Biological Variation of Homeostasis Model Assessment–Derived Insulin Resistance in Type 2 Diabetes

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OBJECTIVE — Individuals with type 2 diabetes are particularly vulnerable to cardiovascular disease. Insulin resistance is a major determinant of this increased risk and is a potential therapeutic target. This study was undertaken to establish the natural biological variation of insulin resistance in individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS — The biological variation of insulin resistance was assessed by measuring insulin resistance at 4-day intervals on 10 consecutive occasions in 12 postmenopausal women with diet-controlled type 2 diabetes and in 11 weight- and age-matched postmenopausal women without type 2 diabetes. Insulin resistance was derived using the homeostasis model assessment for insulin resistance (HOMA-IR) method.

RESULTS — The distribution of HOMA-IR was log Gaussian in the type 2 diabetic study group and Gaussian in the control group. The HOMA-IR in the type 2 diabetic group was significantly greater than that of the control group (mean ± SD: 4.33 ± 2.3 vs. 2.11 ± 0.79 units, $P = 0.001$). After accounting for analytical variation, the mean intraindividual variation was also substantially greater in the type 2 diabetic group than in the control group (mean 1.05 vs. 0.15, $P = 0.001$). Consequently, at any level of HOMA-IR, a subsequent sample must increase by >90% or decrease by >47% to be considered significantly different from the first.

CONCLUSIONS — HOMA-IR is significantly greater and more variable for individuals with type 2 diabetes. Therefore, this inherent variability needs to be accounted for in studies evaluating therapeutic reduction of HOMA-IR in this group.

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Cardiovascular disease is the leading cause of mortality in type 2 diabetes, accounting for ~70–75% of all deaths (1). Through an impaired biological response to insulin, insulin resistance underpins the development of type 2 diabetes, and in the presence of a cluster of atheromatous risk factors comprising hypertension, low HDL cholesterol, raised triglyceride levels, hyperinsulinemia, and dysglycemia, it has been proposed to explain the increased vascular risk associated with type 2 diabetes (2). Therapeutic reduction of insulin resistance has been shown to improve glycemic control (3,4) and has the potential to favorably modify other components of the insulin resistance syndrome, thereby reducing the long-term cardiovascular sequelae. Currently, however, there is no data on the biological variability of insulin resistance for individuals with type 2 diabetes, information that is essential for assessing the benefit from any therapeutic intervention. This study was therefore undertaken to establish whether insulin resistance in individuals with type 2 diabetes remains within narrow biological limits or varies more widely over a given time period.

RESEARCH DESIGN AND METHODS

Subjects

A total of 12 Caucasian postmenopausal women with diet-controlled type 2 diabetes (median age 62 years, range 50–73) and 11 healthy, weight-matched, Caucasian postmenopausal control women (median age 56 years, range 48–70) with normal (<6 mmol/l) fasting blood glucose levels participated in the study. For the subjects with diabetes the diagnosis was based on a fasting venous plasma glucose concentration >7.0 mmol/l or a 2-h postprandial concentration >11.1 mmol/l after a 75-g oral glucose tolerance test. Women were considered postmenopausal if they had amenorrhoea for >1 year and follicle-stimulating hormone levels >20 IU/l. Exclusion criteria included any secondary cause of hyperglycemia, current or previous (in the preceding 6 months) use of estrogen therapy, treatment with insulin or oral hypoglycemic agents, untreated hypothyroidism, history of drug or alcohol abuse, and smoking. Subjects on medication unrelated to diabetes took their medication as usual with no changes reported during the sampling period. All subjects were asked to have an unrestricted diet and instructed not to modify their eating patterns during the period of sampling. The subjects were also advised to refrain from excessive physical exercise and alcohol before each fasting blood test. Fasting plasma glucose, age, BMI, and current smoking status were obtained. The BMI in the group with type 2 diabetes (median 31.6, range 25.1–35.7) was similar ($P = 0.651$) to that in the control group (32.0, 26.6–44.4). Fasting venous blood was

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Abbreviations: HOMA-IR, homeostasis model assessment for insulin resistance.

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

Table 1—*Clinical and biochemical characteristics study participants of subjects*

	Type 2 diabetic patients	Control subjects	*P*
n	12	11	
Age (years)	61.7 ± 7.0	56.2 ± 6.1	NS
Weight (kg)	77.5 ± 8.1	79.9 ± 13.4	NS
BMI (kg/m^2)	31.1 ± 3.3	32.4 ± 5.3	NS
Fasting glucose (mmol/l)	7.6 ± 2.3	5.0 ± 0.5	<0.001
Fasting insulin (μU/ml)	13.0 ± 5.6	9.4 ± 3.4	<0.001

Data are means ± SD.

collected after a 5-min period of rest into serum gel tubes (Becton Dickinson, Oxford, U.K.) and one fluoride oxalate tube at the same time each day (0800–0900) on 10 consecutive occasions at 4-day intervals. Samples were separated by centrifugation at 2000*g* for 15 min at 4°C, and two aliquots of the serum were stored at –20°C within 1 h of collection. Plasma glucose was analyzed in singleton within 4 h of collection. The serum samples were split before assay. All subjects gave their informed written consent before entering the study, which had been approved by the Hull and East Riding Local Research Ethics Committee.

Reagents

Serum insulin was assayed using a competitive chemiluminescent immunoassay, supplied by Euro/DPC, Llanberis, U.K. The assay was performed on a DPC Immulite 2000 analyzer (Euro/DPC, Llanberis, U.K.) using the manufacturer's recommended protocol. The coefficient of variation of this method was 10.6%, calculated using the study samples. The analytical sensitivity was 2 μU/ml, and there was no stated cross-reactivity with proinsulin. Plasma glucose was measured using a Synchron LX 20 analyzer (Beckman-Coulter, High Wycombe, U.K.) using the manufacturer's recommended protocol. The coefficient of variation for this assay was 1.2% at a mean glucose value of 5.3 mmol/l during the study period. Before analysis, all of the serum samples were thawed and thoroughly mixed. The duplicate samples (i.e., two per visit) were randomized and analyzed in a single continuous batch using a single batch of reagents.

Statistical analysis

Statistical analysis was performed using SPSS for Windows NT, version 9.0 (SPSS, Chicago, IL). Insulin resistance was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) method (HOMA-IR = [insulin × glucose]/22.5) (5). Biovariability data were analyzed by calculating analytical, within-subject, and between-subject variances (SD_A2, SD_I2, and SD_G2, respectively) according to the methods of Fraser and Harris (6). By this technique, analytical variance (SD_A2) was calculated from the difference between duplicate results for each specimen ($SD_A2 = \Sigma d^2/2N$, where d is the difference between duplicates, and *N* is the number of paired results). The variance of the first set of duplicate results for each subject on the 10 assessment days was used to calculate the average biological intraindividual variance (SD_I2) by subtraction of SD_A2 from the observed dispersion (equal to $SD_I2 + SD_A2$). Subtracting $SD_I2 + SD_A2$ from the overall variance of the set of the first results determined the interindividual variance (SD_G2). The standard deviation of intraindividual (SD_I) and interindividual (SD_G) variations was estimated as square roots of the respective variance component estimates. The reference change value or critical difference between two consecutive HOMA-IR samples in an individual subject with type 2 diabetes was calculated using the formula $2.77(CV_I)$, where CV_I is the within subject biological coefficient of variation (6).

RESULTS — The clinical and biochemical details of the individual subjects are shown in Table 1. The distribution of HOMA-IR was found to be log Gaussian (by Kolmogorov-Smirnov) in the type 2 diabetic group and Gaussian in the control group. Figure 1 shows the mean and range of insulin resistance for the individuals in the two groups.

The HOMA-IR in the group with type 2 diabetes was greater than that in the control group (4.33 ± 2.3 vs. 2.11 ± 0.79 units, $P = 0.001$ using the Mann-Whitney *U* test). For the group with type 2 diabetes, the analytical variance contributed to 1.5% of the total test variance, 19.2% of the intraindividual variance, and 79.3% of the interindividual variance. For the control group, the analytical variance contributed to 2.5% of the total test variance, 23.7% of the intraindividual variance, and 73.8% of the interindividual variance. After accounting for analytical variation (following log transformation of the type 2 diabetes data), the mean intraindividual variation was sub-

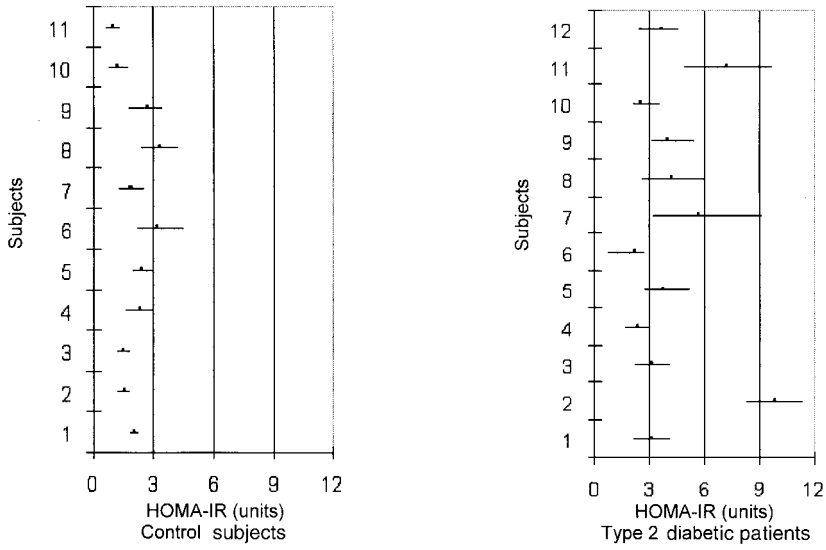

Figure 1—*Means (range) of HOMA-IR in control subjects and postmenopausal women with type 2 diabetes.*

stantially greater in the group with type 2 diabetes than in the control group (mean 1.05 vs. 0.15, $P = 0.001$). The critical difference between two consecutive HOMA-IR samples in an individual patient with type 2 diabetes, calculated using the formula $2.77(CV_I)$ (6) on the log-derived data, was −47% or +90% of any initial level of insulin resistance. This indicates that a subsequent sample must increase by >90% or decrease by >47% to be considered significantly different from the first.

CONCLUSIONS — This is the first study to show that the intraindividual variation in HOMA-derived insulin resistance is much greater in individuals with type 2 diabetes than in nondiabetic individuals. In accord with previous studies, the absolute HOMA-IR in individuals with type 2 diabetes was also significantly greater than in weight-matched individuals without type 2 diabetes (7,8). However, for the subjects with type 2 diabetes, there was a wide degree of variation in the value of HOMA-IR, both as a group and within each individual comprising the group, i.e., the interindividual and intraindividual variation were high. Conversely, for the group without type 2 diabetes, the HOMA-IR remained within a narrow range over a period of time both for the group as a whole and for each individual within the group, i.e., the interindividual and intraindividual variation were relatively low. Therefore, the high degree of intraindividual variability of HOMA-IR seen in individuals with type 2 diabetes means that a change in two single measurements of HOMA-IR in the same subject may be more a consequence of biological variation than any treatment effect. Because of the log Gaussian distribution of insulin resistance in the group with type 2 diabetes, the values must increase by >90% or decrease by >47% before a significant critical difference can be assured.

Until now, there has been no data for the biological variability of insulin resistance in individuals with type 2 diabetes. In healthy individuals, insulin sensitivity does not appear to have seasonal variation (9), and measurement of insulin-mediated glucose disposal has been shown to be stable over time (10). The intraindividual variation of fasting glucose and insulin has previously been evaluated in healthy volunteers (11) and in subjects with type 2 diabetes (12). Mooy et al. (12) analyzed data from two 75-g oral glucose tolerance tests performed 2–6 weeks apart during the Hoorn study, a cross-sectional population-based survey on glucose tolerance in Dutch Caucasians aged 50–74 years without a history of diabetes. The intraindividual variation was assessed by the standard deviation of the test-retest difference. The subgroup of individuals subsequently diagnosed to have type 2 diabetes showed greater total intraindividual variation in both fasting glucose and fasting insulin than the group with normal glucose tolerance. In accord with our data, they found that the biological variation of the measured analytes (glucose and insulin) accounted for most of the intraindividual variation seen with analytical variation, only providing a small contribution to the total variation observed. This means that in their study, as in ours, the variation observed would not be significantly altered, even with improved measurement techniques.

A diurnal variation in insulin sensitivity is known to occur (13,14), and circadian neuroendocrine rhythms of glucocorticoids and prolactin have been implicated in day-to-day variation in insulin sensitivity (15). However, these influences were accounted for here, as the samples were collected at the same time of day to eliminate variations caused by circadian rhythms. Sex hormones, sex hormone–binding globulin, and androgens are known to influence an individuals insulin resistance (16–18), and variations in these hormones either by themselves or in various ratios could have an influence on the variability of the calculated insulin resistance. However, this study was not powered to assess this relation, and further studies are needed for clarification. Interestingly, we observed that the intraindividual variance of HOMA-IR increased linearly with increasing HOMA-IR values for both the control subjects ($r = 0.84$, $P = 0.001$) and the group with type 2 diabetes ($r = 0.60$, $P = 0.039$), suggesting a direct relation between insulin resistance and the degree of variability present in both health and disease. Additional confounding factors to be considered in a study such as ours are the recognized influence of age, BMI, ethnicity, and menopausal status and the concomitant use of estrogen replacement therapy in individuals insulin resistance (19–24). To account for this, the two groups in this study were closely matched for age, menopausal status, and BMI; all subjects were Caucasian; and no subject in either group had been on estrogen replacement therapy for at least 6 months before the study.

The strong correlation previously shown between HOMA and other methods to determine insulin resistance, such as insulin clamps or intravenous glucose tolerance tests (5,8,25), would suggest that the biological variation in HOMA-IR seen here may also be present when insulin resistance is determined by other assessment methods and, indeed, the very low biological variability in HOMA-IR seen in postmenopausal women without type 2 diabetes would indicate that the wider biological variation seen in the group with type 2 diabetes is real rather than a methodological flaw with the HOMA method. Although the variability of HOMA-IR determined here cannot be directly extrapolated to insulin resistance per se, this data highlights the need for further studies (by clamp or other means) to either confirm or refute the HOMA findings. If found to be the case, then this may have implications for the correct interpretation of studies involving intervention in individual subjects.

The reasons for using the HOMA model in this study were twofold. First, it is the technique most likely to be used in clinical practice. Second, the 10 consecutive measurements required over the 36-day sampling period using other more labor intensive and invasive techniques, such as hyperinsulinemic-euglycemic clamps or intravenous glucose tolerance tests, were considered unsuitable for humans in view of the potential associated complications, such as thrombophlebitis, from repetition of these invasive methods.

In conclusion, we have found that insulin resistance measured using the HOMA model is greater and has more intrinsic variability in individuals with type 2 diabetes compared with those without. Whereas the reasons for this high inherent variability remain speculative, it nonetheless needs to be accounted for in studies of insulin resistance for individuals with type 2 diabetes.

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