Importance of Obtaining Independent Measures of Insulin Secretion and Insulin Sensitivity During the Same Test

Results with the Botnia clamp

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OBJECTIVE — To validate and apply a method for independent assessment of insulin secretion and insulin sensitivity (S_I) during the same test; that is, an intravenous glucose tolerance test followed by a euglycemic-hyperinsulinemic clamp, also called the Botnia clamp. This test was then applied to nondiabetic subjects with (FH+) and without (FH−) a first-degree family history of diabetes.

RESEARCH DESIGN AND METHODS — The Botnia clamp measures the first-phase insulin response (FPIR) to 0.3g/kg glucose i.v. and insulin sensitivity (M-value) from a 2-h euglycemic clamp begun 60 min after the glucose bolus. The M-value obtained during the Botnia clamp was compared with M-values obtained during a regular euglycemic clamp without prior glucose bolus. Repeated tests were performed in random order in subjects with normal and abnormal glucose tolerance. Finally, the test was applied to subjects with and without a family history of type 2 diabetes.

RESULTS — S_I and insulin secretion from this test showed a high degree of reproducibility, and the M-value obtained with the Botnia clamp correlated strongly with the M-value from a euglycemic clamp without prior glucose bolus ($r = 0.953$, $P < 0.005$). FH+ subjects showed decreased S_I ($P = 0.02$), but similar FPIR, compared with FH− subjects. However, insulin secretion adjusted for the degree of insulin resistance was significantly impaired ($P = 0.04$).

CONCLUSIONS — In conclusion, the Botnia clamp provides reliable and independent measures of S_I and β-cell function during the same test. As illustrated above, knowledge of the degree of S_I is mandatory when presenting data on insulin secretion.

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Assessment of the degree of insulin resistance and insulin deficiency is often required in metabolic studies of the prediabetic and diabetic state (1,2). Given that insulin secretion is highly dependent on the degree of insulin sensitivity (S_I), it is desirable that these parameters be measured on the same occasion (3). Unfortunately, few tests can fulfill this need. The frequently sampled intravenous glucose tolerance (FSIGT) test provides estimates of both insulin secretion (acute insulin response [AIR]) and S_I, but the S_I is derived from the insulin values during the test and thereby vulnerable to errors during conditions of impaired β-cell function (4–6). In addition, the test cannot be used in insulin-treated patients.

The hyperglycemic clamp has been used for estimation of both S_I and β-cell function in humans (7,8). Although this test may represent the gold standard for the assessment of β-cell function, the test has inherent problems in the assessment of S_I. Glucose uptake is influenced by the ambient plasma glucose concentrations, particularly at low insulin concentrations (9).

The CIGMA test measures insulin sensitivity and integrated insulin response to a low-dosage infusion of glucose, but does not describe the dynamics of insulin secretion (10). The homeostasis model assessment (HOMA) uses fasting values of insulin and glucose to estimate S_I and β-cell function, and thus describes the basal rather than the insulin-stimulated state. Both tests estimate S_I from endogenous insulin secretion and can therefore only be used in subjects with adequate β-cell function (10,11).

Undoubtedly, the combination of the euglycemic and hyperglycemic clamp would represent the gold standard for simultaneous assessment of S_I and β-cell function, but they cannot really be performed on the same occasion. However, the first- or early-phase insulin secretion can be measured during an intravenous glucose tolerance test (IVGTT). To allow assessment of insulin secretion and S_I on the same day for phenotyping purposes of individuals participating in the Botnia study (12), we combined the two tests: a regular IVGTT followed 60 min later by a euglycemic-hyperinsulinemic (45 mU/m^2) clamp for another 120 min (the Botnia clamp) (13). This not only allowed

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Abbreviations: AIR, acute insulin response; CV, coefficient of variation; DI, disposition index; DR, discriminant ratio; EGP, endogenous glucose production; FFM, fat-free mass; FPIR, first-phase insulin response; FSIGT test, frequently sampled intravenous glucose tolerance test; HOMA, homeostasis model assessment; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; NGT, normal glucose tolerance; S_I, insulin sensitivity; WHO, World Health Organization.

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

Table 1—*Clinical characteristics of subjects included in the different tests*

	Botnia clamp reproducibility study	Botnia clamp and euglycemic clamp	Application of Botnia clamp	
			FH^- subjects	FH^+ subjects
n (M/F)	13 (11/2)	9 (5/4)	16 (7/9)	25 (10/15)
Age (years)	46.1 ± 12.1	57 ± 16	42.9 ± 8.6	44.3 ± 11.7
BMI (kg/m^2)	25.5 ± 4.4	27 ± 3	25.3 ± 2.6	26.9 ± 4.3
Fasting plasma-glucose (mmol/l)				
NGT	5.4 ± 0.4 (*n* = 5)	5.1 ± 0.3 (*n* = 4)		
IGT/diabetes	7.2 ± 1.2 (*n* = 8)	6.4 ± 1.3 (*n* = 5)		
Glucose (mmol/l)				
Fasting	—	—	5.4 ± 0.1	5.3 ± 0.4
30 min	—	—	7.8 ± 1.7	7.6 ± 1.6
60 min	—	—	7.1 ± 1.4	6.8 ± 1.1
120 min	—	—	5.4 ± 0.8	5.9 ± 1.7
Waist-to-hip ratio	—	—	0.87 ± 0.07	0.86 ± 0.08
Blood pressure (mmHg)				
Systolic	—	—	123 ± 12	132 ± 17
Diastolic	—	—	80 ± 7	84.5 ± 17
Triglycerides (mmol/l)	—	—	0.96 ± 0.2	1.03 ± 0.8
Cholesterol (mmol/l)	—	—	5.3 ± 0.8	5.4 ± 0.8
HDL cholesterol (mmol/l)	—	—	1.3 ± 0.3	1.25 ± 0.2

Data are *n* or means ± SD.

independent measures of β-cell function and S_I, but also took S_I into account when studying β-cell function (disposition index [DI]). This test was validated in the current study by comparing the results with euglycemic clamps without prior glucose bolus and by performing repeated measurements in the same individuals. We also evaluated the Botnia clamp in subjects with and without a family history of diabetes. The data clearly demonstrated the need to account for the degree of insulin resistance when comparing β-cell function between individuals.

RESEARCH DESIGN AND METHODS

Subjects

Subjects were classified into different stages of glucose tolerance according to the new World Health Organization (WHO) criteria (14). Informed consent was obtained from all subjects, and the studies were approved by the local ethics committees. Subject characteristics are given in Table 1.

Protocol 1: reproducibility of the Botnia clamp

For this protocol, 13 subjects (11 men, 2 women; 5 with normal glucose tolerance [NGT], 8 with type 2 diabetes or impaired glucose tolerance [IGT]) participated in two Botnia clamps performed at a 1-week interval.

Protocol 2: comparison of the Botnia clamp with the euglycemic clamp

The study cohort for this protocol included nine subjects (five men, four

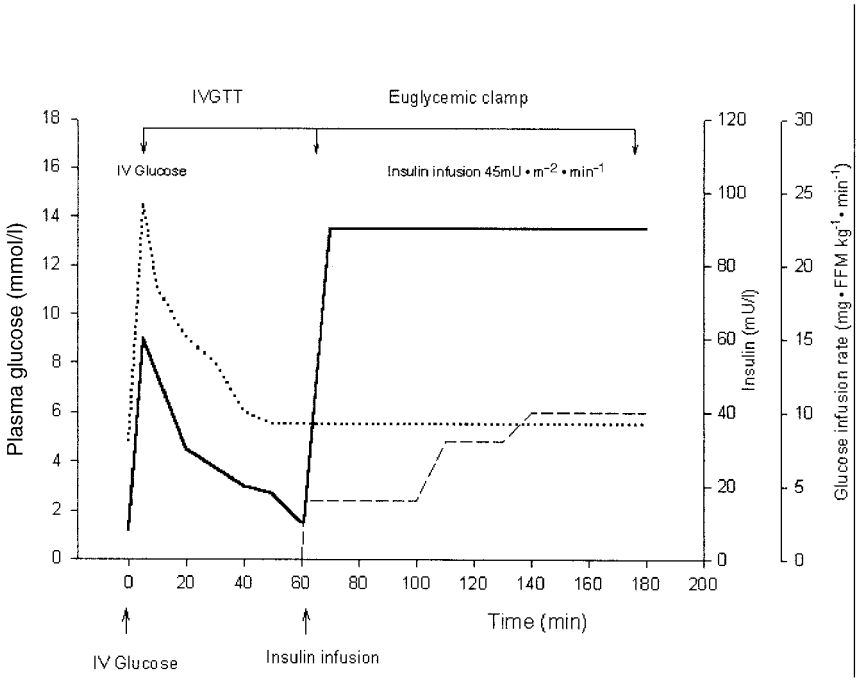

Figure 1—*Schematic description of the Botnia clamp. Plasma glucose (dotted line), insulin concentrations (solid line), and the glucose infusion rates (long dash) during the Botnia clamp. IV, intravenous.*

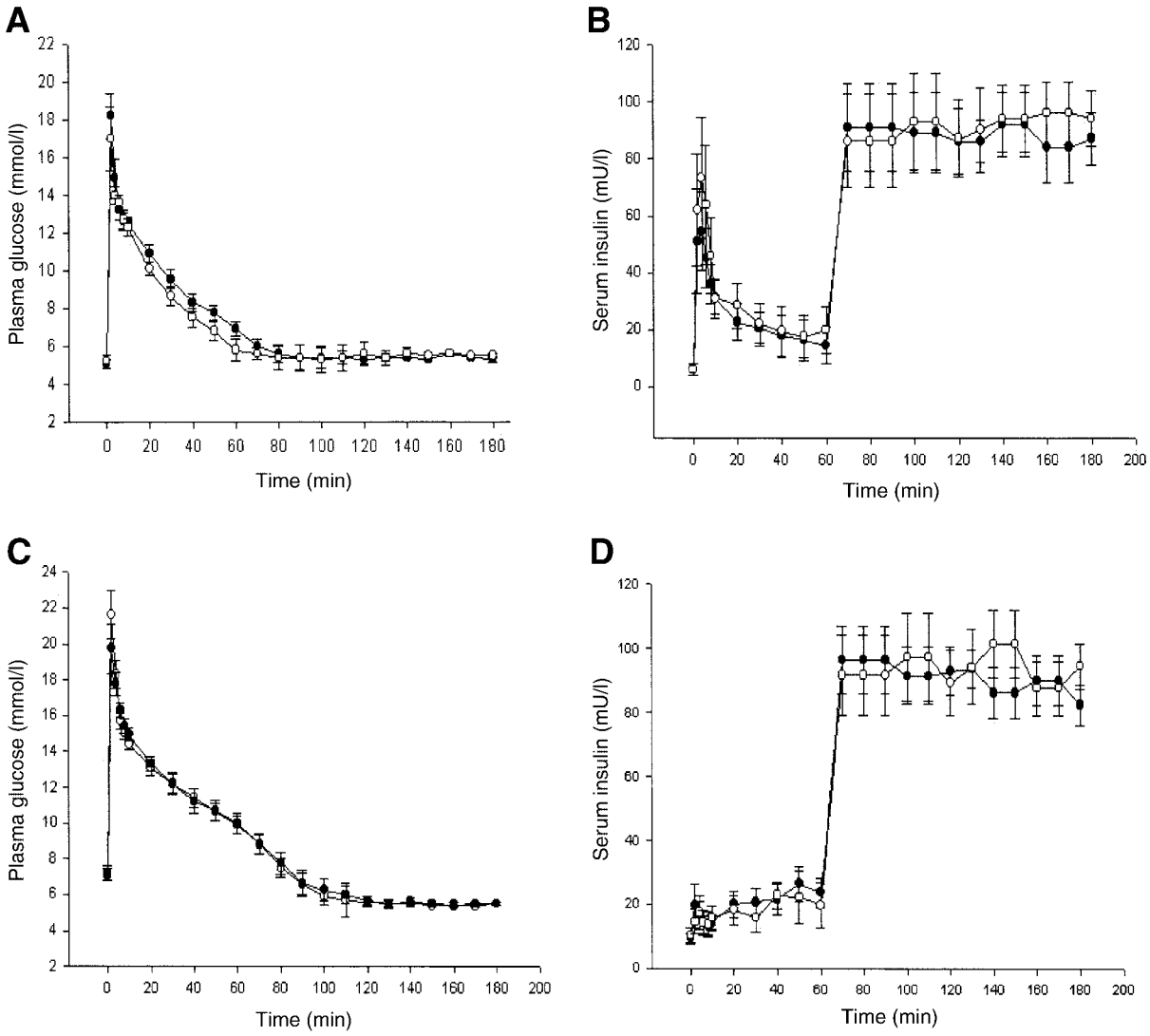

Figure 2—*Plasma glucose (*A *and* C*) and insulin concentrations (*B *and* D*) during repeat Botnia clamps. Clamp 1 (○) and clamp 2 (●) in subjects with NGT (*A *and* B*) and IGT/diabetes (*C *and* D*).*

women; four with NGT, five with type 2 diabetes or IGT) who participated in random order in a Botnia clamp and a euglycemic, hyperinsulinemic clamp at a 1-week interval.

Protocol 3: application of the Botnia clamp in subjects with and without a family history of diabetes

We also applied the Botnia clamp to 41 glucose-tolerant subjects, 25 of whom had a first-degree relative with type 2 diabetes.

Methods

The Botnia clamp was designed to obtain independent measures of insulin secretion and S_I during the same test (Figure 1). In brief, 0.3 g/kg body wt of a 20% glucose solution was given at time 0. Blood samples for the measurement of plasma glucose and serum insulin were obtained at –10, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, 120, and 180 min. The incremental trapezoidal area during the first 10 min was called first-phase insulin response (FPIR). The FPIR was also measured as the sum of three insulin concentrations at 2, 4, and 6 min after the glucose challenge. The incremental insulin secretion during the last 50 min was called the second-phase insulin secretion. After 60 min, a priming dose of insulin was given followed by an infusion (45 mU/m^2) of short-acting human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) for 120 min, thereby achieving steady-state insulin concentrations of 88.5 ± 5.4 and 92.5 ± 5.4 mIU/l during the last 60 min of clamp 1 and clamp 2, respectively. Blood samples for the measurement of plasma glucose were obtained at 5-min intervals throughout the clamp. A variable infusion of 20% glucose was started to maintain the plasma glucose concentration at 5.5 mmol/l. The mean coefficient of variation (CV) of glucose during the clamps was 6%. S_I (M-value) was calculated from the glucose infusion rates during the last 60 min of the euglycemic clamp and as the S_I index (M/I_{clamp}; mg glucose · kg^{-1} body wt · min^{-1}:mU/l insulin) as the ratio of glucose infusion rates during the last 60 min and the mean steady-state insulin levels during the last 60 min of the clamp ([M/I] · 100) (6). The DI is a measure of insulin secretion adjusted for insulin sensitivity

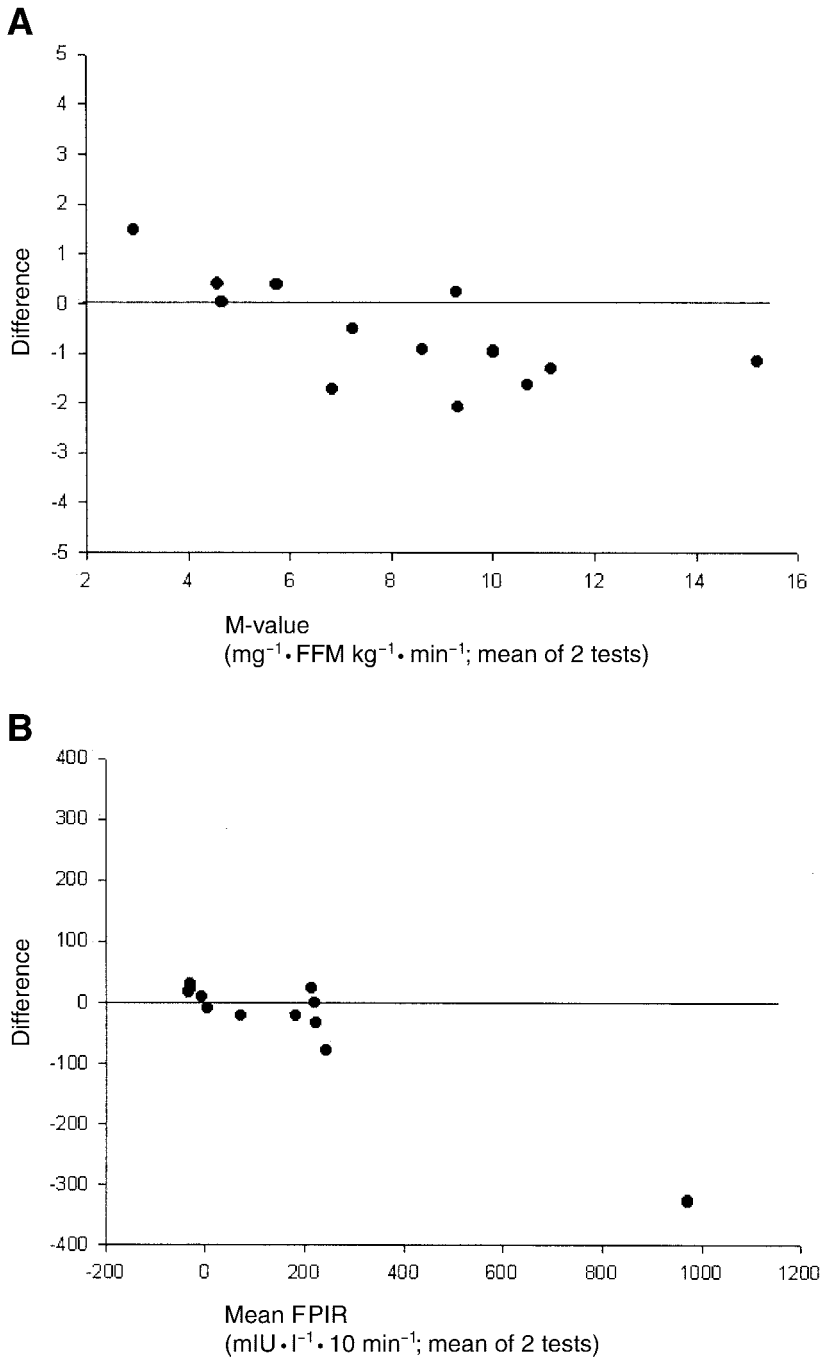

Figure 3—*Altman-Bland plots demonstrating the distribution of variability (first minus second test difference versus mean) of S_I (M-value;* A*) and insulin secretion (FPIR;* B*) across a range of values.*

and was calculated from the product of the FPIR and the M-value (3). In a different group of nine subjects, the Botnia clamp and a euglycemic-hyperinsulinemic clamp without prior glucose infusion was performed to study the potential influence of the glucose bolus on the M-value.

Subjects with and without family history of diabetes (protocol 3) also participated in an OGTT by ingesting 75 g of glucose in a volume of 300 ml (Glucodyn; Leiras, Turku, Finland) after a 12-h overnight fast. Samples for the measurement of glucose and insulin were drawn at −10, 0, 30, 60, and 120 min.

Anthropometric measurements

Body height, weight, and fat-free mass (FFM) were recorded. The FFM was measured with a bioelectrical impedance technique using a two-terminal portable impedance analyzer (BIA 101, RJL; Akern, Copenhagen, Denmark) (15).

Assays

Plasma glucose was measured in duplicate by a glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin concentrations were measured with a double antibody enzyme-linked immunosorbent assay (Dako, Cambridgeshire, U.K.) with an intra-assay CV of 7.5% and an interassay CV of 7%.

Statistical analysis

Data are expressed as means ± SEM or SD. Statistical analyses included Mann Whitney test and Spearman rank correlation and were carried out using the NCSS statistical software (Number Cruncher Statistical System, version 6). Data for insulin were log transformed for normality. Variability and the repeatability of measures of S_I and insulin secretion were assessed using the CV and the recently proposed measure, the discriminant ratio (DR) (16). For the CV, the following formula was used:

$$CV\% = (SD/mean) \cdot 100$$

The DR was measured using the following formula:

$$DR = \sqrt{[MS_B - MS_w)/(k \times MS_w)]}$$

in which MS_B and MS_w are the between- and within-subject error terms derived from a standard repeated measure of ANOVA and k is the number of replicate tests in each subject (16).

RESULTS

Protocol 1: reproducibility of Botnia clamp

The first study examined reproducibility of measures of insulin secretion and S_I obtained from the Botnia clamp. Glucose and insulin values during the repeat Botnia clamps were comparable in subjects with normal (Fig. 2*A* and *B*) and abnormal glucose tolerance (Fig. 2*C* and *D*). The rates of insulin-stimulated glucose uptake did not differ between the two

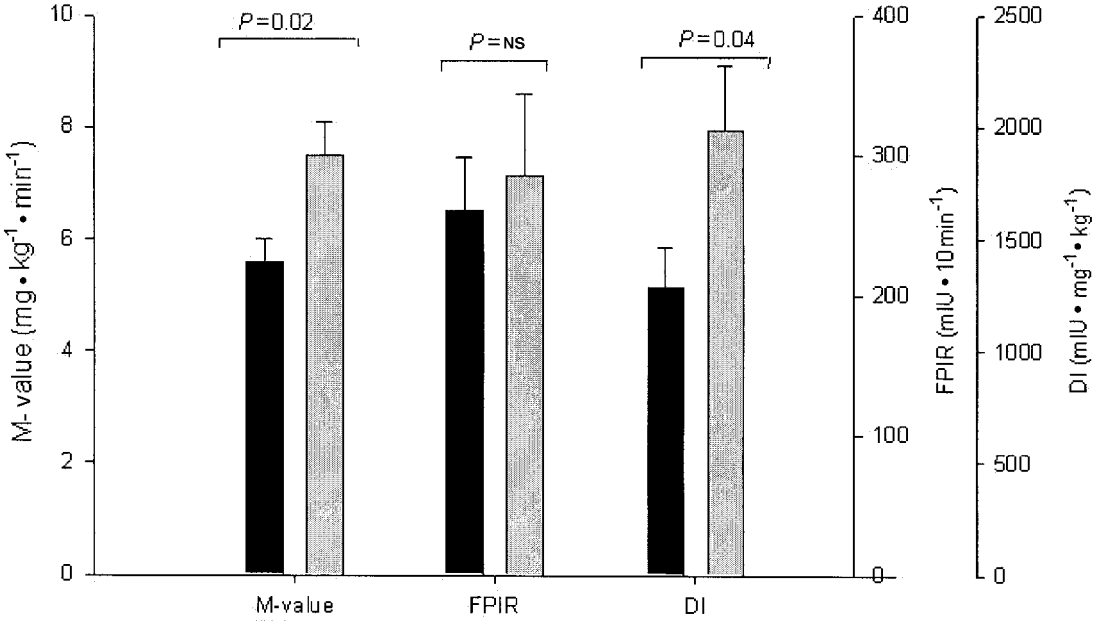

Figure 4—*FPIR, DI, and S_I (M-value) in nondiabetic subjects with (■) and without (▨) family history of diabetes.*

tests, whether measured as the M-value (NGT: 10.4 ± 1.2 and 11.3 ± 1.2 mg · FFM kg^{-1} · min^{-1}; type 2 diabetes/IGT: 6.3 ± 0.9 and 6.7 ± 0.9 mg · FFM kg^{-1} · min^{-1}) or as the S_I index M/I_{clamp} (NGT: 10.2 ± 1.3 and 10.7 ± 1.4 mg · FFM kg^{-1} · min^{-1}/mU/l^{-1}; type 2 diabetes/IGT: 6.3 ± 1.5 and 6.7 ± 1.5 mg · FFM kg^{-1} · min^{-1}/mU/l^{-1}), thereby yielding a mean CV for repeat M-values from the Botnia clamps of 9.0% and a correlation coefficient of $r = 0.983$, $P < 10^{-5}$. The variability and reproducibility of the M-value were also assessed by Altman-Bland plots for the repeat M-values (Fig. 3*A*), and showed a uniform variability across the range of values from NGT to diabetes. The DR for M-value from repeat clamps was 1.81 (CI 1.11–2.78). In contrast, the CV for mean fasting insulin concentrations from three samples on 2 different clamp days was 20%, and therefore the S_I measured as HOMA obtained from the fasting glucose and insulin on the day of the clamp had a CV of 19%.

Although repeat Botnia clamps yielded similar FPIRs regardless of glucose tolerance (152 ± 81 vs. 186 ± 81 mIU · l^{-1} · 10 min^{-1}; NS) and a strong correlation was observed between FPIRs performed on 2 different days ($r = 0.923$, $P < 0.005$), the CV for the repeat tests was 27% in subjects with NGT and 12% in subjects with IGT, thereby yielding a combined CV in NGT/IGT subjects of 23%. The FPIR measured as a sum of the three insulin values at 2, 4, and 6 min also yielded a CV of 25% in NGT/IGT subjects. The CV for FPIR in subjects with diabetes was not included, as all subjects lacked the FPIR on repeat studies. The Altman-Bland plots (Fig. 3*B*) show that the variability is to some extent proportional to the magnitude of the FPIR. Similarly, the CV for second-phase insulin secretion in subjects with NGT/IGT was 22%. The DR for the repeat FPIR was 1.09 and for the second-phase insulin response was 1.45.

Protocol 2: comparison of M-values obtained with the Botnia and euglycemic clamps

The M-values obtained from the Botnia and euglycemic clamps (7.23 ± 1.14 and 7.79 ± 1.14 mg · FFM kg^{-1} · min^{-1}; NS) were comparable and correlated strongly ($r = 0.953$; $P < 0.005$).

Protocol 3: application of the Botnia clamp in glucose-tolerant subjects with and without a family history of diabetes

As previously described (12), FH+ subjects had lower insulin-stimulated glucose uptake compared with FH− subjects (5.6 ± 0.4 vs. 7.5 ± 0.6 mg · kg^{-1} · min^{-1}; $P = 0.02$), whereas no difference was observed in the FPIRs between the two groups (258 ± 37 vs. 285 ± 59 mIU · l^{-1} · 10 min^{-1}; NS). However, when β-cell function was expressed as a DI, FH+ subjects showed a significant reduction in their β-cell function (1,284 ± 181 vs. 1985 ± 288 mUl · mg · kg^{-1} · min^{-1}; $P = 0.04$) (Figure 4).

CONCLUSIONS— The present study validated a means to assess S_I and β-cell function from the same test, the Botnia clamp. S_I measured with the Botnia clamp was highly reproducible, with a CV of 9.0%, and was independent of subjects' glucose tolerance. It may be argued that the glucose bolus given at the start of the study affected the subsequent S_I measured during the clamp. However, this did not occur, as the M-value from the Botnia clamp was similar to and correlated strongly with the M-value obtained from the euglycemic clamp without prior glucose bolus. All subjects with IGT and type 2 diabetes reached the desired steady-state plasma glucose level during the last 60 min of the Botnia clamp.

In addition to the CV, we also estimated the DR (16), which takes into account the within- and intersubject variations. Indeed, the S_I obtained from the Botnia clamp showed a low CV, high DR, and low variability across a wide range of glucose tolerance.

In contrast, a slightly higher variation was observed in the FPIR values between the tests. This finding was in agreement

with previous reports of up to 50% variation in FPIR, even in subjects with NGT (17–19). Given that the mean of three fasting insulin concentrations demonstrated considerable variation, it is not surprising that the glucose-stimulated insulin values showed an even greater variation. This may have represented a biological day-to-day variation rather than a technical one. Also, standard formulas for estimating CV usually underestimate CV at the lower range (16). Of note, all patients with type 2 diabetes lacked the FPIR on repeat studies.

The study of quantitative traits such as S_I and insulin secretion has been proposed as one way to reduce phenotypic heterogeneity in genetic studies. Therefore, there is an urgent need for reliable tests of S_I and β-cell function for phenotyping purposes in genetic studies. The Botnia clamp provides a simple and reproducible tool for such purposes. In fact, the length of the clamp is the same as the FSIGT test, and the number of insulin samples can be reduced to five or six (0, 4, 6, 8, and 10 min and two samples at 150 and 180 min for checking the insulin levels during infusion). It also has the advantage that tracers can be added for estimating endogenous glucose production (EGP), although it remains to be shown whether the preceding glucose bolus affected the EGP during the Botnia clamp. Another advantage is that it can be combined with indirect calorimetry to obtain estimates of intracellular substrate partitioning.

We also applied the Botnia clamp to subjects with and without a family history of diabetes. As expected, the FH+ subjects were insulin resistant, whereas the unadjusted FPIR was similar in subjects with and without a family history of diabetes. However, when the degree of insulin resistance was taken into account (DI), the FH+ subjects demonstrated impaired β-cell function. Several studies have shown that first-degree relatives of patients with type 2 diabetes are insulin resistant (20–22), but few studies have shown the presence of a defect in insulin secretion if the subjects have had NGT (22–25). This is not surprising given the strong relation between insulin secretion and S_I; if both S_I and insulin secretion are impaired, there should be some degree of impaired glucose tolerance (2).

In conclusion, the Botnia clamp provides reliable and independent measures of S_I and β-cell function during the same test. As illustrated above, knowledge of the degree of S_I is important when presenting data on insulin secretion.

A computer program for the Botnia clamp is available on request, www.endo.mas.lu.se.

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