C-Peptide Response to Meal Challenge in Nondiabetic and Diabetic Adults Living in Wadena, Minnesota

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OBJECTIVE — The goal of the study was to provide cross-sectional descriptive data on the response of C-peptide to a vigorous meal stimulus in a population-based sample of nondiabetic adults compared with a population-based sample of adults with NIDDM. Available information is scanty, especially in subjects >50 yr old.

RESEARCH DESIGN AND METHODS — The group under study included 377 adults without previously known diabetes randomly chosen from the population of the city of Wadena, Minnesota, almost all of northern European background, and 88 adults with known diabetes. PCP was measured 90 min after ingestion of 480 ml liquid meal Ensure-Plus, which includes 95 g dextrose, 26 g protein, and 25 g fat. C-peptide also was measured in a 260-min urine collection after the meal challenge. Novo antibody M1221 was used for C-peptide assay throughout the study. Participants whose medical record indicated insulin-dependent diabetes with a history of acetone production were excluded from analyses.

RESULTS — The distribution of UCP and PCP in this group of subjects appears very broad. Both the highest and lowest values for C-peptide were observed in individuals with diabetic glucose tolerance. The mean and median values in the nondiabetic group are higher than in previously published reports. After statistical adjustment for age, sex, BMI, and concomitant plasma glucose, participants with IGT produced significantly more C-peptide than the group with NGT (3.48 vs. 2.96 nM PCP, $P < 0.05$). Participants with diabetic glucose tolerance and who were not taking insulin produced as much or more C-peptide than either the NGT or IGT groups, depending on the statistical model used for adjusting for plasma glucose. Diabetic participants who were taking insulin produced significantly lower amounts of C-peptide than any of the non–insulin-taking groups (~30% of the C-peptide produced by the non–insulin-taking diabetic participants). A decline in PCP production with increasing years since diagnosis (5.7%/yr) was observed exclusively in the insulin-taking NIDDM participants. Effect modification by glucose tolerance classification was observed on the relationship between plasma glucose and PCP: PCP increased with increasing plasma glucose in NGT and IGT groups, but a nonsignificant negative relationship was exhibited in diabetic participants.

CONCLUSIONS — The data suggest that two forms of NIDDM may exist, crudely distinguished by the clinical decision to use insulin to control blood glucose levels. The insulin-taking diabetic individuals may experience a greater likelihood of pancreatic failure, whereas non–insulin-taking diabetic individuals probably experience stable pancreatic function over the course of their disease. Longitudinal observation of the Wadena cohort will provide more insight into this possibility.

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NIDDM, NON-INSULIN-DEPENDENT DIABETES MELLITUS; PCP, PLASMA C-PEPTIDE; BMI, BODY MASS INDEX; UCP, URINE C-PEPTIDE; IGT, IMPAIRED GLUCOSE TOLERANCE; NGT, NORMAL GLUCOSE TOLERANCE; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; SRS, STRATIFIED RANDOM SAMPLE; CV, COEFFICIENT OF VARIATION; WHO, WORLD HEALTH ORGANIZATION.

Measurements of the small molecule designated as C-peptide have been shown to provide a valid and useful measure of the rate of insulin secretion from the human pancreatic β-cell (1–4). Assay of C-peptide in plasma and urine has been extensively used in research on diabetic subjects to distinguish between those with essentially total loss of insulin secretion (IDDM) and those with persistence of some insulin secretion capacity or reserve (NIDDM) (4–10).

Studies of C-peptide in diabetes are numerous, although they rarely have been reported in defined populations. The outstanding exceptions are investigations of diabetes in small ethnic groups, such as the Pima-Papago Indians of Arizona (7).

In contrast, information on C-peptide secretion in nondiabetic reference populations is extremely limited. Published data are in most cases restricted to groups of ≤20 subjects (Table 1). The distribution of C-peptide response in the physiological range has not been documented extensively, and observations in a defined population of northern European origin appear to be lacking.

At least four reasons exist for extending studies of C-peptide to defined diabetic and nondiabetic populations beyond the range of clinic or in-hospital studies: 1) Among the general population of subjects diagnosed with NIDDM, the approximate proportion with markedly reduced, as opposed to abundant, C-peptide reserve is unknown; 2) the precise role of pancreatic islet exhaustion from overwork has not been documented clearly in longitudinal studies of NIDDM, although often it is assumed to take place (11); 3) among the general population without diabetes, it is unknown whether pancreatic islet exhaustion occurs as a part of normal aging (12); and 4) the relationship between endogenous insulin secretion/reserve and arterial hypertension, blood lipid

Table 1—*Some published UCP and PCP measurements in nondiabetic subjects (1977–1988)*

			UCP			PCP (nM)	
			nmol/collection				
Ref.	Antibody	Subjects (*n*)	4 to 5-h collection	24-h collection	nmol/mmol creatinine	Fasting	Fed (600 kcal; 1h)
31	M1230	14		34.6 ± 9.0	2.3 ± 0.5	(0.25–1.2)*	
		7	5.1 ± 0.6/5h		1.9 ± 0.4		
33	M1230	6		19.1 ± 9.2	1.2 ± 0.4		
		8		21.8 ± 13.0	1.4 ± 0.6		
34	Shionogi	10		16.4 ± 1.5			
		8	2.7 ± 0.2/5h				
19	M1230	41	5.4 ± 0.6/4h				
		31				0.5 ± 0.1	1.8 ± 0.2
54	Not stated	8 (lean)		11.7 ± 1.3			
		8 (obese)		14.9 ± 2.8			
55	Daiichi	26		26.7 ± 2.3	2.1 ± 0.2	0.6 ± 0.1	
4	Yanaihara	25		11.9 ± 1.3			

Values are means ± SE.
*Range.

levels, and the development of atherosclerosis needs elucidation (13).

All of these questions bear on the central problem of the nature of NIDDM: Is the primary defect in most cases a failure of insulin secretion with secondary loss of responsiveness to insulin, or is the primary defect a deficit of tissue response to insulin, with decline in insulin secretion as a secondary consequence?

To address these questions and problems in a defined population of northern European origin, a study of PCP and UCP response to a standard meal stimulus has been organized in Wadena, Minnesota (14). Two cohorts of adult residents have been recruited: One cohort of 377 participants constitute a random sample of residents ≥20 yr of age, in 12 strata according to age, sex, and use of prescription medications (SRS); the other cohort of 88 subjects consists of all available physician-diagnosed diabetic subjects living in the community. The study plan is longitudinal, in three phases, with repeat testing of the two cohorts at intervals of 2.5 yr.

This report presents from phase I of the Wadena Study the cross-sectional C-peptide response to 480 ml (710 kcal) of standard liquid nourishment Ensure-Plus in relationship to diabetic status and duration of diabetes, controlling for the known major modifiers of insulin secretion (age, sex, concurrent plasma glucose level, and BMI). The results provide a perspective on the distribution of pancreatic insulin response to a meal in a randomly selected sample of nondiabetic individuals from a mid–North American population and a comparison of the responses of diabetic and nondiabetic individuals: The range of responses in both cohorts is extremely broad, and insulin secretion appears to be decreased primarily among participants with NIDDM who take insulin to control their blood glucose.

RESEARCH DESIGN AND METHODS

Population sample

A detailed description of the recruitment of a stratified random sample of adults in Wadena has been published previously (14). Wadena is a city of 4699 (1980 census—the 1990 census counted 4131 residents) in west-central Minnesota. The residents are almost entirely of northern European origin. Adults ≥20 yr old were enumerated in a special census conducted in November and December of 1985. Nursing home residents and technical institute students were included in the 2962 residents who were registered. More than 99% of eligible households participated in the census.

After elimination of 123 previously known physician-diagnosed diabetic individuals, the remaining 2839 individuals were divided into 12 strata according to age-group (20–39, 40–59, and >60 yr old), sex, and a dichotomous classification (yes/no) of prescription medication use. A random sample of 50 was generated by computer from each stratum (SRS). Out of 600 potential SRS participants thus generated, 377 individuals (63%—range 50–76% by stratum) and 88 of the physician-diagnosed diabetic group (72%) actually completed most of the protocol. Fewer than 465 subjects are presented in some of the

tables and figures because of omission of 9 participants with IDDM and instances of missing data. A detailed in-person interview was completed for each subject, including information on general health and the use of prescription and nonprescription medications. The health history was validated later by review of medical records, with written permission from the subjects.

The study protocol was reviewed and approved by the Committee on the Use of Human Subjects in Research of the University of Minnesota. Written informed consent was secured from all participants.

C-peptide assay

All urine and plasma samples were assayed for C-peptide with a procedure slightly modified from that of Heding (15), which uses 95% ethanol for precipitation, followed by centrifugation to separate bound from free labeled peptide (^{125}I-tyrosylated human C-peptide); synthetic human C-peptide was the standard. Novo C-peptide antibody M1221 was used throughout; all samples were assayed in duplicate by the same technologist. All samples were checked for endogenous C-peptide–binding antibody; none was found. The minimum sensitivity of the method was 0.024 nM, requiring dilution of urine 1:50–1:200 for most specimens. Linearity was preserved with dilution of urine. Recovery of synthetic human C-peptide standard added to C-peptide–free urine was 88–125%. The intra-assay CV of split samples was 4.0% in the midrange of the assay and the between-assay CV 5.7–8.5% in the midrange (for control samples) over the past 5 yr.

Novo antibody M1221 has been adopted for the entire Wadena study as a substitute for the well-known M1230 (16), because supplies of M1230 are nearly exhausted. We compared the two antibodies with 10-fold replicate assays of four plasma samples and one urine sample. The results with M1221 were systematically higher in all comparisons, with a M1221:M1230 ratio of 1.35 for samples in the higher range of the assay, increasing to 1.65 for samples in the lower range. This observation is in general agreement with comparisons in the Novo-Nordisk Laboratories (unpublished observations, B. Tronier).

As an overall check on the performance of the assay, a masked study was conducted in which coded duplicate samples of previously assayed urine or plasma were resubmitted to the technologist 2–4 wk later along with a batch of new samples. The agreement was good between the first and second assays of the specimens ($r = 0.997$).

Collection protocol

Study subjects participated in two mornings of testing and data collection, with the 2nd morning occurring within 10 days of the first session. All subjects were instructed in a diet containing at least 150 g carbohydrate to be taken each day for 3 days before each test day, to abstain from alcohol for 3 days, and to fast from 2000 on the evening before testing. They were asked to record their food intake; the carbohydrate content was calculated from their food diaries by a research dietitian. More than 90% of participants reported taking at least 150 g carbohydrate on each of the 3 days before testing.

One of two different meal stimuli was offered on each test day, with a random order of testing: 75 g of glucose (cola or orange flavored) or 480 ml (16 oz) of Ensure-Plus. A complete glucose tolerance test was performed with the glucose drink, according to the procedure of the National Diabetes Data Group (17). Ensure-Plus is a mixed liquid meal; 480 ml contains 95 g carbohydrate as dextrose, 26 g protein, and 25 g fat, providing 710 kcal. This dose was chosen because it was close to the composition of a 600-kcal hot breakfast used in previous studies from our research unit (18). The glucose or mixed meal was consumed within 20 min, and supervised 260-min urine specimens were collected, with t_0 at 20 min before the meal was presented. A venous blood sample for PCP and creatinine measurement was drawn 90 min after beginning the drink of Ensure-Plus into tubes containing EDTA (as an anticoagulant) and Trasylol (an inhibitor of proteolysis).

The 90- and 260-min intervals were chosen on the basis of previous experience, indicating that the peak and the total rise and fall of PCP corresponded respectively to these times (19).

Plasma aliquots were frozen immediately after collection; urine aliquots were brought to pH 7.0 with 1 N NaOH and centrifuged before freezing. Samples were stored at −20°C until assay within 2–4 wk. Plasma and urine creatinine were measured with an automated method based on the Jaffe reaction in the central chemistry laboratories, University of Minnesota Hospital and Clinic. UCP is reported as both nanomoles/260 min collection and as nanomoles per millimoles creatinine. The latter adjustment has been proposed as a way of allowing for variation in body size from subject to subject and also for minor errors in urine collection (20).

Definitions

Diabetic status. Participants were classified as having NGT, IGT, or diabetic glucose tolerance based on WHO criteria (21). Review of medical records was used to classify whether diabetic individuals had IDDM. Hospital and clinic records that were available in Wadena were abstracted onto standard forms by study staff, and identical forms were mailed to physicians and hospitals outside of Wadena. Individuals whose medical records indicated *1*) a physician statement of IDDM with continuous insulin therapy; 2) a history of elevated fasting (or grossly elevated nonfasting) blood glucose, an abnormal glucose tolerance test, or glycosuria; and 3) a history of either ketoacidosis or ketonuria were classified as IDDM. All other diabetic participants were considered NIDDM, regardless of their use of insulin.

Medication use. Participants completed an interview describing all the medications they had taken in the previous month, including information on dose and frequency of use for both prescription and over-the-counter medications. The type and frequency of use for each medication were coded, and then the data were grouped into one of three classes: medications known to raise blood glucose levels, medications known to lower blood glucose levels, and medications without any established effect on blood glucose levels (17,22,23). Participants who reported taking a medication ≥10 days of the previous 14 days were classified as regular users.

BMI. Obesity was approximated with BMI, which is the weight (kg) divided by the square of the height (m^2).

Duration of diabetes. The duration of diabetes was estimated by averaging the number of years since diagnosis as reported by the participant with the number of years since diagnosis as recorded in the medical record. Although the date of diagnosis of diabetes is probably not coincident with the onset of diabetes, but rather follows it—possibly by quite a few years, the date of diagnosis was the best proxy for the date of onset of diabetes available in this study. Participants who had no prior diagnosis of diabetes, but who had a diabetic glucose tolerance test in this study, were assigned a duration of 0 yr. Participants with a history of diabetes were included in analyses of the relationship between duration of diabetes and C-peptide production, regardless of their glucose tolerance status at the time of testing.

Statistical methods

Unless otherwise stated, all analyses were restricted to participants for whom a diagnosis of IDDM could not be found on review of medical records (see above). Analysis of variance and analysis of covariance were used to compare means and adjusted means between groups. Linear regression models were developed from theoretical considerations, and analysis of variance was used to screen for interactions between the predictor variables. Interactions that were important in the analysis of variance models were tested for inclusion in the final linear regression models. If the logarithm of the C-peptide variables was analyzed rather than the actual levels, the regression models fit the data somewhat better (adjusted R^2 increased by ~25%), and the residuals appeared to be distributed more normally and homoscedastically (thus the assumptions for significance testing were better met). Analysis of the logarithm of an entity corresponds to comparing the geometric means between groups. (In general, the geometric mean is close to the median and is a good central measure for skewed variables.) In addition, it makes sense that insulin secretion would change along a multiplicative rather than a linear scale, because the pancreas is part of a biological system, and other biological and chemical relationships are best described with nonlinear scales (e.g., drug half-life, bacterial doubling time, decibels of sound).

Adjusted geometric means were computed with the grand means for all participants. Adjustment in this case represents a form of extrapolation, because it is unrealistic to assume that the 90-min postchallenge glucose would be as high as 137 mg/dl (7.6 mM) in normal, nondiabetic individuals or that low in individuals with diabetes. However, the differences between the groups would be the same regardless of the exact number used for age, sex, BMI, and 90-min plasma glucose in estimating the adjusted values. Statistical analyses were performed on a SUN computer with SPSS, and graphing was accomplished with Cricket Graph on a Macintosh computer.

RESULTS

Acceptance of the liquid-meal test

The liquid meal, consisting of 480 ml Ensure-Plus, was generally well accepted by subjects of all age-groups. A few older subjects had abdominal distress and diarrhea, but this was avoided by lengthening the allowed time for drinking the mean to 20 min and serving the drink at room temperature. Diabetic subjects preferred Ensure-Plus to the glucose drink.

Glucose tolerance

Table 2 contains the cross-tabulation of glucose tolerance categories using WHO criteria. Among the SRS participants with complete data available, 28 had a diabetic classification, 68 were classified as IGT, and the remaining 272 had NGT. Among the physician-diagnosed diabetic patients with complete data available, only 14 did not have diabetic glucose tolerance at the time of testing: 8 had IGT, and 6 were classified as NGT.

Table 3 contains the means, medians, and SDs, by glucose tolerance category and insulin status, for the variables used herein. Participants with IGT or diabetic glucose tolerance were on average older and more obese than those with NGT, and the participants with abnormal glucose tolerance also had poorer mean creatinine clearance than those with NGT. The mean absolute C-peptide levels were highest in the NIDDM participants who were not taking insulin and lowest in the insulin-taking NIDDM participants.

General distribution of UCP and PCP responses

Histograms of the distribution of UCP responses are shown in Fig. 1 (nmol/260 min) and Fig. 2 (nmol/mmol creatinine) and of the 90-min postmeal PCP in Fig. 3. The distribution of urine responses appears broad, with modal values between 6.0 and 9.0 nmol/260-min specimen or from 3.0 to 6.0 nmol/mmol creatinine. Results are skewed toward higher values. The general distribution of the meal-stimulated PCP values closely resembles the pattern of the UCP responses. The modal interval is 2.0–3.0 nM.

Although the general shape of the histogram of PCP values is much like

those of the UCP values, this does not necessarily mean that the participants were in the same relative positions in the PCP and UCP distributions. Figure 4 presents a scatter plot, plotting UCP versus PCP drawn at 90 min during the Ensure-Plus test. UCP/260-min collection increased ~2 nmol for each nM increase in PCP ($r = 0.476$, $P < 0.00001$). However, there is a great deal of scatter and one value cannot be precisely predicted from the other. An outlying point among the diabetic participants may be noted in passing.

For the remainder of the results section, PCP will be the primary variable of interest; UCP will be described in terms of how they compare with PCP concentrations.

C-peptide's association with use of medications

Insulin, which is only taken by individuals who have diabetes, was the only medication found to be associated with C-peptide levels. This observation was developed via a top-down approach. Analysis of variance indicated that the only major group of medications significantly related to PCP levels were those known to lower blood glucose. The effect was apparent only among diabetic patients. When the specific medications that lower blood glucose were compared between diabetic and nondiabetic participants, it was obvious that the predominant forms of glucose-lowering drugs taken by the diabetic patients were insulin and oral hypoglycemic agents, whereas the predominant form of glucose-lowering drugs taken by nondiabetic subjects were salicylates (which are thought to lower blood glucose only when taken in very large doses) (22,24). PCP levels in diabetic participants taking oral hypoglycemic agents did not differ from those in diabetic participants not taking any medications known to lower blood glucose.

Thiazide diuretics are thought to affect glucose metabolism (22), but there were no significant differences in C-peptide responses between participants who were regular users of a thiazide diuretic, and those who were not.

C-peptide and age, sex, and BMI

PCP levels increased significantly with age: If age was the only predictor considered, then PCP increased ~0.4% with each year of increased age in this cross-sectional study ($P = 0.003$). In contrast, urine C-peptide production decreased with age; ~0.5% less C-peptide appeared in the urine for each additional year of age ($P = 0.01$). The C-peptide:creatinine ratio in the urine was related to age similarly to the plasma concentration: the ratio increased by ~0.5% for each year of additional age ($P = 0.01$).

Men and women had very similar geometric mean PCP levels 90 min postchallenge (2.92 vs. 2.94 nM, respectively, $P = 0.881$). On average, men produced a somewhat greater amount of UCP than the women (geometric means of 9.72 and 8.44 nmol/260 min, respectively, $P = 0.035$); however, when the ratio of C-peptide to creatinine in the urine was considered, women had higher levels than men (geometric means of 5.45 and 3.95 nmol C-peptide/mmol creatinine, respectively, $P < 0.0001$).

Insulin resistance increases in obesity (25,26); thus, it was expected that C-peptide production would increase with increasing BMI. We observed that PCP increased by ~2.5% ($P < 0.00001$), UCP increased by ~2.8% ($P < 0.00001$), and the C-peptide:creatinine ratio increased by 0.6% ($P = 0.30$) with each unit increase in BMI.

Adjusted mean C-peptide by glucose tolerance category

Multiple linear regression was used to calculate equations estimating C-peptide levels for the three levels of glucose tolerance and two levels of insulin use with age, sex, BMI, and the 90-min plasma glucose level as covariates. The regression model accounted for 27% of the variance in PCP, 17% of the variance in UCP, and 21% of the variance in C-peptide:creatinine ratio in the urine. Estimated geometric means before and after adjustment for covariates are presented in Table 4. The differences between adjusted and unadjusted geometric means were primarily because of differences between the groups in plasma glucose levels.

Table 2—*Cross-tabulation of sample strata and WHO glucose tolerance classification, Wadena City Health Study*

	WHO GLUCOSE TOLERANCE CLASSIFICATION (N)		
STRATUM	NGT	IGT	DIABETIC
SRS	272	68	28
PREVIOUS DIABETES	6 (0)	7 (1)	62 (7)

Values in parentheses are N for IDDM by medical record review.

Participants with IGT produced significantly more PCP compared with those who had NGT ($P < 0.05$), and the non–insulin-taking diabetic participants produced levels of PCP that were intermediate, but not significantly different from either of the two other non–insulin-taking groups. The insulin-taking diabetic participants had significantly lower PCP levels ($P < 0.0001$) than any of the non–insulin-taking groups. Participants whose medical records indicated that they had IDDM had adjusted geometric mean C-peptide levels that were significantly lower than the insulin-taking NIDDM participants (0.32 vs. 1.07 nM, $P < 0.00001$ in a regression model including a term for IDDM).

With minor exceptions, the same set of variables for PCP was useful in predicting UCP and C-peptide:creatinine ratio. Insulin-taking diabetic participants had significantly lower geometric mean UCP and C-peptide:creatinine ratio than any of the other three non–insulin-taking groups. As with the geometric mean PCP, the geometric mean C-peptide:creatinine ratio in the urine was higher in those with IGT than in those with NGT ($P < 0.05$). However, in

Table 3—*Distribution of age, sex, BMI, 90-min plasma glucose, creatinine clearance, PCP, UCP, and C-peptide:creatinine ratio in urine by WHO glucose tolerance and insulin status, Wadena City Health Study*

	NGT (*N* = 278)	IGT (*N* = 75)	NIDDM* (*N* = 69)	NIDDM† (*N* = 21)	*P*
AGE (YR)	48.7 ± 16.18 (47.0)	60.7 ± 15.35 (63.0)	66.1 ± 12.96 (67.0)	68.1 ± 11.73 (70.0)	<0.0001
WOMEN (%)	53	53	62	62	0.5090
BMI (KG/M^2)	26.6 ± 5.25 (25.8)	28.2 ± 5.65 (27.2)	29.9 ± 6.24 (29.5)	28.4 ± 6.72 (27.9)	0.0001
90-MIN PLASMA GLUCOSE (MG/DL)	100.2 ± 23.89 (97.0)	131.7 ± 31.93 (124.0)	238.4 ± 74.32 (228.0)	306.6 ± 48.72 (301.0)	<0.0001
CREATININE CLEARANCE (ML · MIN^{-1} · 1.73 M^{-2})	101.9 ± 25.12 (103.0)	91.7 ± 24.73 (88.5)	78.9 ± 30.30 (76.5)	78.7 ± 25.63 (77.5)	<0.0001
PCP (NM)	3.00 ± 1.235 (2.70)	3.95 ± 1.562 (3.70)	4.19 ± 2.228 (3.70)	1.74 ± 1.091 (1.30)	<0.0001
UCP (NMOL/260 MIN)‡	10.2 ± 5.33 (9.07)	12.0 ± 6.52 (11.20)	13.8 ± 11.21 (11.06)	5.4 ± 3.94 (4.33)	<0.0001
UCP (NMOL/MMOL CREATININE)§	5.2 ± 3.12 (4.49)	6.6 ± 4.01 (5.75)	7.8 ± 6.18 (6.11)	2.9 ± 2.20 (2.34)	<0.0001

Values are means ± SD with medians in parentheses.
*Diabetic glucose tolerance test, not IDDM by medical records (see METHODS); not taking insulin.
†Diabetic glucose tolerance test, not IDDM by medical records (see METHODS); taking insulin.
‡*N* = 270, 73, 65, and 20 for 4 groups.
§*N* = 278, 74, 69, and 21 for 4 groups.

contrast to PCP, the UCP:creatinine ratio was also higher in those with diabetic glucose tolerance (not taking insulin) than the NGT group ($P < 0.01$ vs. nondiabetic participants; NS vs. IGT) (Table 4). The pattern of UCP levels across the three non–insulin-taking groups was the same as the pattern of UCP:creatinine ratio, although the differences were not statistically significant for UCP.

Association of C-peptide production with concurrent plasma glucose level

A second regression model was examined, which allowed for effect modification on the relationship between C-peptide production and 90-min plasma glucose by the level of glucose tolerance (Table 5). The percentage of the variance explained by this model was 35.3, 18.0, and 21.6% for PCP, UCP, and C-peptide/creatinine, respectively. In the participants with normal or impaired glucose tolerance, PCP increased 0.6 and 0.5%, respectively, with each milligram per deciliter increase in 90-min plasma glucose; the relationship was not significantly different between the two subgroups. In contrast, among the diabetic participants (insulin taking or not), the relationship was nonsignificant and inverse between C-peptide production and the 90-min plasma glucose level (Fig. 5). (Results are shown for PCP; results for UCP were similar.)

With the regression model that allowed for effect modification, the adjusted geometric means were obtained (Table 6, Fig. 6). Table 6 presents adjusted geometric mean C-peptide levels for a plasma glucose held constant at 7.6 mM (137 mg/dl). After controlling for the plasma glucose levels via the statistical model that better fit the data, the non–insulin-taking diabetic participants

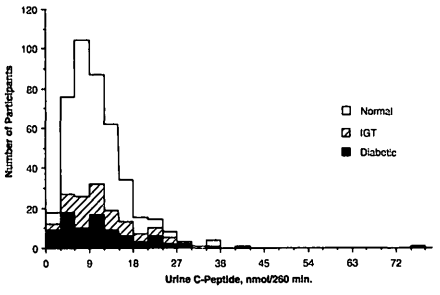

Figure 1—*Frequency distribution of UCP response to 480 ml Ensure-Plus by WHO glucose tolerance classification, 428 Wadena City Health Study participants.* □, *NGT;* ▨, *IGT;* ■, *diabetic.*

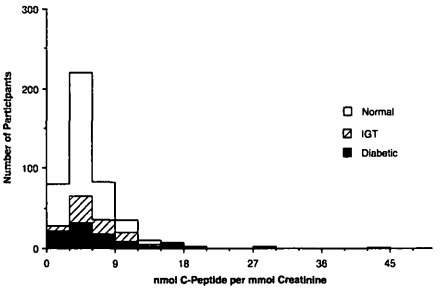

Figure 2—*Frequency distribution of UCP response to 480 ml Ensure-Plus by WHO glucose tolerance classification, 442 Wadena City Health Study participants.* □, *NGT;* ▨, *IGT;* ■, *diabetic.*

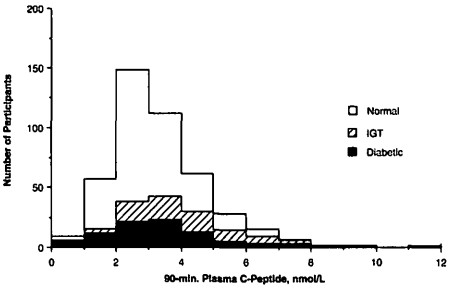

Figure 3—*Frequency distribution of PCP 90 min after consuming 480 ml Ensure-Plus by WHO glucose tolerance classification, 443 Wadena City Health Study participants.* □, *NGT;* ▨, *IGT;* ■, *diabetic.*

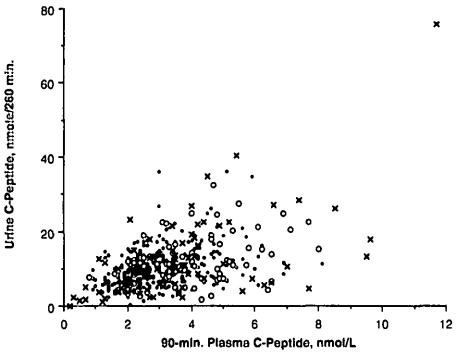

Figure 4—*PCP 90 min after Ensure-Plus plotted against simultaneously collected UCP by WHO glucose tolerance category, 428 Wadena City Health Study participants. ●, NGT; ○, IGT; x, diabetic.*

had higher average PCP levels than the participants with either NGT or IGT and higher UCP and C-peptide/creatinine levels than those with NGT. Figure 6 depicts the geometric mean PCP levels at various constant levels of plasma glucose. At any level of plasma glucose, it appears that the insulin-taking diabetic participants would produce on average significantly less C-peptide than the non–insulin-taking groups, and that the participants with IGT would have geometric mean C-peptide levels statistically indistinguishable from the participants with NGT.

C-peptide production and duration of diabetes

Among participants with a diagnosis of diabetes before participation in the Wadena study, the mean number of years since diagnosis was greater among the insulin-taking participants than among those not taking insulin (12.8 vs. 7.9 yr, respectively, $P = 0.014$). However, a great deal of overlap was noted in duration of diabetes between insulin-taking and non–insulin-taking participants: 3.0–34.5 vs. 0.5–25.0 yr, respectively.

Because residual analysis indicated a better linear fit when the logarithm of PCP was the dependent variable, the log model was used. A significant ($P < 0.01$) inverse relationship was noted between the number of years since diagnosis and log PCP.

Although we observed an overall inverse relationship between years since diagnosis and log PCP, the proportion of variance explained by the regression (R^2) was only 0.13. If insulin-taking status was added as a predictor, with separate slopes calculated for use or nonuse of insulin, R^2 increased to 0.42. PCP declined significantly (5.7%/yr, $P < 0.001$) with years since diagnosis of diabetes only among the participants who were taking insulin. Among the non–insulin takers, no relationship was evidenced between the number of years since diagnosis and the PCP response to a meal stimulus (Fig. 7). Similar results were obtained with UCP and C-peptide/creatinine.

CONCLUSIONS

General distribution of C-peptide values among Wadena adults: comparison with other reports

The range of meal-stimulated UCP and PCP in nondiabetic Wadena participants is surprisingly broad: from 0.44 to 37.0 nmol/260 min for UCP and 0.9 to 8.1 nM for PCP. The minimum values approach those reported for IDDM subjects (6–10). Estimated mean C-peptide levels for the general adult population of Wadena, stratified by age and sex, have been reported elsewhere (27).

The range of meal-stimulated UCP and PCP in diabetic Wadena participants was also very broad. Both the highest and the lowest observations occurred in the diabetic group. The highest values were observed in a newly identified diabetic participant (11.7 nM in plasma and 75.7 nmol/260 min in urine).

The mean UCP and PCP values reported here are two to three times as high as values reported by others in nondiabetic subjects (mostly adults <40 yr old) (Table 1). A partial explanation could be the potency of the 710-kcal meal challenge and the timing of sample collection at the approximate peak postmeal. Furthermore, the range of age and body weight encountered in this population is broad, with the likely result that insulin sensitivity also covers a broader range than that encountered in previous studies.

It has been proposed that cross-reaction of immunoassays with proinsulin may account for the failure to distinguish the insulin responses in NIDDM from those in control subjects, because proinsulin may account for ~50% of what is measured by immunoassay of plasma insulin in NIDDM but <33% of

Table 4—*Unadjusted and adjusted geometric mean levels of PCP (nM) 90 min after 2-can meal of Ensure-Plus, and unadjusted and adjusted geometric mean UCP (nmol/collection) and C-peptide:creatinine ratio (nmol UCP/mmol creatinine), 260-min urine collection after 2 cans Ensure-Plus*

Glucose tolerance category	Unadjusted geometric mean			Adjusted* geometric mean		
	PCP	UCP	C-peptide/creatinine	PCP	UCP	C-peptide/creatinine
NGT	2.76	8.98	4.48	2.96	8.81	4.48
IGT	3.66	10.20	5.71	3.48†	10.22	5.51†
Diabetic (no insulin)	3.70	10.75	6.34	3.14	11.21	6.33‡
Diabetic (insulin)	1.39	3.20	1.81	1.13§	3.61§	1.93§

*Adjusted for age, sex, 90-min postchallenge plasma glucose level, and BMI (see Table 3).
†$P < 0.05$, ‡$P < 0.01$, vs. NGT.
§$P < 0.0001$ vs. non–insulin-taking groups.

Table 5—*Regression coefficients for relationship of ln(PCP) with 90-min plasma glucose level in linear regression model including age and BMI as continuous variables and sex, WHO classification, and use of insulin as indicator variables (U are proportion change in PCP/mg/dl increase in 90-min plasma glucose)*

Glucose tolerance category	Adjusted regression coefficient*	95% Confidence interval
NGT	0.0063	0.0042–0.0084
IGT	0.0052	0.0006–0.0097
Diabetic (no insulin)	−0.0020	−0.0064–0.0023
Diabetic (taking insulin)	−0.0023	−0.0084–0.0037

*Adjusted for age, sex, and BMI.

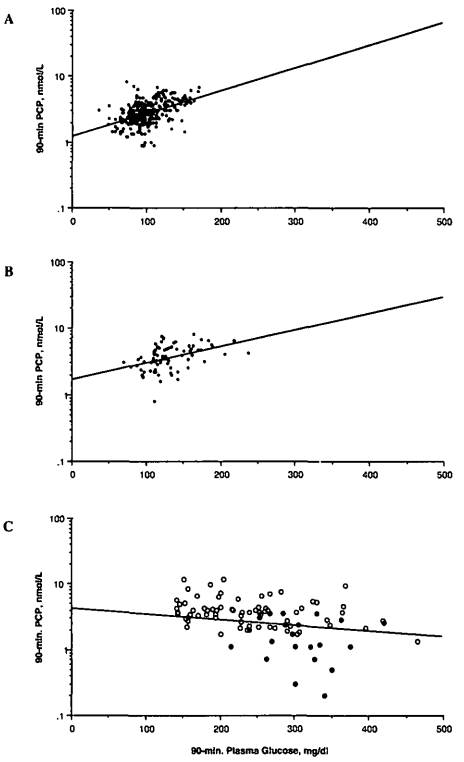

Figure 5—*PCP 90 min after 480 ml Ensure-Plus plotted against simultaneously collected plasma glucose, 443 Wadena City Health Study participants. A: Participants with NGT. Slope is +0.6%/mg/dl increase in plasma glucose. B: Participants with IGT. Slope is +0.5%/mg/dl increase in plasma glucose. C: Participants with diabetic glucose tolerance. Slope is −0.2%/mg/dl increase in plasma glucose. ○, non–insulin-takers; ●, insulin-takers.*

measured insulin in control subjects (28). However, cross-reaction of the C-peptide assay with proinsulin is unlikely to account for the fairly high levels of C-peptide reported herein because, even with an antibody that fully recognized both proinsulin and C-peptide, proinsulin in peripheral blood would constitute only 1–2% of measured C-peptide (28). In addition, none of the cases of high UCP level would be caused by high levels of proinsulin, because proinsulin does not appear in urine (29).

Meal-stimulated UCP

The relative abundance of UCP (compared with urine insulin) has made it a naturally attractive substance for study (4), especially with respect to integrated β-cell response to physiological and pharmacological stimuli (30). An analogy to other tests of endocrine function and reserve suggests itself, particularly the long-established clinical and research value of urinary steroid measurements in the study of adrenal cortical function.

Definitive C-peptide infusion studies have been conducted in humans at the University of Chicago concerning the 24-h fractional urinary excretion of C-peptide (the amount appearing in urine divided by the integrated C-peptide secretion as measured by the infusion technique): an average of 8% in 14 nondiabetic control subjects (range 1.1–27.9%) and 11.3% in 13 NIDDM subjects (range 3.9–20.8%) (31). Thus, on average, only ~10% of secreted C-peptide appears in the urine, and considerable subject-to-subject difference occurs in the fractional excretion. Within subjects, on two trials, the mean CV of the fractional urinary excretion was 28.4% (31), implying a within-subject range for fractional urinary excretion of ~5–15%. Gjessing and colleagues (10,32) have estimated the within-subject CV for 24-h UCP to be 45% in insulin-taking (10) and 22% in non–insulin-taking (32) diabetic subjects. The mean within-subject CV among 8 nondiabetic subjects studied by Hoogwerf et al. (33) was 20.3%. These observations imply that the within-subject variance is approximately equal between the two diabetic groups, although the mean UCP level is lower among insulin takers; and that the within-subject variance does not play a large role in the estimation of UCP in NGT.

Although PCP has a smaller CV than UCP and thus allows more precise comparisons between groups (smaller *P* values for a fixed difference between groups), the two methods for measuring pancreatic function resulted in very similar observations in this study. The 260-min UCP measurement in studies of groups of subjects of moderate-to-large size should yield useful information, on the average, for between-group comparisons and for within-group comparisons over time.

Diabetic subjects are often more willing to undergo a meal challenge than a glucose challenge; thus, meal challenge increases the proportion of diabetic participants from whom data can be obtained on pancreatic reserve. Consumption of a meal including fat also permits the study of postmeal lipemia. Takai et al. (34) and Hoogwerf et al. (33) found that carbohydrate and protein but not fat led to additive acute increases in UCP. Clearly, studies with food as the stimulus to insulin secretion must address the

Table 6—*Adjusted geometric mean 90-min PCP (nM), UCP (nmol/260 min collection), and C-peptide:creatinine ratio (nmol UCP/mmol creatinine) after 2 cans Ensure-Plus, including an interaction between level of glucose tolerance and 90-min plasma glucose (which was set at 137 mg/dl)*

	ADJUSTED GEOMETRIC MEAN*		
GLUCOSE TOLERANCE CATEGORY	PCP	UCP	C-PEPTIDE/ CREATININE
NGT	3.61 (278)	10.04 (270)	5.10 (278)
IGT	3.60 (75)	10.23 (73)	5.56 (74)
DIABETIC (NO INSULIN)	4.20 (69)†‡	12.70 (65)§	7.27 (69)¶
DIABETIC (TAKING INSULIN)	1.80 (21)‖	4.38 (20)‖	2.40 (21)‖

Values in parentheses are (*N*) of observations.
*Adjusted for age, sex, 90-min postchallenge plasma glucose level and BMI.
†$P < 0.001$ vs. IGT.
‡$P < 0.0001$, §$P < 0.01$, ¶$P < 0.0001$, vs. NGT.
‖$P < 0.0001$ vs. non–insulin-taking groups.

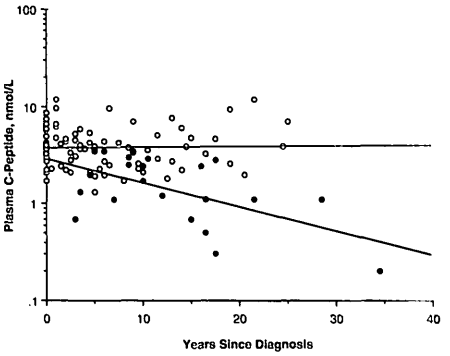

Figure 7—*PCP 90 min after 480 ml Ensure-Plus plotted against number years since diagnosis of diabetes, Wadena City Health Study participants with either history of diabetes or diabetic glucose tolerance test by insulin-taking status. Slope among non–insulin-taking participants is +0.1%/yr, and slope in insulin-taking participants is −5.7%/yr.* ○, *non–insulin takers;* ●, *insulin takers.*

need to standardize the mix of carbohydrate and protein to be used.

Pancreatic function in NIDDM

This cross-sectional study of insulin reserve and NIDDM provides evidence that pancreatic failure may not occur in most NIDDM patients. Provided they were not taking insulin to control their blood glucose, Wadena participants with diabetic glucose tolerance produced as much, if not more, C-peptide and—by logical extension—insulin compared with those with IGT or NGT. This observation held irrespective of the statistical method used to control for confounding or effect modification. The assumption was made that the relationship between plasma glucose and pancreatic output was a simple linear function (the higher the plasma glucose, the greater the C-peptide production; 35) and that it was the same regardless of glucose tolerance status. Under this assumption, the non–insulin-taking NIDDM participants produced just as much C-peptide as did the participants with NGT (Table 4). Alternatively, it can be assumed on the basis of other studies that the insulin response to an oral challenge is not a simple linear function of the blood glucose (36–38). With a regression model that allowed the association between the C-peptide response and blood glucose to vary between the levels of glucose tolerance, the non–insulin-taking NIDDM participants produced as much or more C-peptide than the participants with NGT, at least standardized to the normal range of plasma glucose (Table 6, Fig. 6).

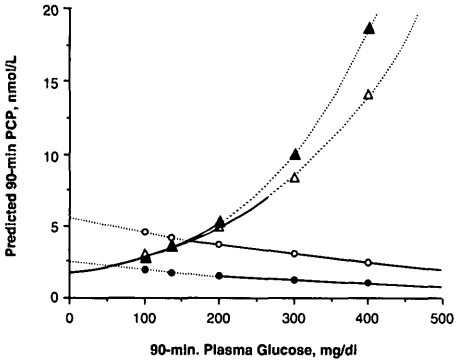

Figure 6—*Predicted geometric mean PCP (adjusted for age, sex, BMI, and 90-min plasma glucose level) by level of glucose tolerance and insulin-taking status, Wadena City Health Study. Effect modification by level of glucose tolerance results in changing relative differences between levels of glucose tolerance, depending on adjusting value used for 90-min plasma glucose. Table 6 is calculated with 137 mg/dl as imputed glucose level.* ▲, *normal;* △, *IGT;* ○, *NIDDM-no ins;* ●, *NIDDM-ins.*

In contrast, C-peptide production was decreased among insulin-taking NIDDM participants. Also, a decline in C-peptide production was associated with a proxy for duration of diabetes among the insulin takers only. This suggests that the clinical decision to use insulin to control blood glucose but not to prevent ketosis may be an indicator for a separate subtype of NIDDM: one that is characterized by lower mean insulin secretion and by a decline in pancreatic function over time. The differences in geometric mean C-peptide levels were not explained by age, sex, BMI, or concurrent blood glucose levels. It is unlikely that insulin therapy would cause a decline in β-cell function, because careful control of blood glucose with insulin increases insulin secretion in NIDDM (39). It has been estimated that 14.2% of NIDDM patients between 45 and 64 yr old use insulin to control their blood glucose (40), and data from the National Health Interview Survey (1976) and the second National Health and Nutrition Examination Survey were used to esti-

mate that 21.3 and 26.1%, respectively, of all diabetic participants 20–74 yr old take insulin (41). This suggests that only a minority of individuals with NIDDM experience a deficit in pancreatic function.

Alternative explanations for the failure to observe a deficit in C-peptide production among the non–insulin-taking diabetic participants in this study is the shorter average length of time since onset of diabetes compared with the insulin-taking group. Perhaps a decline in C-peptide production would also be observed among the non–insulin-taking diabetic group, given a longer period of observation. However, we observed a great deal of overlap in the estimated time since diagnosis between the insulin-taking and non–insulin-taking diabetic groups, and little evidence suggests a sudden drop in pancreatic function occurs after a certain number of years have elapsed since the onset of disease (Fig. 7). Groop and Tolppanen (42) observed no overall decline in C-peptide production with duration of NIDDM, although 24% of their patients were using insulin. In another paper, Groop et al. (43) suggested that deterioration of pancreatic function occurred only in subjects with the HLA antigens generally associated with IDDM (DR3/DR4). HLA-DR typing in the Wadena study is in progress and will be reported later.

Another alternative explanation for the observed results is the possibility that some IDDM individuals were mistakenly included in the insulin-taking group labeled as NIDDM. Because IDDM usually is diagnosed at a young age, individuals with IDDM in a cross-sectional study are more likely to have had their diabetes for a longer period of time than age-matched NIDDM individuals. It is difficult to differentiate the truly IDDM individual from the insulin-taking but not insulin-dependent one, but the definition of established IDDM customarily includes the production of ketone bodies in the absence of exogenous insulin (17). In this study, medical records were reviewed for all but 11 of the diabetic participants. Acetonuria and/or ketoacidosis was documented in 8 participants, and these were excluded from analyses. The medical review records of 3 diabetic participants, treated as NIDDM in these analyses, indicated probable or possible IDDM: The record included a physician statement of IDDM, but no documentation of acetone production. One of these 3 probable/possible IDDM participants had no detectable levels of C-peptide in the urine; the other 2 had levels of C-peptide within the range of the nondiabetic participants. When the 3 probable/possible IDDM participants were excluded from analyses, the insulin-taking NIDDM participants still had significantly lower C-peptide production than the non–insulin-taking participants. Also, the magnitude of the decline in PCP with years since diagnosis among the insulin-taking diabetic participants was the same, although statistical significance was no longer achieved. The relationship between the UCP variables and years since diagnosis nearly disappeared when the participants with probable/possible IDDM were excluded (Fig. 8). If the clinical decision to use insulin to control blood glucose is associated with a predisposition for progressive pancreatic failure, then it is not surprising that in some cases it is difficult to distinguish between IDDM and NIDDM.

On average, diabetic participants had somewhat poorer kidney function than did the nondiabetic participants. Metabolic handling and disposal of C-peptide occur mostly in the kidney so that declining kidney function could affect observed levels of C-peptide in the blood and urine. Elevated fasting PCP levels have been observed in uremic patients (44). However, the observed relationships between glucose tolerance and C-peptide production are unlikely to be explained by differences in kidney function between the groups: The association between creatinine clearance and C-peptide production disappeared after controlling for age, which was used as a covariate in the analyses presented here. In addition, excluding participants with abnormal (<80 ml/min) creatinine clearance resulted in nearly identical results as did including them (data not shown). Finally, the most marked differences in C-peptide production observed in this study were between the insulin-taking and the non–insulin-taking diabetic participants, and these two groups had almost identical mean creatinine clearance.

A cross-sectional study gives only a limited view of the progression of disease. The same individuals should be studied over time to eliminate any possible biases (such as confounding due to differential mortality) of studying a cross-sectional sample. However, the cross-sectional study can provide a longer-term perspective on a disease state than is usually possible in a cohort study. Thus, the data suggest that a decline in pancreatic reserve does not occur inexorably in individuals who have diabetes, but it is impossible to say that conclusively. The Wadena study group is continuing to follow phase 1 participants over time.

Pancreatic function in IGT

Consistent with previous reports (36,38), participants with IGT demonstrated C-peptide production that was somewhat greater than those with NGT, even (in this report) after controlling for age, sex, and BMI with a linear term for concurrent plasma glucose. The difference reached statistical significance with plasma C-peptide and the ratio of C-peptide to creatinine in the urine. Individuals with IGT are at increased risk of developing diabetes (45), although many individuals with IGT revert to NGT (17,46). Thus, the observation that individuals with IGT displayed greater C-peptide production than individuals with NGT may indicate that insulin resistance occurs first, before any decline in pancreatic function occurs (if it ever does). The relationship between C-peptide and plasma glucose among IGT participants was statistically indistinguishable from either the normal, nondiabetic or di-

abetic groups (Table 5, Figs. 5 and 6). This is consistent with the theory that IGT is an intermediate condition between NGT and what is labeled diabetes. Again, following individuals over time may confirm or discredit this observation.

C-peptide production and plasma glucose

Another question raised by this investigation is whether a proper way to control for the effect of blood glucose on measurements of pancreatic function exists. In a community-based research study, many variables are not under the direct control of the researchers. The C-peptide response to a meal challenge is a function not only of the β-cell reserve, but also of gastrointestinal hormones, the glucose load, the tissues' response to insulin, and feedback to the β-cell of the resulting blood glucose level (30,37,47–49). The size of the challenge was held constant in this study, although variations in how the meal was absorbed from the intestinal tract could change the effective size of the caloric load. Insulin-clamp techniques are not feasible outside of a clinical research center; and even with insulin clamps, it is often not possible to achieve equivalent blood glucose levels in diabetic and nondiabetic individuals (50). This makes statistical adjustment the only method available for controlling confounding and effecting modification. The assumption that there is a simple linear relationship between blood glucose and pancreatic output leads to the first type of analysis used in this report. The observation that the response of the β-cell to plasma glucose actually varies with the level of glucose tolerance leads to the second type of analysis reported here and theoretically may be more consistent with the hypothesis of glucose toxicity (51,39). If insulin secretion indeed is suppressed by chronically elevated blood glucose levels, then it may be appropriate to adjust upwards the C-peptide level in subjects with elevated blood glucose. Because blood glucose level is the basis for classification of diabetic status, confounding by blood glucose is probably impossible to avoid completely, as has been pointed out by Savage et al. (38).

The association of the 90-min plasma glucose with UCP was much weaker than with PCP. This is probably because urine values are integrated measures of pancreatic activity during the entire 4-h postmeal period and so would be less tightly correlated with a single plasma glucose measurement than would a simultaneously collected PCP measurement. It probably also explains the observation that adjustment for covariates including plasma glucose had a smaller effect on the geometric mean UCP and C-peptide/creatinine levels than it did on PCP levels (Table 4).

Comments

Numerous methods are now available for estimation of human insulin secretion: among them are measurement of insulin or C-peptide in a peripheral blood vessel or in urine, fasting or after an oral or intravenous glucose load, after a mixed meal, or after glucagon injection. Although all of the methods will be likely to agree with each other over broad areas of contrast (e.g., comparing IDDM with normal islet function), they will differ in special instances. For example, the rapid, first-phase insulin response to i.v. glucose can be shown clearly by measurement of peripheral venous insulin levels. Loss of this response is characteristic of NIDDM (52), but it is not reflected by PCP because of its longer half-life and somewhat delayed rise and fall (53). On the other hand, C-peptide more closely reflects total prehepatic insulin secretion (1–3).

UCP, although it carries a higher CV than PCP, is convenient, noninvasive, and as an integrated measure of pancreatic response may have a place in longitudinal and group studies. We have focused our study on postmeal C-peptide values rather than fasting values because we wish to explore the top range of secretion response in nondiabetic and NIDDM subjects. The inclusion of protein in the meal increases its potency as a stimulus; this has been a useful approach also in studying IDDM, as in the Diabetes Control and Complications Trial study (8). This potency, together with analytical differences attributable to the choice of antibody, accounts in part for our relatively high C-peptide values compared with those reported by others.

We speculate that useful new ways of defining the diabetic state may be developed eventually, based on mixed-meal stimuli and moving away from dependence on glucose-stimulated tests alone.

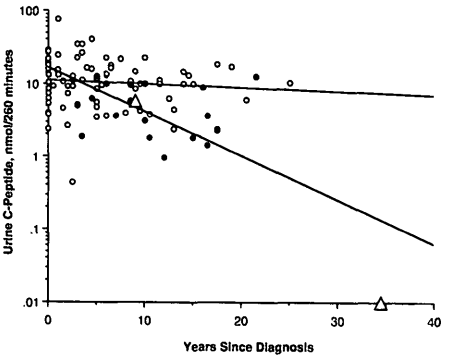

Figure 8—*UCP after 480 ml Ensure-Plus plotted against number years since diagnosis of diabetes, Wadena City Health Study participants with either history of diabetes or diabetic glucose tolerance test by insulin-taking status. Slope among non–insulin-taking participants is −1.2%/yr (P = 0.50); and slope in insulin-taking participants is −14.0%/yr (P = 0.0001). Two participants with UCP and designation as probable or possible IDDM on medical record review are indicated. Without these 2 participants, slope among insulin-taking participants is −2.8%/yr (P = 0.66). ○, non–insulin-takers; ●, insulin-takers; △, prob/poss IDDM.*

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