 (10), while morbidly obese subjects with type 2 diabetes may exhibit >20-fold greater values of the HOMA-IR index (11).

It is unknown whether HOMA-IR can accurately quantify longitudinal improvements in, or deteriorations of,

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insulin sensitivity. HOMA-IR is calculated from fasting glucose and insulin, but resistance often develops in the absence of elevated glycemia (12). If insulin resistance was accompanied by a β-cell defect, fasting hyperinsulinemia may not occur, and hormone levels may not exceed normal values (13). Furthermore, when HOMA-IR is used under conditions when fasting glucose is unchanged, the index is equivalent to fasting insulin, and it is unclear whether the surrogate measure offers any significant advantage over the hormone measurement alone.

In this study, we test the accuracy of HOMA-IR to detect longitudinal development of insulin resistance in dogs fed a fat-supplemented diet. Resistance was quantified by euglycemic clamps and minimal model analysis and compared with diet-associated changes in HOMA-IR.

RESEARCH DESIGN AND METHODS

Animals
Procedures were performed on 36 male mongrel dogs (28.2 ± 0.5 kg); data were pooled from previous published studies (14–16). All procedures were approved by the University of Southern California Institutional Animal Care and Use Committee.

Diet
Dogs were fed a weight-maintaining diet of 3,885 kcal/day (38% carbohydrates, 26% protein, and 35% fat). After 2 to 3 weeks of baseline metabolic testing, animals were switched to a hypercaloric high-fat diet (5,236 kcal/day, derived from 28% carbohydrates, 19% protein, and 51% fat). Animals were fed at 9 A.M. and given 3 h to consume available ration.

Experimental Design
All dogs underwent comprehensive assessment of insulin sensitivity prior to and after a 6-week period of high-fat feeding, with both the hyperinsulinemic-euglycemic clamp (EGC) and minimal model analysis of the IVGTT. Also, HOMA-IR was calculated from fasting glucose and insulin at each of these two study periods. Development of diet-induced obesity was confirmed by magnetic resonance imaging. All procedures were performed after overnight fasting, and all sensitivity tests occurred in conscious, unstressed animals.

EGC
Tracer (25 μCi + 0.25 μCi/min; PerkinElmer NEN) was infused into a peripheral vein and continued for a 90-min equilibration period. After basal blood sampling, somatostatin (1 μg/min/kg; Bachem) was infused i.v. along with insulin (regular purified pork; Lilly) from $t = 0$ to 180 min at either 0.75 mU/min/kg ($n = 19$) or 1.15 mU/min/kg ($n = 17$) to induce hyperinsulinemia. Euglycemia was maintained by variable rate 50% dextrose infusion, spiked with [3-^{3}H] glucose (specific activity: 2.2 μCi/g). Blood samples were obtained from a jugular vein catheter ($n = 8$) or sampling port ($n = 17$) surgically implanted >1 week prior to testing

or via peripheral limb intracatheters ($n = 11$). Samples were drawn every 10–15 min for 180 min and assayed for glucose, insulin, and tracer.

IVGTT
Glucose (0.3 g/kg) and insulin (0.03 units/kg) were injected intravenously at $t = 0$ and 20 min, respectively, and 31 blood samples were drawn from $t = -30$ to 180 min and assayed for glucose and insulin.

Blood Sampling and Assays
Blood was collected in lithium- and heparin-coated tubes containing EDTA, centrifuged, and plasma stored at $-80°C$. Glucose was assayed by the glucose oxidase technique (YSI Model 2300; YSI Life Sciences), with intra-assay coefficient of variation (CV) <1%. Insulin was measured in duplicate by ELISA (Linco Research, St. Charles, MO), with detection limit of 5 pmol/L and intra- and interassay CVs of 2 ± 1 and 5 ± 1%, respectively.

Calculations and Data Analysis
Pooled data from multiple studies with similar feeding regimens were used to test the ability of HOMA-IR to detect changes in insulin sensitivity induced by fat feeding, as described below.

Insulin Sensitivity
EGC. EGCs were used to quantify whole-body insulin sensitivity (SI_{CLAMP}), as well as sensitivity of periphery and liver (17). SI_{CLAMP} was calculated as:

$$SI_{CLAMP} = \Delta GINF/(\Delta INS \times GLU_{ss}), \quad (1a)$$

where $\Delta GINF$ and ΔINS are respective increments in glucose infusion rate and insulin during exogenous insulin infusion, and GLU_{ss} is the steady-state glucose concentration (final 30 min of the EGC). In a subset of animals ($n = 31$), rates of glucose uptake (R_d) and hepatic glucose output (HGO) were calculated using Steele's equations modified for use with labeled glucose infusion (18). Peripheral (SIp_{CLAMP}) and hepatic ($SIHGO_{CLAMP}$) sensitivity indices were defined as:

$$SIp_{CLAMP} = \Delta R_d/(\Delta INS \times GLU_{ss}), \quad (1b)$$

$$SIHGO_{CLAMP} = |\Delta HGO/(\Delta INS \times GLU_{ss})|, \quad (1c)$$

where ΔR_d and ΔHGO are the changes in R_d and HGO from basal to steady state.

IVGTT. The insulin sensitivity index (S_{Imm}) was calculated from minimal model analysis of the IVGTT (MINMOD Millennium, ver. 6.02; Ref. 19).

HOMA-IR.

$$HOMA\text{-}IR = [insulin \times glucose]/22.5 \quad (2)$$

where insulin and glucose are plasma concentrations after an overnight fast (8) (in μU/mL and mmol, respectively; mean of three samples, assayed in duplicate).

Glucose-Stimulated Insulin Response

We calculated glucose-stimulated insulin response (AIR_G) from the IVGTT, as the incremental insulin area under the curve from 0–10 min after glucose injection.

Statistics

Statistics (t test and ANOVA, with Tukey post hoc analysis when overall significance was detected) were performed using MINITAB (ver. 13.32; State College, PA). Statistical significance was set at $P \leq 0.05$.

RESULTS

Baseline Assessment

Dogs had fasting glucose and insulin concentrations in the normal range (89.4 ± 1.2 mg/dL and 11.6 ± 1.0 µU/mL, respectively), with body weight within a narrow range (28.2 ± 0.5 kg; CV = 10%). Body adiposity varied widely (three- to sixfold range for visceral and subcutaneous fat mass, respectively). SI_{CLAMP} ranged from 5.9–72.9 dL/min/kg/µU/ml (12.4-fold variation; mean: 34.5 ± 2.6), and similar variability was observed in SI_{mm} (9.1-fold range; mean: $4.53 ± 0.39 × 10^{-4} min^{-1}/µU/mL$). HOMA-IR was 2.67 ± 0.16, and the range from most-sensitive (0.86) to most-resistant animal (4.64) was narrow (5.4-fold). Baseline HOMA-IR did not correlate with either clamp- or IVGTT-based indices (Fig. 1).

Effect of Fat Feeding

Consumption of a high-fat diet induced substantial increases in both total (64 ± 7%) and regional adiposity (44 ± 5 and 110 ± 12% for visceral and subcutaneous depots, respectively; $P < 0.0001$). Diet-induced obesity caused significant insulin resistance (Fig. 2A). SI_{CLAMP} declined from 34.5 ± 2.6 to 22.9 ± 1.6 dL/min/kg/µU/mL ($P < 0.0001$), and resistance developed in 32 of 36 animals tested, both in insulin action on R_d (SIp_{CLAMP}; −51 ± 12%; $P < 0.0001$) and HGO ($SIHGO_{CLAMP}$; −96 ± 45%, $P < 0.0001$). Insulin resistance was confirmed by IVGTT (SI_{mm}: 4.5 ± 0.4 to $2.9 ± 0.2 × 10^{-4} min^{-1}/µU/mL$; $P < 0.0001$; Fig. 2B). Clamp-based whole-body insulin sensitivity and SI_{mm} were well-correlated ($P = 0.001$).

Diet-induced resistance was not detected by HOMA-IR (Fig. 2C). Twenty-three of 36 animals showed an increase in HOMA-IR, denoting increasing insulin resistance (ΔHOMA-IR = 1.1 ± 0.2); 13 (36% of all animals) showed an artifactual decrease (ΔHOMA-IR = −0.8 ± 0.1). The overall change in HOMA-IR was minimal, with a large SE (ΔHOMA-IR = 0.4 ± 0.2); change in HOMA-IR failed to attain statistical significance despite the large number of animals ($P = 0.22$).

The small increase in HOMA-IR with fat feeding was, by definition, the result of observed effects of diet on fasting glucose and insulin (Eq. 2). Glycemia was unaffected by fat feeding, but animals did develop modest fasting hyperinsulinemia (15.1 ± 1.3 µU/mL; $P = 0.0005$). HOMA-IR offered no additional advantage over insulin values per se in predicting development of insulin resistance.
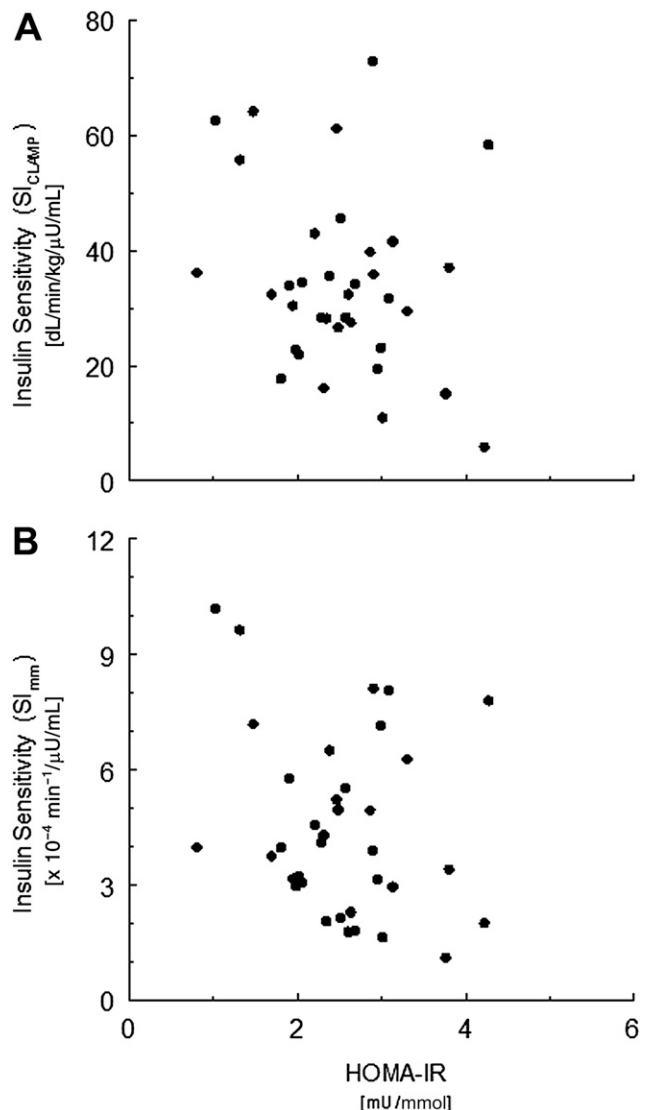
Figure 1—Relationship between baseline (prefat feeding) HOMA-IR values and either clamp-based (*A*) or IVGTT-based (*B*) indices of insulin sensitivity. There was no significant correlation between HOMA-IR and either measure ($P > 0.1$).

Impact of AIR_G on HOMA-IR Accuracy

Development of diet-induced insulin resistance led to increased AIR_G (prefat: 609 ± 38; postfat: 850 ± 52 µU/mL). HOMA-IR displayed a strong, positive correlation with AIR_G (Fig. 3), consistent with the known reciprocal relationship between insulin sensitivity and insulin responsiveness (20).

The ability of HOMA-IR to detect insulin resistance is centered on the development of hyperinsulinemia, which is proportional to insulin responsiveness (i.e., AIR_G). Using both baseline and post–fat feeding data, we examined the influence of AIR_G on HOMA-IR accuracy by segregating data into groups below or above the mean AIR_G (730 µU/mL, mean of 72 assessments; Fig. 4). In animals with a robust AIR_G (Fig. 4A), HOMA-IR was correlated with
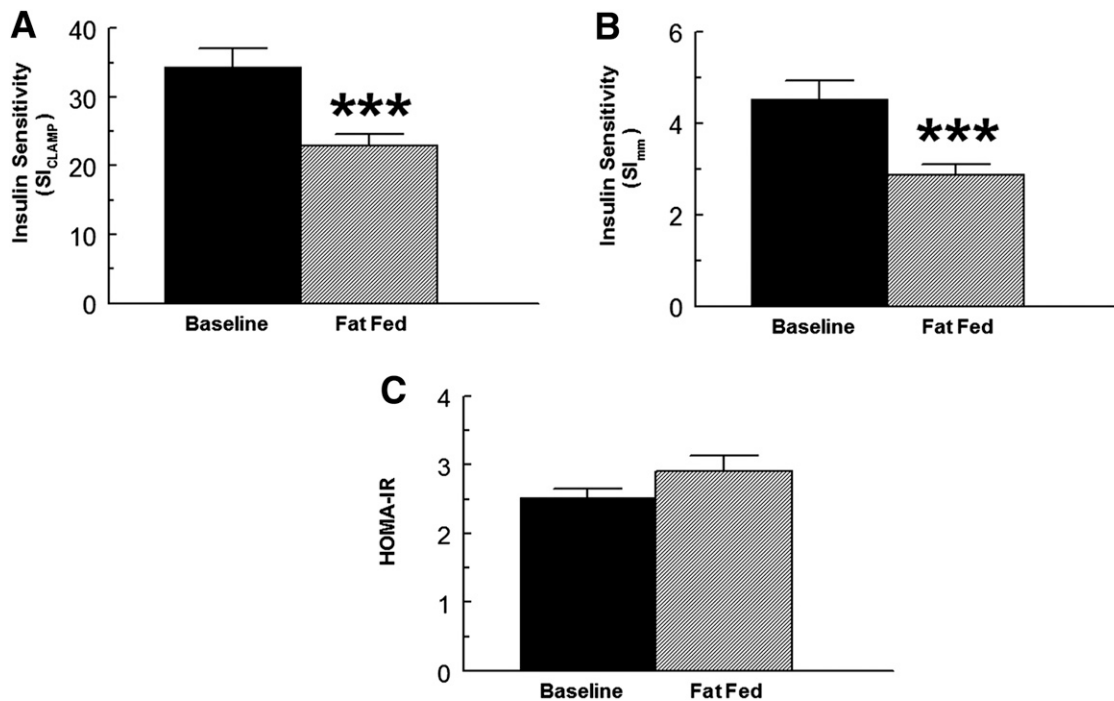
Figure 2—Diet-induced changes in insulin sensitivity, measured from EGC (*A*), IVGTT (*B*), or HOMA-IR (*C*). Both clamp and IVGTT detected substantial diet-induced insulin resistance. In contrast, changes in HOMA-IR did not reflect development of resistance after fat feeding. ***, $P < 0.0001$.

SI_{CLAMP} ($r = -0.36$; $P = 0.046$), but HOMA-IR failed to accurately estimate insulin sensitivity in animals with lesser β-cell function ($P = 0.575$; Fig. 4*B*). Failure to detect changes in sensitivity was also observed with the
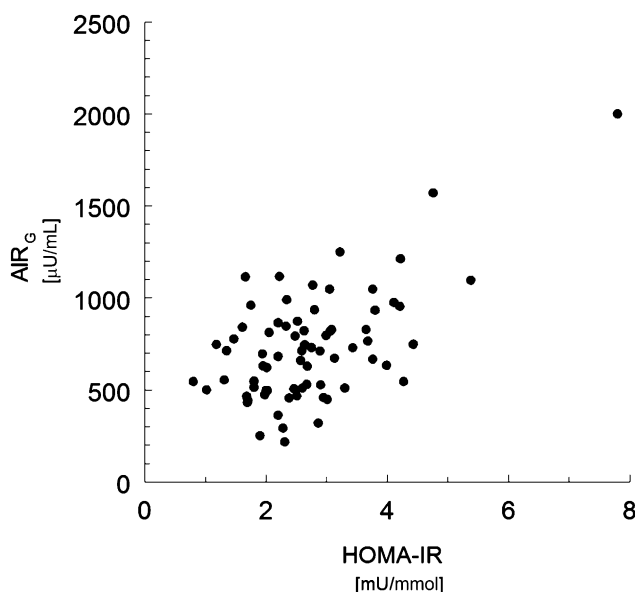

Figure 3—Correlation between HOMA-IR and AIR_G. Using both baseline and postfat data ($n = 72$ assessments), we observed a strong linear relationship between HOMA-IR and the acute insulin response ($r = 0.592$; $P < 0.0001$).

quantitative insulin sensitivity check index (QUICKI) (21), a related surrogate ($P = 0.014$ and $P = 0.268$ for correlations in animals with high and low AIR_G, respectively), and detection was not improved with second-generation HOMA2-IR estimates (http://www.dtu.ox.ac.uk/homacalculator). The ability of HOMA-IR (or QUICKI) to detect development of insulin resistance is dependent upon β-cell function.

DISCUSSION

Accurate quantitation of insulin sensitivity is critical for comprehensive metabolic phenotyping essential in the search for genetic loci for diabetes risk. The euglycemic clamp (1) measures the steady-state response to hyperinsulinemia to quantify whole-body insulin sensitivity, as well as indices of both peripheral and hepatic sensitivity. The IVGTT-based minimal model (2,19) analyzes the dynamic glucose-insulin relationship following glucose injection to yield the insulin sensitivity index, as well as measures of β-cell function and insulin clearance (e.g., Refs. 22,23). Equivalence of the clamp- and IVGTT-based indices of sensitivity has been demonstrated (17). Surrogate measures of insulin sensitivity (8,21) require only measurement of fasting glucose and insulin and represent the simplest approach. But the current study reveals serious shortcomings of surrogate measures HOMA-IR, as well as QUICKI, to detect confirmed development of insulin resistance.

At baseline, animals exhibited a wide range of insulin sensitivity, and clamp-based values were well-correlated
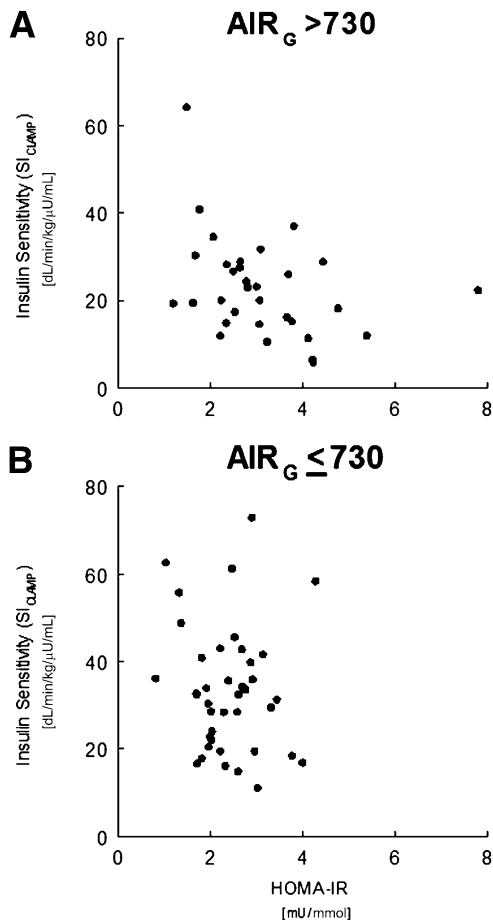
A AIR$_G$ >730

B AIR$_G$ ≤730

Figure 4—Impact of AIR$_G$ on the ability of HOMA-IR to accurately estimate insulin sensitivity. All data (baseline and postfat; $n = 72$ assessments) were separated according to high AIR$_G$ (higher than mean of 730 μU/mL; $n = 32$) (A) and low AIR$_G$ (less than or equal to mean; $n = 40$) (B). HOMA-IR provided an accurate reflection of SI$_{CLAMP}$ only when a robust insulin response was evident ($P = 0.008$ vs. $P = 0.597$ when AIR$_G$ is low).

with minimal model indices. But there was no significant relationship between either clamp- or minimal model–based measures of baseline sensitivity and corresponding HOMA-IR (Fig. 1). HOMA-IR detected insulin resistance in animals where clamps revealed normal sensitivity. The median value of HOMA-IR was 2.50, but animals considered resistant by virtue of HOMA-IR exceeding the median were not resistant by clamp-based SI$_{CLAMP}$ (32.7 ± 3.8 vs. 36.2 ± 3.6 dL/min/kg/μU/mL for animals with HOMA-IR values below the median; $P = 0.81$).

The interventional methods were both effective in detecting deterioration of whole-body insulin sensitivity induced by high-fat diet. SI$_{CLAMP}$ and SI$_{mm}$ decreased by 34 and 37%, respectively (Fig. 2A and B), and clamp data indicate resistance developed at both hepatic and peripheral tissues. In stark contrast, HOMA-IR was of surprisingly limited utility in detecting this diet-induced resistance (Fig. 2C). There was a modest increase in HOMA-IR after fat feeding; the mean change was small, with a large SE,

due in part to small, diet-induced reduction in fasting glycemia in 18 of 36 animals (ΔHOMA-IR = 0.2 ± 0.4), but this change was not statistically significant ($P = 0.51$). Moreover, 13 of 36 animals tested showed an artifactual decrease in HOMA-IR (ΔHOMA-IR = -0.8 ± 0.1).

We observed a strong correlation between HOMA-IR and the AIR$_G$ (Fig. 3). We segregated HOMA-IR and SI$_{CLAMP}$ data into two groups, reflecting animals with high (higher than mean; $n = 32$) or low AIR$_G$ (lower than or equal to mean; $n = 40$). HOMA-IR accuracy was indeed dependent on the magnitude of the insulin secretory response (Fig. 4). When β-cell function was robust (i.e., AIR$_G$ exceeded the mean), HOMA-IR yielded an accurate estimation of insulin sensitivity. The limitations of HOMA-IR are exposed in subjects that display a lesser insulin secretory response. No animals in this study were diabetic; the low AIR$_G$ is a likely reflection of high insulin sensitivity in those animals.

HOMA-IR accuracy is weakest for estimating insulin sensitivity under conditions in which insulin secretory function is in the normal range, but less robust. Thus, HOMA-IR should be viewed with skepticism if β-cell function is not known a priori in all individuals. Relationship between HOMA-IR as a quantitative trait and specific variants may not indicate genetic signals for insulin resistance per se, but may more represent signals for β-cell function, as HOMA-IR may reflect islet cell function or metabolic clearance of insulin, rather than insulin resistance itself (24).

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