Annals of Internal Medicine | ORIGINAL RESEARCH

Diagnostic Accuracy of an "Amended" Insulin–Glucose Ratio for the Biochemical Diagnosis of Insulinomas

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Background: Recent biochemical diagnostic guidelines for insulinomas require demonstration of hypoglycemia with inappropriately elevated (nonsuppressed) insulin, C-peptide, or proinsulin, but these criteria may overlap with those in patients without insulinomas. Use of an "amended" insulin–glucose ratio that accounts for the normal variation in insulin secretion according to prevailing glycemia may improve diagnostic accuracy.

Objective: To compare the diagnostic accuracy of current diagnostic guideline criteria with the amended insulin–glucose ratio in patients with a suspected insulinoma.

Design: Retrospective cohort study.

Setting: 2 specialized university departments in Germany.

Patients: 114 patients with suspected hypoglycemia over 10 years having diagnostic prolonged fasts.

Measurements: Glucose, insulin, C-peptide, and the amended insulin–glucose ratio were measured during and at discontinuation of prolonged fasts.

Results: Of 114 patients who were evaluated, 49 had surgical resection of histologically confirmed insulinomas. Insulinoma was excluded in 65 patients; follow-up for a mean of 10 years (range, 0 to 16 years) showed no progressively severe hypoglycemic events or diagnoses of insulinoma. Patients with insulinoma had lower glucose levels and higher insulin and C-peptide levels overall than did control patients at the end of prolonged fasts, but there was considerable overlap. The amended insulin–glucose ratio correctly identified 48 of 49 patients with insulinoma and excluded the diagnosis in 64 of 65 control patients, resulting in positive and negative predictive values of 0.98 (95% CI, 0.89 to 1.00) and 0.99 (CI, 0.92 to 1.00), respectively, compared with 0.75 (CI, 0.63 to 0.85) and 0.98 (CI, 0.89 to 1.00), respectively, for glucose, insulin, and C-peptide concentration criteria.

Limitation: The study had a retrospective design, no proinsulin concentrations were available, and a nonspecific insulin immunoassay (crossreactive with proinsulin) was used.

Conclusion: The amended insulin–glucose ratio showed improved diagnostic accuracy over established criteria that use glucose, insulin, and C-peptide concentrations.

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Insulinoma is a rare endocrine pancreatic tumor characterized by autonomous insulin and proinsulin secretion that leads to inappropriately elevated plasma insulin or proinsulin levels (1–4). This elevation suppresses hepatic glucose output (5), thereby causing hypoglycemia, especially during periods of fasting. Hypoglycemic symptoms may be rare in patients with insulinoma (6). Supervised prolonged fasts are widely used to provoke hypoglycemic episodes and have been advocated as the diagnostic gold standard for documenting hypoglycemia and differentiating between hyperinsulinemic and nonhyperinsulinemic forms of fasting hypoglycemia (2, 3, 7). Insulin and proinsulin concentrations remain inappropriately elevated even in the presence of hypoglycemia, which normally suppresses insulin secretion (8), in patients with insulinoma (2–4). Evaluation of insulin and proinsulin concentrations must consider the simultaneously measured glucose concentrations (9, 10).

The Endocrine Society recently issued clinical practice guidelines for the diagnosis of hypoglycemic disorders based on hypoglycemia (glucose level <3.1 mmol/L [<55 mg/dL]), elevated insulin level (≥18 pmol/L), and elevated C-peptide level (≥0.2 nmol/L [≥0.61 ng/mL]), supplemented with a proinsulin level greater than 5 pmol/L and a suppressed β-hydroxy-butyric acid level (11). However, the guidelines do not specify how many of these criteria must be met to support the biochemical diagnosis of an insulinoma. It is also not clear whether using these criteria can safely exclude the diagnosis in patients with suspected but not confirmed insulinoma. Furthermore, diagnostic accuracy may be lost if the effect of the prevailing glucose concentration on insulin secretion, which is evaluated by calculating insulin–glucose ratios, is not considered (9). Accordingly, an "amended" insulin–glucose ratio has been proposed that is derived from the simple insulin–glucose ratio by subtracting 1.7 mmol/L (30 mg/dL) from the measured glucose concentrations (12), based on the assumption that human β cells secrete negligible amounts of insulin at a glucose concentration less than 1.7 mmol/L (<30 mg/dL). However, the amended insulin–glucose ratio has been evaluated and diagnostic cutoff values defined only in samples obtained after an overnight fast in 5 patients with histologically confirmed insulinoma (12).

We aimed to determine the diagnostic accuracy of the amended insulin–glucose ratio (3, 12) compared with the criteria in the recently published Endocrine Society guidelines (11).

METHODS

Study Design

We performed a retrospective chart review to compare the diagnostic performance of the Endocrine Society

Context

The most accurate approach to the diagnosis of insulinoma in patients with suspected hypoglycemic disorders is not clear, and practice guidelines do not indicate precisely which biochemical indices are required.

Contribution

In a review of results from prolonged fasts in patients with suspected hypoglycemia, an "amended" insulin–glucose ratio that accounts for prevailing plasma glucose concentrations was more accurate than current practice guideline definitions for the diagnosis of insulinoma.

Implication

This approach to diagnosis may be useful in evaluating patients with a suspected hypoglycemic disorder.

—The Editors

guidelines with the amended insulin–glucose ratio in a cohort of patients in whom insulinoma had been either confirmed or excluded.

Patients

Charts of all consecutive patients who presented with spontaneous hypoglycemic episodes at 2 large German referral centers (Department of Medicine, Division of Metabolic Diseases at the University of Düsseldorf and Department of Medicine, Division of Gastroenterology and Endocrinology at the University of Göttingen) between April 1985 and January 1995 were identified by manual searches for insulin and C-peptide measurements among patients who had prolonged fasts. Patients had, as a rule, been referred by primary care physicians or psychiatrists who had observed hypoglycemia without having performed any tests. In Düsseldorf, some patients had outpatient C-peptide–suppression tests (13), and those with positive or equivocal results were subsequently admitted for supervised prolonged fasting.

Diagnostic Procedures

Supervised prolonged fasts were done according to a standardized protocol at both participating centers and began at 8:00 a.m. The time of ingestion of the last meal ($n = 15$) or a 75-g oral glucose load ($n = 99$ [86.8% of the total patient population]) was designated as hour 0. Capillary blood for the measurement of plasma glucose (Glucose Analyzer 2; Beckman Coulter, Munich, Germany; glucose oxidase method) and venous blood for the measurement of insulin and C-peptide were drawn at regular intervals, at least every 4 hours. The frequency of blood sampling was increased to every 2 hours, every hour, or every half hour if a decrease in plasma glucose concentrations was seen or when symptoms of hypoglycemia developed and termination had to be considered. Bedside glucose measurements were taken immediately to guide the decision to discontinue the fasts, but all measurements reported are laboratory determinations. Prolonged fasts were discontinued if symptoms of hypoglycemia were present at a plasma glucose level less than 2.5 mmol/L (<45 mg/dL), if glucose concentrations were less than 2.2 mmol/L (<40 mg/dL) in successive blood samples, or if more than 48 hours elapsed without hypoglycemia.

Plasma insulin was measured by radioimmunoassay (Insulin RIA 100; Pharmacia, Freiburg, Germany). C-peptide was measured by using commercial radioimmunoassays (Byk-Sangtec Diagnostica [Dietzenbach, Germany] or RIAgnost HC-Peptid [Behringwerke, Marburg, Germany]). For details, see the **Appendix** (available at www.annals.org). None of the procedures or assay methods was changed during the 10-year period of our analysis.

The patients' respective physicians made the clinical decision to proceed to surgery independent of the present analysis. The diagnostic accuracy of suggested criteria for the biochemical diagnosis of an insulinoma is compared post hoc in the present study according to the approach described in **Figure 1**. We considered patients with no evidence of an insulinoma to be control patients, and we obtained information to assess symptoms of recurrent hypoglycemia by contacting them, their family, or their primary care physician. We used patients who were diagnosed with insulinoma who had repeated fasting studies after successful surgical resection as a second control group but did not include them in any statistical comparisons.

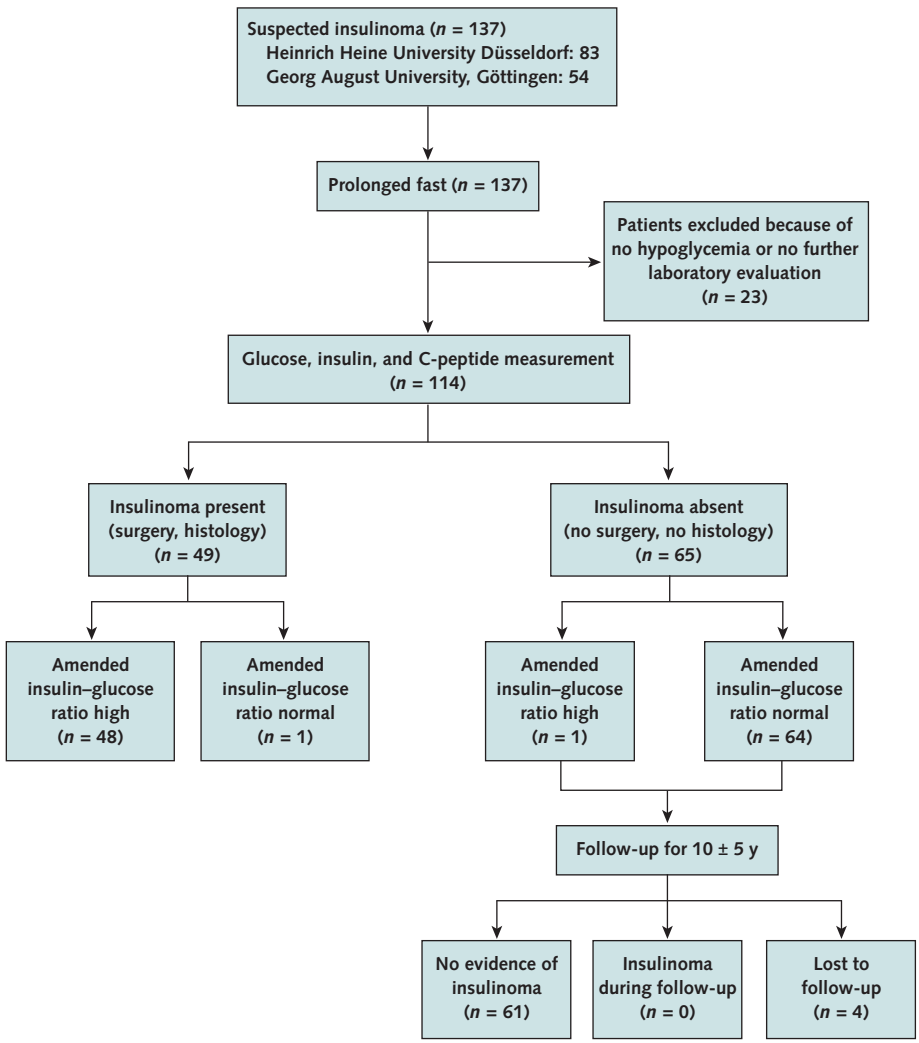
Diagnostic Criteria

Criteria for inappropriately high insulin secretory activity relative to the ambient glucose concentration were evaluated in samples taken at the time of discontinuation because of hypoglycemia or because 48 hours had elapsed. Insulin–glucose ratios were calculated with the concentrations expressed in pmol/L and mmol/L, respectively, with a normal range of 32.2 (pmol/L)/(mmol/L) or less. We derived amended insulin–glucose ratios by subtracting 1.7 mmol/L (30 mg/dL) from the glucose concentration, with a normal range of 53.6 (pmol/L)/(mmol/L) or less (12). We assessed Endocrine Society criteria for diagnostic accuracy based on single variables or on combinations of 2 or 3 fulfilled criteria.

Statistical Analysis

We present patient characteristics and experimental results as means with SDs and differences between patient samples as mean differences with 95% CIs. We assessed significances of differences in the comparison of patients with insulinoma and control patients with 1-way analysis of variance (continuous variables), the Fisher exact test (2×2 tables with categorical variables), or the chi-square test (larger contingency tables with categorical variables) by using Statistica, version 5.0 (StatSoft, Tulsa, Oklahoma). Analysis of time to discontinuation of prolonged fasts or to

Figure 1. **Study flow diagram.**



All diagnosed insulinomas were confirmed by surgery and histologic evaluation. Amended insulin–glucose ratios of at least 53.6 (pmol/L)/(mmol/L) were considered abnormally high (12).

low glucose concentrations was performed using the Kaplan–Meier method and the log-rank test with GraphPad Prism, version 5.02 (GraphPad Software, La Jolla, California). We calculated diagnostic sensitivity and specificity, positive and negative predictive values, and positive and negative likelihood ratios, as well as their 95% CIs, using MedCalc, version 12.2.1.0 (MedCalc Software, Mariakerke, Belgium).

Role of the Funding Source

This study received no specific funding.

RESULTS

We identified 114 consecutive patients with symptoms suggestive of spontaneous hypoglycemia who had undergone prolonged fasts (**Table 1**). No patient received antidiabetic or other drugs that affected glucose metabolism. None had advanced renal insufficiency (serum creatinine level ≥133 μmol/L [≥1.5 mg/dL]). A larger proportion of patients at Düsseldorf was diagnosed with insulinoma than at Göttingen.

Forty-nine of the patients received a biochemical diagnosis of insulinoma and had surgery. Presence of an insulinoma was confirmed by histologic evaluation in each case. Tumor characteristics are presented in **Appendix Table** (available at www.annals.org).

Sixty-five control patients in whom biochemical criteria provided evidence against insulinoma were diagnosed with various conditions (**Table 2**). No case of insulinoma was identified during a mean of 10 years (range, 0 to 16 years). Four control patients were lost to follow-up. Seven control patients died; 6 had no evidence of recurrent hypoglycemic symptoms, and no information could be obtained for the seventh. Eight patients with insulinoma were re-

Table 1. **Patient Characteristics***

Characteristic	Patients With Insulinoma (n = 49)	Control Patients (n = 65)	Difference (95% CI)	P Value†	Patients With Insulinoma After Successful Surgery
Female, *n (%)*	33 (67.4)	45 (69.2)	−1.8 (−19.2 to 15.4)	0.84	6 (75.0)
Mean age (SD), *y*	51 (15)	41 (15)	10 (4 to 15)	<0.001	47 (13)
Mean height (SD), *cm*	169 (9)	169 (9)	−1 (−4 to 3)	0.67	170 (7)
Mean weight (SD), *kg*	74 (13)	72 (17)	2 (−3 to 8)	0.43	70 (8)
Mean BMI (SD), kg/m^2	26.2 (3.9)	25.0 (4.5)	1.2 (−0.4 to 2.7)	0.152	24.2 (2.3)
Baseline OGTT measurements					
Patients with OGTT performed, *n (%)*	38 (77.6)	61 (93.8)	16.2 (2.3 to 31.1)	0.023	6 (75.0)
Mean FPG level (SD), *mmol/L*	2.8 (1.2)	4.4 (0.8)	−1.6 (−2.0 to −1.1)	<0.001	4.2 (1.2)
FPG level <3.1 mmol/L, *n (%)*	23 (62.1)	1 (1.6)	60.5 (41.9 to 75.8)	<0.001	0 (0.0)
120-min glucose level, *n (%)*	–	–	–	0.60	–
Normal (<7.8 mmol/L)	27 (71.1)	46 (75.4)	−4.4 (−13.6 to 22.2)	–	5 (83.3)
Impaired (7.8–11.1 mmol/L)	11 (29.0)	14 (23.0)	6.0 (−11.6 to 23.6)	–	1 (16.7)
Diabetic (≥11.1 mmol/L)	0 (0.0)	1 (1.6)	−1.6 (−7.8 to 8.7)	–	0 (0.0)
Results of prolonged fasts					
Mean time to first plasma glucose measurement <3.1 mmol/L (SD), *h*	8 (7)	39 (12)‡	−31 (−25 to −28)	<0.001	48 (2)‡
Mean time to discontinuation of prolonged fast (SD), *h*	19 (11)	48 (0)	−29 (−26 to −22)	<0.001	48 (0)
Mean glucose concentration at discontinuation (SD), *mmol/L*	1.3 (0.5)	3.2 (0.7)	−1.4 (−1.6 to −1.1)	<0.001	3.2 (1.0)
Mean insulin concentration at discontinuation (SD), *pmol/L*	142 (145)	32 (17)	110 (69 to 152)	<0.001	36 (25)
Mean C-peptide concentration at discontinuation (SD), *nmol/L*	0.89 (0.40)	0.34 (0.20)	0.55 (0.34 to 0.67)	<0.001	0.34 (0.21)

BMI = body mass index; FPG = fasting plasma glucose; OGTT = oral glucose tolerance test.
* To convert glucose values from mmol/L to mg/dL, divide by 0.0555. To convert C-peptide values from nmol/L to ng/mL, divide by 0.331.
† Analysis of variance (continuous variables), Fisher exact test (2 × 2 tables, categorical variables), or chi-square test (larger tables, categorical variables) comparing patients with insulinoma and control patients. Patients with insulinoma after successful surgery were not included in this analysis.
‡ For patients not reaching this end point within 48 h, a duration of 49 h was used in the calculation.

studied after successful surgery. In general, biochemical results were the same as those for control patients in all respects.

Prolonged Fasting

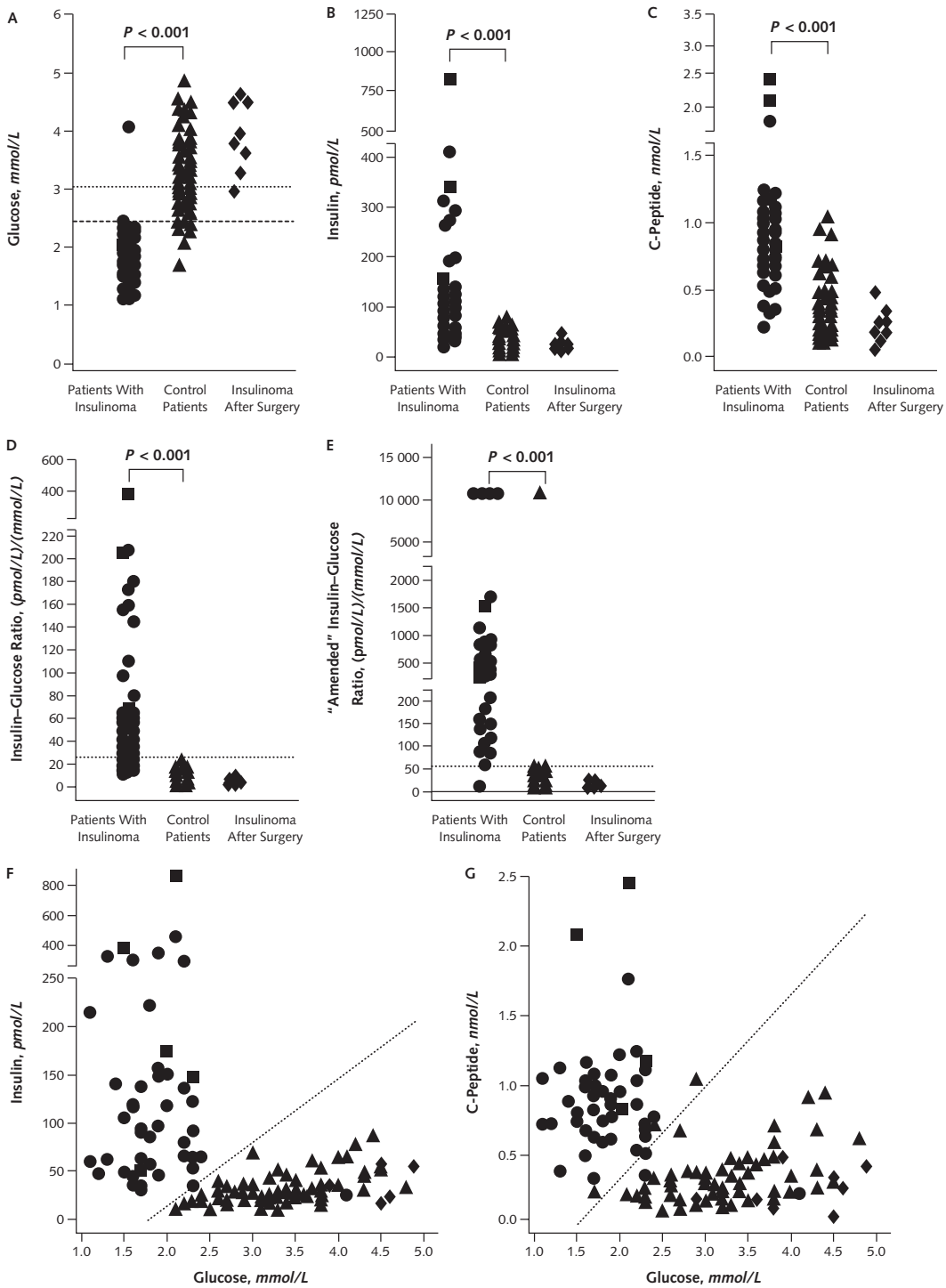
In all patients with insulinoma, prolonged fasts had to be terminated because of chemical (mean glucose level, 1.9 mmol/L [34 mg/dL] [range, 1.1 to 4.1 mmol/L {20 to 73 mg/dL}]) and clinical hypoglycemia within 48 hours (mean, 19 hours), whereas none of the control patients or patients with insulinoma who had successful surgery required early discontinuation (**Appendix Figure 1** [*top*], available at www.annals.org).

Table 2. **Diagnoses and Follow-up in Control Patients**

Diagnostic Category	Patients, *n (%)**	Deaths, *n (%)*†	Lost to Follow-up, *n (%)*†	Mean Duration (SD), *y*‡	Relevant Information Available, *n (%)*§	No Symptoms, *n (%)*†	Characteristic Symptoms Without Hypoglycemia, *n (%)*†	Severe Hypoglycemia, *n (%)**
Hypoglycemia associated with early type 1 or type 2 diabetes	6 (9.2)	1 (16.7)	1 (16.7)	8 (6)	5 (83.3)	3 (50.0)	1 (16.7)	1 (16.7)
Erroneously low glucose measurement	4 (6.2)	0 (0.0)	0 (0.0)	8 (1)	4 (100.0)	4 (100.0)	0 (0.0)	0 (0.0)
Postprandial (reactive) hypoglycemia	12 (18.5)	1 (16.7)	0 (0.0)	10 (5)	11 (91.7)	2 (16.7)	6 (50.0)	3 (25.0)
Pancreatic/hepatic mass compatible with endocrine tumor	2 (3.1)	0 (0.0)	0 (0.0)	11 (5)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0.0)
Psychosomatic disorders with symptoms suggestive of hypoglycemia	5 (7.7)	0 (0.0)	0 (0.0)	13 (3)	5 (100.0)	2 (40)	3 (60.0)	0 (0.0)
Cardiovascular dysregulation	8 (12.3)	1 (12.5)	1 (12.5)	10 (4)	7 (87.5)	3 (37.5)	3 (37.5)	1 (12.5)
Seizures	3 (4.6)	1 (33.3)	0 (0.0)	9 (8)	3 (100.1)	2 (66.7)	0 (0.0)	1 (33.3)
Alcohol-related hypoglycemia	1 (1.5)	0 (0.0)	0 (0.0)	12	1 (100.0)	1 (100.0)	0 (0.0)	0 (0)
Factitious hypoglycemia	2 (3.1)	0 (0.0)	0 (0.0)	12 (1)	2 (100.0)	2 (100.0)	0 (0.0)	0 (0)
No organic disorder compatible with symptoms of hypoglycemia	22 (33.8)	2 (9.1)	2 (9.1)	10 (4)	20 (90.9)	16 (80)	4 (18.2)	0 (0)
Total (all control patients)	65 (100)	7 (10.8)	4 (6.2)	10 (5)	60 (92.3)	37 (56.9)	17 (26.2)	6 (9.2)

* Relative to the total number of patients.
† Relative to the number of patients in this diagnostic category.
‡ Includes patients who had died or were not available for follow-up (counted as 0 *y*).
§ Based on direct contact or information provided by patients' primary care physicians or families; thus, the number could be higher than the number in this category minus those who died or were lost to follow-up.

Figure 2. **Plasma values at discontinuation of prolonged fasts.**



Plasma concentrations of glucose (*A*), insulin (*B*), and C-peptide (*C*); insulin–glucose ratios (*D*); and "amended" insulin–glucose ratios (insulin [pmol/L]/[glucose {mmol/L} − 1.7 mmol/L]) (*E*) are shown, as well as insulin (*F*) and C-peptide (*G*) values plotted versus plasma glucose value measured at the time of discontinuation of prolonged fasts in patients with insulinoma (*circles*: benign insulinomas; *squares*: malignant insulinomas), control patients (*triangles*), and patients with insulinoma who were restudied after successful tumor removal (*diamonds*). *P* values indicate results of analysis of variance (comparison between patients with insulinoma and control patients; patients after successful surgery were not included in this analysis). In *A*, the dotted line indicates a glucose concentration of 3.1 mmol/L (55 mg/dL) (one of the criteria advocated by the Endocrine Society), and the dashed line indicates a glucose concentration of 2.4 mmol/L (43 mg/dL), below which prolonged fasts were discontinued in this study. In *D* and *E*, dotted lines indicate the upper normal limits as published previously (9, 10, 12). In *F* and *G*, the dotted lines were drawn to best separate patients with and without insulinoma. To convert glucose values from mmol/L to mg/dL, divide by 0.0555. To convert C-peptide values from nmol/L to ng/mL, divide by 0.331.

Table 3. **Diagnostic Accuracy***

Criterion	Patients Meeting Criteria, *n* (%)		Sensitivity†	Specificity†
	Patients With Insulinoma (*n* = 49)	Control Patients (*n* = 65)		
Variables derived from insulin, C-peptide–glucose ratio, or insulin–C-peptide ratio				
Insulin–glucose ratio >32.2 (pmol/L)/(mmol/L)	36 (73.5)	0 (0.0)	0.73 (0.59 to 0.85)	1.00 (0.95 to 1.00)
Amended insulin–glucose ratio >53.6 (pmol/L)/(mmol/L)	48 (98.0)	1 (2.0)	0.98 (0.89 to 1.00)	0.98 (0.92 to 1.00)
C-peptide–glucose ratio >0.24 (nmol/L)/(mmol/L)	44 (89.8)	3 (4.6)	0.90 (0.78 to 0.97)	0.96 (0.88 to 0.99)
Amended C-peptide–glucose ratio >0.61 (nmol/L)(mmol/L)‡	47 (95.9)	4 (6.2)	0.95 (0.86 to 1.00)	0.94 (0.86 to 0.98)
Insulin–C-peptide ratio >1.00 (pmol/L)/(nmol/L)‡	49 (100)	65 (100.0)	1.00 (0.93 to 1.00)	0.00 (0.00 to 0.06)
Variables suggested by the Endocrine Society clinical practice guideline				
Glucose level <3.1 mmol/L	48 (98.0)	26 (40.0)	0.98 (0.89 to 1.00)	0.60 (0.47 to 0.72)
Insulin level ≥17.9 pmol/L	49 (100.0)	55 (84.6)	1.00 (0.93 to 1.00)	0.15 (0.07 to 0.26)
C-peptide level ≥0.2 nmol/L	49 (100.0)	52 (80.0)	1.00 (0.93 to 1.00)	0.20 (0.11 to 0.32)
All 3 criteria met	48 (98.0)	16 (24.6)	0.98 (0.89 to 1.00)	0.75 (0.63 to 0.85)
2 criteria met§	1 (2.0)	37 (56.9)	0.02 (0.00 to 0.11)	0.43 (0.31 to 0.56)
1 criterion met§	0 (0.0)	11 (16.9)	0.00 (0.00 to 0.07)	0.83 (0.72 to 0.91)
No criteria met	0 (0.0)	1 (2.0)	0.00 (0.00 to 0.07)	0.98 (0.92 to 1.00)
Glucose level <3.1 mmol/L and insulin level ≥17.9 pmol/L	48 (98.0)	21 (32.3)	0.98 (0.89 to 1.00)	0.68 (0.55 to 0.79)
Glucose level <3.1 mmol/L and C-peptide level ≥0.2 nmol/L	48 (98.0)	18 (27.7)	0.98 (0.89 to 1.00)	0.72 (0.60 to 0.83)

NA = not applicable.
* Comparison of simple and amended insulin–glucose ratios with criteria suggested by the Endocrine Society clinical practice guideline (11). All values were measured at the time of discontinuation of a prolonged fast. to convert glucose values from mmol/L to mg/dL, divide by 0.0555. To convert C-peptide values from nmol/L to ng/mL, divide by 0.331.
† Based on comparison with control patients (excluding successfully operated patients with insulinoma).
‡ Equivalent cutoffs for C-peptide–glucose ratios and amended insulin–glucose ratios were derived from linear regression analyses versus insulin–glucose ratios ($R^2 = 0.716$; $P < 0.001$) and amended insulin–glucose ratios ($R^2 = 0.989$; $P < 0.001$). Values equivalent to ∞ (glucose concentrations <1.7 mmol/L) were excluded from this analysis.
§ Exactly this many criteria were met.

Plasma glucose concentrations less than 2.4 mmol/L (<43 mg/dL) (no prespecified end point) were seen during the course of prolonged fasts in all patients with insulinoma but in only 16 of 65 control patients and in none of the patients with insulinoma who had successful surgery. Plasma glucose levels remained at 3.2 mmol/L (58 mg/dL) and 3.9 mmol/L (70 mg/dL) in the control and successful surgery groups, respectively ($P < 0.001$) (**Figure 2**, *A* and **Appendix Figure 2**, available at www.annals.org). At the termination of fasting due to hypoglycemia symptoms or passage of 48 hours, plasma insulin (**Figure 2**, *B*) and C-peptide (**Figure 2**, *C*) concentrations were significantly higher ($P < 0.001$) in patients with insulinoma than in control patients, although there was considerable overlap.

Performance of Endocrine Society Criteria

A plasma glucose level less than 3.1 mmol/L (<55 mg/dL) was reached in all patients with insulinoma early in the course of prolonged fasts (>90% in 12 hours, in all after ≥25 hours) (**Appendix Figure 1** [*bottom*]). However, 26 of 65 (40.0%) control patients also met this threshold, usually later in the time course ($P < 0.001$) (**Table 3** and **Appendix Figure 1** [*bottom*]).

On the basis of the recently proposed Endocrine Society criteria (plasma glucose level <3.1 mmol/L [<55 mg/dL], insulin level ≥18 pmol/L, and C-peptide level ≥0.2 nmol/L [≥0.61 ng/mL]) (11), 48 of 49 patients with insulinoma fulfilled all 3 criteria and were correctly identified (**Table 3**). Sixteen of 65 control patients, however, also fulfilled all 3 criteria.

Performance of the Amended Insulin–Glucose Ratio

An abnormally high insulin–glucose ratio (>32.2 [pmol/L]/[mmol/L]) at the time of discontinuation of prolonged fasts was seen in 36 of 49 patients with insulinoma compared with none of the control patients (**Table 3**). Insulin–glucose ratios at the time of discontinuation of fasting were significantly higher in patients with insulinoma than in control patients ($P < 0.001$) (**Figure 2**, *D*), but some overlap remained.

An abnormally elevated amended insulin–glucose ratio (>53.6 [pmol/L]/[mmol/L]) at the time of discontinuation of prolonged fasts was seen in 48 of 49 patients with insulinoma and in only 1 of 65 control patients ($P < 0.001$) (**Table 3**). A similar separation was obtained when insulin levels were plotted versus plasma glucose concentrations at the time of termination of prolonged fasts (**Figure 2**, *F*). Panel G of **Figure 2** presents a similar plot relating C-peptide and glucose concentrations.

The molar insulin–C-peptide ratio did not have good diagnostic accuracy for distinguishing patients with insulinoma from control patients (**Table 3**).

Diagnostic Accuracy of Endocrine Society Criteria Compared With Amended Insulin–Glucose Ratios

The amended insulin–glucose ratio had a high diagnostic sensitivity and specificity and a high positive predictive value (**Table 3**). The Endocrine Society criteria had high sensitivity but lower specificity (highest when glucose, insulin, and C-peptide criteria were all met), resulting in a lower positive predictive value (0.75 [95% CI, 0.63 to

Table 3—**Continued**

Positive Predictive Value	Negative Predictive Value	Positive Likelihood Ratio	Negative Likelihood Ratio
1.00 (0.90 to 1.00)	0.83 (0.73 to 0.91)	NA	0.27 (0.17 to 0.42)
0.98 (0.89 to 1.00)	0.99 (0.92 to 1.00)	63.7 (9.1 to 445.4)	0.02 (0.00 to 0.14)
0.94 (0.82 to 0.99)	0.93 (0.84 to 0.98)	20.4 (6.7 to 61.8)	0.11 (0.05 to 0.25)
0.92 (0.81 to 0.98)	0.97 (0.89 to 1.00)	16.3 (6.2 to 42.3)	0.04 (0.01 to 0.17)
0.43 (0.34 to 0.53)	NA	1.00 (1.00 to 1.00)	NA
0.65 (0.53 to 0.76)	0.98 (0.87 to 1.00)	2.5 (1.8 to 3.1)	0.03 (0.00 to 0.24)
0.47 (0.37 to 0.57)	1.00 (0.69 to 1.00)	1.2 (1.1 to 1.3)	0 (NA)
0.49 (0.39 to 0.59)	1.00 (0.75 to 1.00)	1.3 (1.1 to 1.4)	0 (NA)
0.75 (0.63 to 0.85)	0.98 (0.89 to 1.00)	4.0 (2.6 to 6.1)	0.03 (0.00 to 0.19)
0.02 (0.00 to 0.14)	0.37 (0.26 to 0.49)	0.04 (0.01 to 0.25)	2.3 (1.7 to 3.0)
0.00 (0.00 to 0.29)	0.52 (0.42 to 0.61)	0.0 (NA)	1.2 (1.1 to 1.3)
0.00 (0.00 to 0.98)	0.57 (0.47 to 0.66)	0.0 (NA)	1.0 (1.0 to 1.1)
0.70 (0.57 to 0.80)	0.98 (0.88 to 0.99)	3.0 (2.1 to 4.3)	0.03 (0.00 to 0.21)
0.73 (0.60 to 0.83)	0.98 (0.89 to 1.00)	3.5 (2.4 to 5.3)	0.03 (0.00 to 0.20)

0.85]) than the amended insulin–glucose ratio (0.98 [CI, 0.89 to 1.00]).

DISCUSSION

This study shows that the amended insulin–glucose ratio calculated at the time of discontinuation of prolonged fasts has high diagnostic accuracy for confirming or ruling out insulinoma. In contrast, the recently proposed criteria from the Endocrine Society practice guideline (11) were equal in terms of sensitivity and negative predictive value but failed to provide similar diagnostic specificity and positive predictive value because the same criteria were met by a substantial proportion of control patients (**Table 3**).

In the absence of reliable imaging tools to detect or rule out small insulinomas, the decision to proceed to exploratory surgery often relies on an accurate biochemical diagnosis. The amended insulin–glucose ratio—alone or as an adjunct to other criteria—should help to correctly classify patients with suspected insulinoma.

To ascertain the absence of a subclinical insulinoma in the control group, patients were contacted a mean of 10 years after the initial diagnostic work-up. No insulinoma had become clinically apparent among the 92.3% of patients in whom relevant information could be obtained (**Table 2**), thereby confirming that these patients did not have subclinical insulinoma at the time of the diagnostic prolonged fast. This is relevant because pancreatic adenomas are found in autopsy series in as many as 10% of patients (14) and could progress from a clinically silent (non–hormone-producing) to a hormone-producing stage with clinical symptoms.

The main reason for the difference in diagnostic accuracy between the amended insulin–glucose ratio and Endocrine Society criteria may be that a glucose concentration cutoff of 3.1 mmol/L (55 mg/dL) is too high. Thus, complete suppression of insulin and C-peptide secretion cannot be expected at this concentration in healthy persons (**Figure 2**, *D* and *E*). Furthermore, in clinical practice prolonged fasts are typically discontinued at glucose concentrations less than 2.5 mmol/L (<45 mg/dL) to achieve better diagnostic discrimination, especially because patients with insulinoma typically do not display symptoms of hypoglycemia above this range (6).

Although C-peptide levels are believed to be more suitable than insulin levels for assessment of insulin secretory rates (15), C-peptide seems to be less ideal for diagnostic validation of insulinoma (**Figure 2**) (16). Reasons may include variations in C-peptide levels depending on the respective renal function or cross-reactivity of proinsulin and conversion intermediates in C-peptide assays. In accordance with previous studies (16), the role of measuring C-peptide during prolonged fasts is limited, and it may, at best, confirm findings based on insulin and proinsulin measurements.

We retrieved pertinent literature by performing an English-language MEDLINE search (using the terms "insulinoma" in conjunction with "fast," "fasting," "insulin," "C-peptide," "proinsulin," "diagnostic," or "procedure") up to 2011. We confirmed the high diagnostic accuracy of the amended insulin–glucose ratio by using data from case reports. The amended insulin–glucose ratio identified 44 of 46 patients with confirmed insulinoma (sensitivity, 0.96), similar to the performance of the Endocrine Society criteria (43 of 46 patients). Control patients were not reported in this study (17).

Vezzosi and colleagues (18) used specific insulin assays that did not crossreact with proinsulin. The amended insulin–glucose ratio identified 26 of 33 patients, whereas

the Endocrine Society criteria identified only 28 of 33 patients. However, proinsulin levels were greater than 5 pmol/L in all patients. Single-patient data were not available for control patients.

In previous analyses, the amended insulin–glucose ratio has been found to produce false-positive results (2). However, these analyses were done in comparison with control patients who developed hypoglycemia due to endocrine deficiencies (for example, from corticosteroids). Such patients were not represented in our cohort of control patients (**Table 2**).

A word of caution must be mentioned about the insulin assay used in this study: It was chosen also to detect changes in insulin and proinsulin, because intact proinsulin, 32–33 split proinsulin, and des-31,32-proinsulin represent a large proportion of insulin-like immunoreactivity in the tumors and plasma of patients with insulinoma (1, 19–21), whereas des 64/65 split proinsulin seems to comprise only a minor component (20, 21). Current commercial insulin immunoassays are highly specific and do not crossreact with proinsulin or its split forms. Therefore, when such a specific assay is used, it is necessary also to measure proinsulin (22, 23), as proposed by the Endocrine Society (11). Nevertheless, a nonspecific assay offers the advantage of detecting insulin and proinsulin at the same time (24).

For patients in whom a prolonged fast provides an equivocal result, additional suppression tests (for example, a C-peptide–suppression test [13] or a hyperinsulinemic, hypoglycemic clamp test) may still be needed to establish a definitive diagnosis (25, 26). Furthermore, application of more sensitive imaging procedures, such as endoscopic ultrasonography (27, 28) or glucagon-like peptide-1 receptor scintigraphy (29, 30), in combination with biochemical tests, may improve diagnostic accuracy. However, such tests may be more helpful for locating tumor formations before surgery than for ruling out the presence of an insulinoma (14).

Given the low incidence of insulinomas, clinical experience with these tumors is limited even in specialized institutions, which often delays accurate diagnosis and treatment. The development of standardized protocols, including a consensus on assay procedures, would allow multicentric analyses based on greater patient numbers.

In conclusion, the diagnostic accuracy of the amended insulin–glucose ratio was compared with that of Endocrine Society clinical practice guideline criteria in a large cohort of patients who had prolonged supervised fasts to confirm or rule out the diagnosis of an insulinoma. Both criteria offered high diagnostic sensitivity. However, the amended insulin–glucose ratio had higher specificity and positive predictive value and may therefore have advantages in confirming the diagnosis of an insulinoma.

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Appendix: Methodological Details and Additional Results

Details of the Insulin and C-Peptide Immunoassays

Blood was drawn into heparinized tubes (Monovette NH_4 lithium heparinate; Sarstedt, Nümbrecht, Germany). Samples were stored at 4 °C until centrifugation. Plasma was frozen and stored at less than −20 °C until analysis.

Plasma insulin was measured by radioimmunoassay. At both centers, the Insulin RIA 100 (Pharmacia, Freiburg, Germany) was used. The antiserum that was used cross-reacts with proinsulin and all conversion intermediates to a similar degree as fully processed insulin (details not shown). The detection limit is 9 pmol/L, and intra-assay and interassay coefficients of variation were 6% and 8%, respectively.

C-peptide was measured using commercial radioimmunoassays obtained from Byk-Sangtec Diagnostica (Dietzenbach, Germany) (Göttingen) or by using RIAgnost HC-Peptid (Behringwerke, Marburg, Germany) (Düsseldorf). Coefficients of variation were less than 8% for both the intra-assay and the interassay.

Details of Prolonged Fasts in the Patient With Insulinoma and the Control Patient Misclassified on the Basis of the Amended Insulin–Glucose Ratio at the Time of Discontinuation

The patient with insulinoma who was misclassified because of a falsely low (that is, normal) amended insulin–glucose ratio showed a secretory burst with a peak insulin increment to 101 pmol/L at 11 hours of fasting, followed by hypoglycemia (1.8 mmol/L [32 mg/dL]) at 12 hours. The fast was terminated at 14 hours, when plasma glucose had already recovered to 4.2 mmol/L (75 mg/dL). The control patient who was misclassified because of an abnormally high (that is, diagnostic) amended insulin–glucose ratio had a plasma glucose level of 1.7 mmol/L (30 mg/dL) and an insulin concentration of 51 pmol/L at the time of discontinuation. Insulin had increased above the previous level of 23 pmol/L without an accompanying increment in C-peptide (details not shown). Retrospective evaluation of the 2 misclassified patients (**Figure 2**) shows that the evaluation of previous time points (before discontinuation of the prolonged fasts) would have helped to interpret these results correctly, or at least to suggest additional diagnostic studies (13, 28, 29) in these patients.

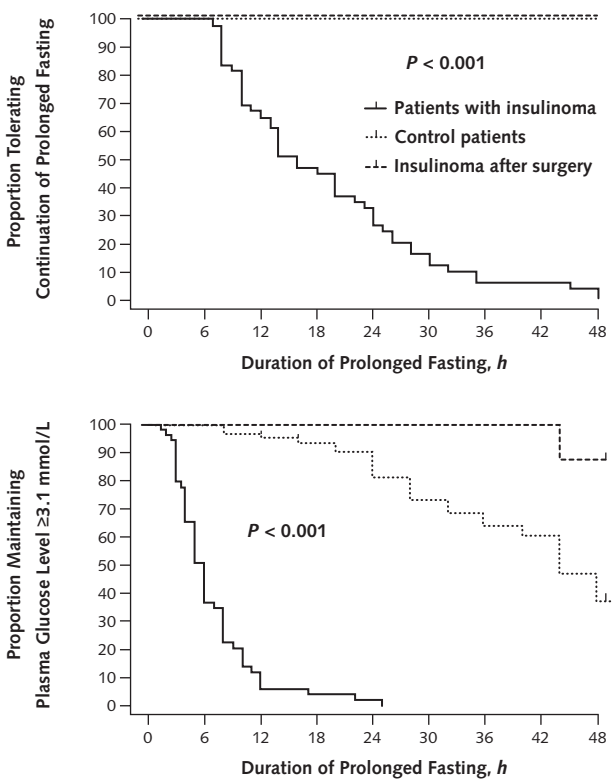
Persistence of Diagnostic Criteria With Prolonged Fasting Beyond Their First Occurrence

Once a plasma glucose level of 2.4 mmol/L (43 mg/dL) was reached, 90% of all subsequent time points ($n = 5$) were also characterized by a plasma glucose level less than 2.4 mmol/L (<43 mg/dL) in patients with insulinoma, whereas this number was 69% (of $n = 3$) in control patients ($P = 0.001$). After a high insulin–glucose ratio (>32.2 [pmol/L]/[mmol/L]) was reached, 80% of all subsequent time points ($n = 6$) were also characterized by a high ratio in patients with insulinoma, whereas this number was 9% ($n = 12$) in control patients ($P < 0.001$). Once a high amended insulin–glucose ratio (>53.6 [pmol/L]/[mmol/L]) was reached, 94% of all subsequent time points ($n = 6$) were also characterized by an abnormally elevated amended insulin–glucose ratio in patients with insulinoma, whereas this number was 24% (of $n = 10$; $P < 0.001$) in control patients. All values presented are means.

Appendix Table. **Characteristics of 52 Tumors From 49 Patients With Insulinoma Who Had Prolonged Fasting**

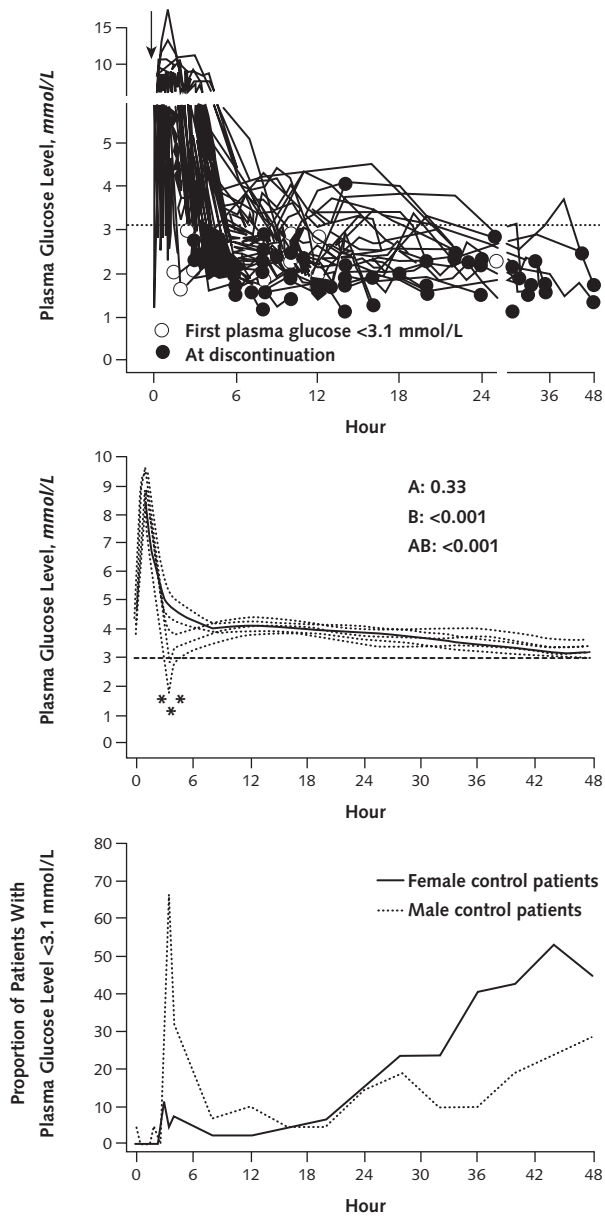
Tumor Characteristic	Tumors, *n*
Malignant	4
Benign	45
Single adenoma	40
Multiple adenomas	5
Diameter	
0–0.5 cm	3
0.6–1 cm	10
1.1–2 cm	25
>2 cm	7
Location	
Pancreatic head	21
Pancreatic body	15
Pancreatic tail	16
Extrapancreatic	0

Appendix Figure 1. **Proportion of patients with insulinoma, control patients, and patients who had successful surgical removal of the insulinoma tolerating prolonged fasts for as long as 48 h (*top*) or maintaining a plasma glucose level of at least 3.1 mmol/L (≥55 mg/dL) (*bottom*).**



Analysis was performed using the Kaplan–Meier method and the Mantel–Cox log-rank test.

Appendix Figure 2. **Glucose concentrations during prolonged fasts over 48 h or until discontinuation.**



Patients with insulinoma are shown in the top panel and control patients are shown in the middle panel separated by sex (mean [95% CI]). The proportion of female and male control patients with a glucose concentration less than 3.1 mmol/L (<55 mg/dL) is shown in the bottom panel. In the top panel, individual glucose concentrations over time are shown, highlighting the first glucose concentration less than 3.1 mmol/L (<55 mg/dL) (*open circles*) and the glucose concentration at the time of discontinuation of prolonged fasts (*solid circles*). In the middle panel, solid lines are means, and dotted lines are 95% CIs; statistical analysis was performed using repeated-measures analysis of variance, and asterisks indicate significant differences (by analysis of variance) between female and male control patients at single time points. To convert glucose values from mmol/L to mg/dL, divide by 0.0555. A = female vs. male control patients; B = changes over time; AB = interaction.