The Day-to-Day Variation in Insulin Sensitivity in Non–Insulin-Dependent Diabetes Mellitus Patients Assessed by the Hyperinsulinemic-Euglycemic Clamp Method

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The objective was to study the day-to-day variation in insulin sensitivity in non–insulin-dependent diabetes mellitus (NIDDM) and to analyze within- and between-person variances in the glucose infusion rate during steady state (M value). Ten NIDDM patients attending the outpatient clinic at Aarhus Amtssygehus were studied three times under standardized conditions. Each time, a 120-minute hyperinsulinemic-euglycemic clamp was performed. Similar M values were found on the 3 study days, with difference between M values on the 3 days of (mean ± SD) 0.3 ± 1.8 mg glucose/kg lean body mass (LBM)/min. The total coefficient of variation (CV) for M values was 57% after the first clamp, 55% after the second, and 53% after the third. Ninety percent of the total day-to-day variation in M values could be ascribed to between-person variation and 10% to within-person variation. Within-person components of variance included all sources of variation other than between-person variation. The within-person CV for M values was 11.9% ± 7.2% after two clamp studies and 12.1% ± 7.3% after three ($P < .55$). In conclusion, under standardized conditions, a valid estimate of insulin sensitivity assessed by the hyperinsulinemic-euglycemic clamp in NIDDM patients is obtained after a single measurement. Because of large between-person variation, paired data should be used when comparing insulin sensitivity in NIDDM patients.

INSULIN RESISTANCE is a main feature of non–insulin-dependent diabetes mellitus (NIDDM). The glucose clamp technique, generally considered the "gold standard" in measuring insulin sensitivity, is an important tool in physiologic and pathophysiologic studies of metabolism. The glucose clamp technique originally introduced by Andres et al[1] has been developed to the present form by DeFronzo et al.[2] In their classic study, DeFronzo et al[2] found almost identical amounts of insulin-stimulated glucose uptake during two repeated hyperinsulinemic-euglycemic clamp procedures in healthy persons. The question arises as to whether insulin sensitivity in insulin-resistant states such as NIDDM varies to a greater extent and therefore requires more than one clamp study to produce a valid and representative estimate of insulin sensitivity. The aim of this study was to measure the day-to-day variation in insulin sensitivity in NIDDM subjects studied three times with the hyperinsulinemic-euglycemic clamp, and to analyze within- and between-person variance in the glucose infusion rate during steady state (M value).

SUBJECTS AND METHODS

Ten NIDDM patients were recruited from the outpatient clinic (Table 1). Inclusion criteria were diet or sulfonylurea treatment, stable body weight during the prior 3 months, fasting blood glucose level less than 8 mmol/L, fasting triglyceride level less than 4 mmol/L, and known diabetes duration longer than 3 years. Exclusion criteria were diabetic complications except simplex retinopathy, atherosclerotic symptoms, hypertension, and treatment with angiotensin-converting enzyme inhibitors or β-adrenergic blockers.

The patients participated three times at least 1 week apart. A 24-hour urine collection was made for analysis of glucose. Lean body mass (LBM) was estimated by bioimpedance analysis[3] (Animeter; HTS-Engineering, Odense, Denmark). During the 24 hours before the study days, the patients ingested high-carbohydrate food delivered from the dietitian. Food amounts corresponded to individual energy requirements estimated from the Harris-Benedict equation with adjustment for physical activity.[4] The patients were required to standardize and minimize their physical activity on the study mornings, that is, get dressed without any washing procedures, be transported to the experimental setting by taxi cab, and immediately get into bed upon arrival at 7:30 AM. The two cigarette smokers were not allowed to smoke after 7:00 PM the evening before. Upon arrival, two intracatheters were placed, one in an antecubital vein and the other in a dorsal vein on the opposite hand, which was then placed in a heating chamber to arterialize the blood,[5] and the patients rested for 60 minutes. Basal blood samples were drawn, and the hyperinsulinemic-euglycemic clamp study was performed according to the method of DeFronzo et al.[2] At time zero of the clamp procedure, a constant infusion of insulin (40 mU/m^2, Actrapid; Novo Nordisk, Copenhagen, Denmark) was initiated, and a variable glucose (20% glucose with added KCl, 20 mmol/L glucose) infusion was adjusted every 5 to 10 minutes according to glucose determinations in arterialized blood, aiming at a blood glucose level of 5 mmol/L. The period from 90 to 120 minutes with a stable glucose infusion rate was defined as the steady-state period of the clamp.

Urinary glucose level was measured using a Technicon RA-1000 analyzer. Blood glucose levels were measured with a YSI Model 2300 STAT PLUS glucometer (Yellow Springs Instruments, Yellow Springs, OH; coefficient of variation [CV], 4%). Serum insulin was determined by an enzyme-linked immunoassay method[6] (CV, 1.7%). Results are expressed as the mean ± SD. The M value is presented as milligrams glucose per kilogram LBM per minute corrected for urinary glucose loss during the clamp. The insulin to glucose ratio is calculated as the ratio between the fasting levels of insulin and glucose. The data presented are all consistent with the hypothesis of normality. Multiple comparisons between mean values were made by ANOVA (repeated measures) followed by Student's t test with Bonferroni correction[7] (RM-ANOVA; BMDP Statistical Software, Berkeley, CA). Correlation analysis was performed using the Spearman rank-order correlation. The

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Table 1. Clinical Characteristics of 10 NIDDM Patients (six men and four women; mean ± SD)

Study Day	Weight (kg)	Fasting Blood Glucose (mmol/L)	Fasting Serum Insulin (pmol/L)	Body Mass Index (kg/m²)	LBM (kg)	Age (yr)
1	79.8 ± 9.9	6.6 ± 1.6	44 ± 34	28.2 ± 5.5	37.8 ± 7.3	63.5 ± 2.7
2	79.9 ± 9.9	6.5 ± 1.6	40 ± 22			
3	80.0 ± 10.0	6.5 ± 1.5	43 ± 18			
	$P = .51$	$P = .91$	$P = .72$			

CV was calculated as the standard deviation expressed as a percentage of the mean. $P < .05$ was considered statistically significant.

RESULTS

No differences were seen in body weight, fasting blood glucose, or fasting serum insulin levels on the three occasions (Table 1). The CV for fasting blood glucose was 6.6% ± 1.5%. Of the total variation in fasting blood glucose levels, 9% could be ascribed to within-person variation. During steady state, similar blood glucose levels were achieved (Table 2). Urinary glucose excretion during steady state was negligible and did not differ on the 3 study days. Similar M values were found on the 3 study days (Table 2), with a mean difference between M values on the 3 days of 0.3 ± 1.8 mg glucose/kg LBM/min. The total CV for M values was 57% after the first clamp, 55% after the second, and 53% after the third. Ninety percent of the total day-to-day variation in M values could be ascribed to between-person variation and 10% to within-person variation (Fig 1). The within-person CV for M values was 11.9% ± 7.2% after two clamp studies and 12.1% ± 7.3% after three ($P > .55$). No influence was found for gender, LBM, or fasting blood glucose levels on the CV for M values. Furthermore, no systematic period effect was found (Fig 1).

The insulin to glucose ratio was $6.9 \pm 5.3 \times 10^{-3}$, $6.6 \pm 3.9 \times 10^{-3}$, and $7.3 \pm 4.0 \times 10^{-3}$, respectively. The total CV for the insulin to glucose ratio was 64%, and 95.5% of the total day-to-day variation in the insulin to glucose ratio could be ascribed to between-person variation and 4.5% to within-person variation. No correlation was found between M values and insulin to glucose indices ($r = -.154$, $P = .41$).

DISCUSSION

In this study, insulin sensitivity in NIDDM subjects was measured three times by the classic hyperinsulinemic-euglycemic clamp method.[2] The study conditions were standardized to minimize possible interactions on insulin sensitivity by weight changes,[8,9] daily diet, stimulants (tobacco[10] or alcohol[11]), physical activity,[12] stress,[13] and medication. These actions are probably responsible for the similarity in the basal conditions with similar fasting blood glucose levels. Fasting blood glucose levels near 6.5 mmol/L ensured that the blood glucose decline to steady-state levels near 5.2 mmol/L would not induce hormonal responses, which have been shown to begin at less than 4.2 mmol/L in diabetic persons with fasting blood glucose levels of about 7 to 8 mmol/L.[14] Furthermore, we did not use an insulin priming dose, since stable insulin levels are hardly obtained earlier with this approach[15] and constant insulin infusion reduces the risk of abrupt glucose declines initially. During clamping, both similar blood glucose levels and M values were obtained on the 3 study days, the latter with a mean difference (Table 2) near the values obtained in healthy subjects.[2] The total CV for M values was approximately 50% and did not change by increasing the number of clamps from one to three. This relatively large and constant CV is mainly due to the large between-person variation component of 90% of the total variance of the M value. The corresponding within-person CV was only approximately 12%, being unchanged by increasing the number of clamps from two to three. The relatively large and constant total CV increases the risk of a statistical type 2 error in studies comparing insulin sensitivity by the hyperinsulinemic-euglycemic clamp method; however, with a paired study design, this risk is minimized. For example, to demonstrate a 25% improvement in insulin sensitivity with a statistical power greater than 80%, the required number of subjects would

Table 2. Results From Three Hyperinsulinemic-Euglycemic Clamps in 10 NIDDM Patients (mean ± SD)

Study Day	Steady-State Blood Glucose (mmol/L)	Steady-State Serum Insulin (pmol/L)	M Value (mg/kg LBM/min)
1	5.2 ± 0.3	395 ± 70	6.1 ± 4.0
2	5.2 ± 0.3	411 ± 78	5.4 ± 3.0
3	5.1 ± 0.2	415 ± 64	5.7 ± 3.7
	$P = .26$	$P = .85$	$P = .97$

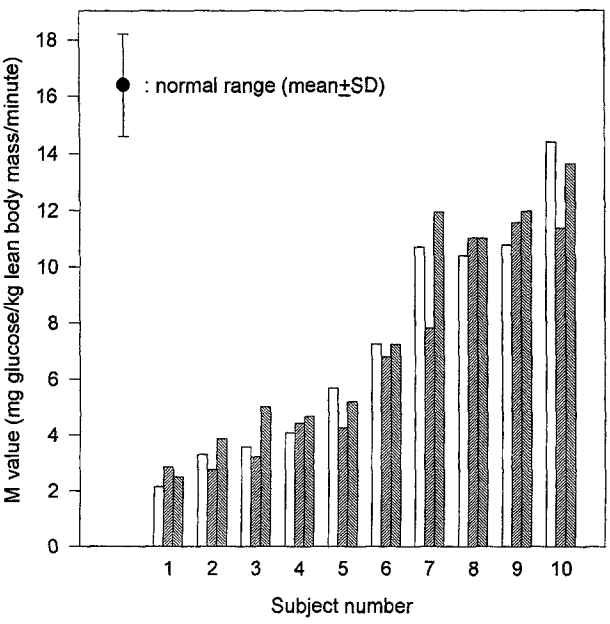

Fig 1. Individual M values from three hyperinsulinemic-euglycemic clamps in 10 NIDDM subjects.

be eight in a paired study design (crossover), whereas at least 12 subjects would be necessary in an unpaired study design. Due to the low within-person variation (10% of the total variance in M values), a good estimate of the M value is obtained after only a single clamp study under the conditions present in this investigation. The duration of the clamps in this study was 120 minutes, as described by DeFronzo et al.[2] It is known that activation by insulin is significantly slower in obese subjects than in lean subjects[16]; however, the increment of increase in the glucose infusion rate is only modest after 120 minutes of insulin exposure.[17] The within-person variation found in our study primarily reflects physiological variation, since there is only a small tolerance in adjusting the glucose infusion during steady state. Thus, it has been demonstrated that a systematic increase or decrease in the glucose infusion rate, amounting to only 10% from the predicted infusion, results in considerable hyperglycemia or hypoglycemia.[2]

A commonly used substitute for insulin sensitivity is the insulin to glucose ratio, which is more convenient to obtain, especially in larger-scale studies. The CV for the insulin to glucose ratio was 64%, and the within- and between-person components constituted 4.5% and 85.5%, respectively. However, no correlation was found between M values and insulin to glucose ratios in our study.

In conclusion, under standardized conditions, a valid estimate of insulin sensitivity assessed by the hyperinsulinemic-euglycemic clamp in NIDDM patients is obtained after only one clamp procedure. Because of a large between-person variation, paired data should be applied when comparing insulin sensitivity in NIDDM patients.

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