Are Specific Serum Insulin Levels Low in Impaired Glucose Tolerance and Type II Diabetes?: Measurement With a Radioimmunoassay Blind to Proinsulin, in the Population of Wadena, Minnesota

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It has been suggested that serum insulin levels in subjects with recently diagnosed type II diabetes have been overestimated, and that after correction for proinsulin, true insulin levels are depressed rather than elevated. We tested this possibility in a cross-sectional study of a population-based sample of 328 adults living in Wadena, a Minnesota community in which residents are of northern European background. Specificity of insulin measurements was provided by an antibody blind to proinsulin and its major metabolite. Oral glucose tolerance and liquid mixed-meal (Ensure-Plus) tests were performed on separate days. Mean insulin levels before and 90 minutes after the mixed meal were as follows. Among 302 randomly ascertained adults not previously known to have diabetes, both fasting and postmeal levels in subjects with impaired glucose tolerance (IGT) and newly identified type II diabetes were equal to or greater than levels in subjects with normal glucose tolerance (fasting: normal 52 pmol/L, IGT 78, new type II 87; postmeal: 317, 565, and 406, respectively). The fasting insulin to glucose ratio was significantly increased in IGT and new type II diabetes subjects. Among 26 established (previously known) type II diabetic subjects not taking insulin, fasting levels were elevated and postmeal levels were normal in absolute terms (75 and 328), but were normal or low with respect to plasma glucose. Relationships among the groups were not materially changed by adjustment for body mass index (BMI), sex, age, or blood pressure. There was marked overlap of individual insulin levels from group to group. In summary, randomly selected adults in Wadena with IGT or asymptomatic diabetes showed, on average, elevated insulin levels, but physician-diagnosed diabetes was associated with relative diminution of serum insulin. In this population, the current view of insulin resistance in "early" diabetes was supported by insulin-specific measurements.

MEASURABLE AMOUNTS of insulin in the plasma of many diabetic humans have been reported since the 1950s.[1-4] It is now generally accepted on the basis of many studies (reviewed by DeFronzo et al[5]) that persons who are developing type II diabetes pass through a stage in which serum insulin levels are elevated in comparison to those of nondiabetic control subjects. This pattern has been shown with particular clarity in certain ethnic groups with especially high prevalences of diabetes, such as Pima Indians[6] and Nauruan islanders.[7]

However, it has more recently been shown that measurement of insulin levels by conventional immunoassay may be inaccurate because of lack of specificity for insulin of the polyclonal antibodies ordinarily used. In diabetic subjects reported by Temple et al,[8,9] proinsulin and its major metabolite accounted for an important proportion of the sum of fasting insulin plus proinsulin. When a highly specific insulin assay system was used, Temple et al found lower absolute insulin levels in newly diagnosed type II diabetic patients than in nondiabetic control subjects. This led them to suggest that the importance of insulin deficiency in type II diabetes may have been significantly underestimated.

This observation could reflect not only differences in laboratory methods but also differences in methods of ascertainment and testing of subjects and in the populations from which they were drawn. For these reasons, we studied a population-based sample of 328 adults living in a Minnesota community in which residents are entirely of northern European background. An insulin-specific assay was used, depending on an antibody demonstrated to be "blind" to proinsulin and its major metabolite. Cross-sectional results are presented here.

SUBJECTS AND METHODS

Subjects

The subjects were residents of Wadena, MN, a city of 4,699 residents (1980 US census) in west-central Minnesota. Over 99% were white (< 1% Hispanic and Native American); more than 85% of the subjects indicated that their families came from Germany, Scandinavia (including Finland), or the British Isles. The sampling frame for the study included all known physician-diagnosed diabetic residents of Wadena and all other residents at least 20 years of age living within the city limits of Wadena. Greater than 99% of eligible households participated in a special census conducted by the study staff between November 18 and December 31, 1985. All known diabetic persons identified from health care sources during a previous survey (1979 to 1981)[10] were eligible for the study if they still lived in the city; the list of diabetic subjects was updated using methods similar to those used in the previous survey. In addition, a stratified random sample (SRS) was drawn from the list of 2,989 adult residents of Wadena who had not been identified as diabetic from health care sources. The list was stratified according to sex and age group (20 to 39, 40 to 59, and ≥ 60) and balanced

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according to use or nonuse of miscellaneous medications, with 100 names per age and sex group, for a total of 600 names, an average of one of five residents. The initial participation rate was 71% (87 of 123) for the known diabetic subjects and 65% (389 of 600) for the SRS. Details of the design of the Wadena City Health Study have been previously published.[11-13] The prevalence of type II diabetes in Wadena adults over age 20 in 1986 to 1987 was estimated using National Diabetes Data Group (NDDG) criteria to be 7.8%, and of impaired glucose tolerance (IGT), 5.2%, for a combined prevalence of 13.0%, standardized to the 1980 US census for whites from 20 to ≥ 60 years of age.

Cross-sectional data are presented here from 5-year follow-up studies. Results were available for a total of 302 SRS subjects, divided into four groups according to increasing hyperglycemia on glucose tolerance testing as defined by the NDDG[14]: 202 with normal glucose tolerance (NGT); 60 with intermediate glucose tolerance (nondiagnostic); 21 with IGT; and 19 new type II subjects identified at baseline, designated as new type II diabetes. There were additionally 26 previously diagnosed diabetic subjects who had never taken insulin, designated here as known type II diabetes. Subjects who gave a history of present or past use of insulin were excluded from this report, to avoid spuriously high insulin measurements due to endogenous insulin-binding antibody in their sera.

We use the term "intermediate" as a substitute for the relatively unfamiliar "nondiagnostic" to designate glucose tolerance curves higher than normal but less than the NDDG minimum cutoff points for IGT. These cutoff points are a 30-, 60-, or 90-minute glucose level greater than 200 mg · dL^{-1} (11.1 mmol/L) and a 2-hour value greater than 140 mg · dL^{-1} (7.8 mmol/L) but less than 200 mg · dL^{-1}.

Testing Procedure

Glucose tolerance testing was performed using the procedure reported by the NDDG for epidemiologic studies,[14] using an oral dose of 75 g anhydrous glucose. On a later morning within 1 week, subjects returned for measurement of serum insulin and plasma glucose levels during fasting and 90 minutes after a liquid-formula mixed meal. The meal used was Ensure-Plus (Ross Laboratories, Columbus, OH) 480 mL, containing 95 g carbohydrate as dextrose, 26 g protein, and 25 g fat, and providing 710 kcal.

For both tests, subjects came to the testing center in Wadena at 7:30 AM after having been instructed to follow a diet of at least 150 g carbohydrate daily for 3 days and to keep a diet record for review; 94% of subjects indicated taking at least 150 g carbohydrate. Participants were instructed to fast for at least 12 hours before testing. They were seated quietly while blood samples were drawn from an antecubital vein, at least 20 minutes after arrival in the clinic.

Laboratory Methods

Venous blood samples for insulin assay were allowed to clot at room temperature; serum was promptly frozen and stored at −20°C for assay within 12 to 18 months. Insulin level was measured in triplicate at the University of Minnesota, using a human insulin-specific radioimmunoassay kit furnished by Linco Research, St Louis, MO. Specificity of the assay is provided by a unique insulin antibody that reacts with an epitope presenting a free NH_2 terminus on the insulin A chain. Intact human proinsulin and Des 31,32 human proinsulin (the two forms found in significant levels in human serum[15]) are not reactive in this assay, since the required epitope is blocked by the lysine/arginine dibasic linkage connecting insulin with C-peptide. Cross-reactivity to intact human proinsulin and to Des 31,32 human proinsulin is 0.2% and $<0.2\%$, respectively. The lack of response to proinsulin was confirmed in our University of Minnesota laboratory with a sample of proinsulin produced by recombinant DNA technology (Eli Lilly Research Laboratories, Indianapolis, IN). All samples were tested for endogenous insulin-binding activity; any sample showing such antibody activity was excluded from the study. The lower limit of sensitivity for the method was 12 pmol/L (2 μU/mL). The within-assay coefficient of variation was 5.0% ($r = .63$); the between-assay coefficient of variation was 8.0%.

Urine for C-peptide measurement was collected under supervision for 260 minutes, beginning just before taking the liquid meal. The method reported by Heding[16] was used for assay, using antibody K6 from Novo Laboratories, Copenhagen, Denmark.

Venous blood for glucose assay was collected in fluoride tubes and chilled, and the plasma was analyzed in triplicate within 2 hours using the Yellow Springs Institute (Yellow Springs, OH) glucose analyzer (glucose oxidase method). To evaluate insulin concentration in relation to simultaneous plasma glucose concentration, the ratio of insulin to glucose (picomoles to millimoles) was calculated for each subject.

Data Analysis

For descriptive and analytical statistics, we used SPSS 4.0 software (SPSS Inc, Chicago, IL) on the Sun Sparc System (Sun Microsystems Computer, Mountainview, CA). Tukey's honestly significant difference procedure was chosen as a conservative test for multiple comparisons among groups of varying size.[17] Statistical significance was set at .05 or less. Logarithmic transformation of serum insulin and urine C-peptide values was performed before analysis, to normalize their skewed distributions. The means and confidence intervals were then transformed back to the original scale. Thus, reported means for these variables are geometric means.

RESULTS

Descriptive Information

Descriptive information on subject groups is listed in Table 1. Subjects with NGT were significantly younger than those with intermediate glucose tolerance and those with either new or previously diagnosed type II diabetes. Subjects with IGT were younger than type II diabetic subjects. Body mass index (BMI) tended to be greater with increasing glucose intolerance, but the differences were not statistically significant except for the known type II diabetes group as compared with those with NGT or intermediate glucose tolerance. Fasting plasma glucose, as expected, was significantly higher for the two type II diabetic groups as compared with the other three. The five glucose tolerance groups were well separated from each other by postnutrient plasma glucose levels, not only with respect to mean levels 2 hours after the glucose drink but also with regard to mean levels 90 minutes after the liquid meal: for both measurements, each group was significantly different from the other four, with little overlap of 95% confidence intervals (CIs). Systolic blood pressure was lower in the NGT group than in any other group. For diastolic blood pressure, the IGT group had the highest value. The table also shows the proportion of subjects reporting a family history of diabetes in a parent, siblings, or offspring. This proportion increased from 32% for individuals in the NGT group, to 50% for

Table 1. Descriptive Data (mean and 95% CI) for the Five Groups of Wadena Adults by Glucose Tolerance Status: Four Groups Comprising the SRS, and the Cohort of Previously Known Diabetic Persons Not Taking Insulin

Characteristic	Normal		Intermediate		IGT		New Diabetes		Known Diabetes	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
No.	202		60		21		19		26	
Age (yr)	53.2	51.1, 55.3	62.0	58.0, 65.9	55.4	48.3, 62.4	69.4	64.1, 74.6	71.3	67.1, 75.5
Male/female ratio	0.8		1.0		2.5		0.4		0.9	
BMI	26.6	25.8, 27.3	27.0	25.7, 28.2	29.5	27.5, 31.4	29.3	27.0, 31.6	30.9	28.0, 33.7
FPG (mmol/L)	5.4	5.3, 5.4	5.7	5.6, 5.9	6.0	5.7, 6.3	7.6	6.5, 8.7	9.6	8.3, 10.9
PG 90 minutes postmeal (mmol/L)	5.8	5.6, 6.0	6.9	6.6, 7.2	8.9	8.1, 9.7	11.7	9.8, 13.5	15.8	13.8, 17.9
PG 2 hours postglucose (mmol/L)	5.9	5.7, 6.1	7.8	7.5, 8.2	9.3	8.9, 9.7	14.1	12.7, 15.5	17.3	14.6, 20.0
Systolic BP (mm Hg)	117	115, 119	125	120, 130	132	123, 141	138	131, 146	132	124, 141
Diastolic BP (mm Hg)	72	71, 73	73	70, 75	80	74, 86	78	73, 83	71	67, 76
Family history (% positive)	32		43		50		69		58	

Abbreviations: FPG, fasting plasma glucose; PG, plasma glucose; BP, blood pressure.

IGT subjects, and to a maximum of 69% for the new diabetes group.

Fasting Serum Insulin Levels

Mean fasting insulin levels and 95% CIs are shown in Table 2 in order of increasing hyperglycemia. There is an increase in mean insulin levels as hyperglycemia increases, from 52 pmol/L (9 μU/mL) for those with NGT to 87 pmol/L (15 μU/mL) for newly type II diabetic individuals. By the test of honestly significant difference, the normal group had significantly lower levels than the IGT group and both diabetic groups; the intermediate (nondiagnostic) group had significantly lower levels than the IGT group and newly diabetic group.

Scattergrams for individual subjects are presented in Fig 1 according to glucose tolerance status, with a logarithmic vertical scale. The geometric mean for each group is shown. There is marked overlap of the ranges of all subgroups with each other.

Meal-Stimulated Insulin Levels

Normal subjects showed the expected brisk increase, to a mean value of 317 (53 μU/mL), and the other four groups also showed a brisk increase. Here, the highest mean values were noted in the IGT group and were statistically significantly higher than in normal, intermediate, and known-diabetic groups. Mean values in the two diabetic groups did not differ from values in the normal group. As with fasting levels, there was marked overlap of the ranges of the various groups (Fig 2 and Table 2).

Urine C-peptide

Meal-stimulated urine C-peptide level was measured in parallel with serum insulin and provided an independent measure of pancreatic insulin secretion (Table 2). Differences among groups were not significant at a P level of .05 by the honestly significant difference test, but the pattern of means closely paralleled that of postmeal serum insulin levels.

Ratio of Insulin to Glucose

Table 3 presents mean fasting and postmeal insulin to glucose ratios (picomolar to millimolar) by diabetes status. For the mean fasting ratio, new-diabetes and IGT groups both had significantly higher values than the intermediate (nondiagnostic) and normal groups. For the postmeal ratio, the known-diabetes group had significantly lower values than normal, intermediate, and new-diabetes groups.

Scattergrams for the individual ratios (not shown) again showed substantial overlap among the groups.

Table 2. Fasting and Postmeal Serum Insulin Levels and Meal-Stimulated Urine C-peptide in Wadena Adults According to Glucose Tolerance Status: Four Groups Comprising the SRS, and the Cohort of Previously Known Type II Diabetic Persons Not Taking Insulin (known type II DM)

Group	No. of Subjects	Serum Insulin (pmol/L)				Urine C-peptide (nmol · 260 min^{-1})	
		Fasting		90 Minutes Postmeal			
		Mean	95% CI	Mean	95% CI	Mean	95% CI
NGT	202	52 [54]	48, 55	317 [324]	294, 341	8.0 [7.8]	7.3, 8.6
Intermediate (nondiagnostic)	60	56 [56]	50, 61	345 [347]	297, 400	8.8 [8.9]	7.6, 10.1
IGT	21	78 [69]	63, 98	565 [513]	396, 807	11.2 [10.0]	7.9, 15.8
New type II DM	19	87 [83]	65, 117	406 [380]	299, 551	9.8 [10.7]	7.1, 13.4
Known type II DM	26	75 [69]	56, 100	328 [302]	244, 440	8.1 [8.3]	5.7, 11.6

NOTE. Values are geometric means. Numbers in brackets are mean values adjusted for age, BMI, sex, and systolic blood pressure by analysis of covariance.

Abbreviation: DM, diabetes mellitus.

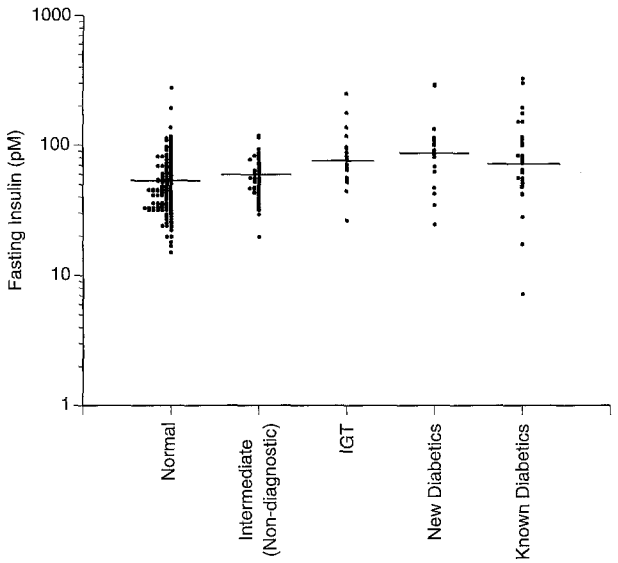

Fig 1. Fasting serum insulin concentrations in residents of Wadena, MN. The figure shows individual values for 302 members of the SRS randomly chosen from all Wadena adults aged 20 to 90. The groups are presented in ascending order of glucose intolerance, from left to right. The additional group, known diabetics, is a separate cohort of 26 subjects representing all physician-diagnosed cases of diabetes in Wadena, omitting those taking insulin. The vertical scale is logarithmic, and the horizontal lines show the geometric mean for each group.

Relationships Between Insulin Levels and Other Variables: Adjustment by Analysis of Covariance

We examined by regression analysis the relationships between log-transformed insulin levels and selected variables of interest: obesity (BMI), age, fasting plasma glucose, and systolic blood pressure. For women in the SRS (n = 161), regression of log fasting insulin on independent variables one at a time was highly significant ($P < .0001$) for BMI ($R^2 = .25$) and fasting plasma glucose ($R^2 = .14$), and for systolic blood pressure ($R^2 = .03$), it was moderately significant ($P = .03$). However, for age, there was no association ($R^2 = .004$, $P = .42$). With multiple regression including all four independent variables, the total R^2 was .34 ($P < .0001$). Corresponding values for men in the SRS (n = 141) were similar, with a somewhat stronger overall association ($R^2 = .43$, $P < .0001$) when all four variables were included in the regression equation.

For both men and women in the SRS, associations between log postmeal insulin levels and independent variables were similar to those for fasting insulin levels, but not as strong (four-variable $R^2 = .20$ for women and .21 for men, $P < .0001$ for both).

Because of the importance of these factors—especially relative obesity as measured by BMI—mean values for insulin and the insulin to glucose ratio were adjusted by analysis of covariance for sex and for age, BMI, and systolic blood pressure measured when insulin levels were determined. The adjusted results are listed in Tables 2 and 3. Although the adjusted values are shifted to some extent—primarily in decreasing the values for IGT subjects somewhat—positions of the groups relative to each other are unchanged.

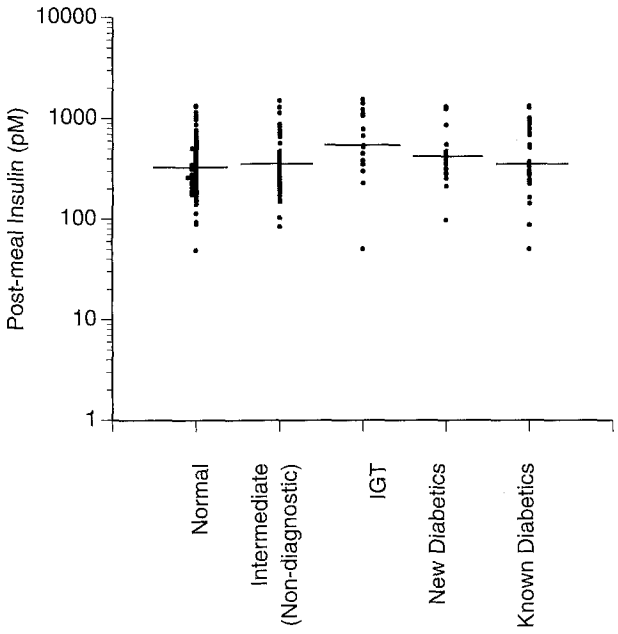

Fig 2. Postmeal serum insulin values for the same subjects shown in Fig 1, measured 90 minutes after consuming the liquid mixed meal, Ensure-Plus. The meal contained 95 g carbohydrate, 26 g protein, and 25 g fat, providing 710 calories. Note the change of scale from Fig 1; otherwise, the figure parallels Fig 1.

DISCUSSION

The most striking aspect of the results of this study of specifically measured plasma insulin levels is the broad range of values in the group with NGT, and the high degree of overlap with the normal range found in groups with varying degrees of impairment of glucose tolerance. It would be difficult or impossible to predict the glucose tolerance status of a given individual from insulin level alone. This is particularly true for insulin values in the fasting state. This result would be expected because of the design of our study, since we studied a random population sample to avoid selection bias and thus would include mild cases. Nearly all of the newly diabetic persons denied any symptoms suggesting diabetes.

The simplest and most straightforward evidence for insulin resistance is the fasting level of insulin as the absolute value or in relation to the simultaneous venous plasma glucose concentration. When mean absolute fasting insulin levels were examined, subjects with IGT and with type II diabetes had statistically significantly higher levels than the normal group. The mildly hyperglycemic intermediate (nondiagnostic) group showed mean results a little above normal, although not statistically significantly so; however, they were no lower than normal. Thus, in this cross-sectional examination, there was no indication of a deficit of circulating insulin as an accompaniment to mild and presumably early glucose intolerance.

However, after the liquid mixed meal (which contained some protein and fat, as well as carbohydrate), there was a clear difference between the response of the IGT group and that of the two diabetic groups: the former responded with

Table 3. Fasting and 90-Minute Postmeal (Ensure Plus) Insulin to Glucose Ratio in Wadena Adults According to Glucose Tolerance Status: Four Groups Comprising the SRS, and the Cohort of Previously Known Type II Diabetic Persons Not Taking Insulin (known type II DM)

Group	No. of Subjects	Insulin to Glucose Ratio (pmol/L to mmol/L)			
		Fasting		90 Minutes Postmeal	
		Mean	95% CI	Mean	95% CI
NGT	202	10.6 [10.9]	9.9, 11.4	63.7 [64.6]	58.3, 69.0
Intermediate (nondiagnostic)	60	10.3 [10.4]	9.4, 11.2	59.1 [59.5]	49.1, 69.1
IGT	21	14.7 [13.2]	11.0, 18.4	79.8 [76.6]	57.7, 101.9
New type II DM	19	14.9 [14.5]	9.1, 20.7	49.0 [46.6]	28.0, 70.1
Known type II DM	26	10.3 [9.6]	7.0, 13.6	28.6 [25.2]	18.6, 38.6

NOTE. Values are arithmetic means. Numbers in brackets are mean values adjusted for age, BMI, sex, and systolic blood pressure by analysis of covariance.

the highest average levels, but the latter were on a par with the normal group. Thus, the inverted-U pattern of insulin against time, found in studies of other populations,[6,7] is suggested by our cross-sectional data.

Relationships suggested by the absolute insulin levels were generally supported by the insulin to glucose ratios: for the fasting observations, these were highest in the IGT and newly type II diabetic subjects (consistent with insulin resistance), and for postmeal observations, lowest in the known–type II diabetic subjects (consistent with insulin deficit). The 90-minute postmeal (post–Ensure-plus) glucose level (Table 1) is suggested as a potentially useful measure of relative hyperglycemia, since it separated the glucose tolerance groups just as effectively as the 2-hour postglucose value, on which both the NDDG and World Health Organization classifications are mainly based.

Urine C-peptide values, representing an integrated postmeal pancreatic insulin response, qualitatively agreed closely with postmeal serum insulin average values. We have previously reported urine C-peptide levels in clinically defined type II diabetic subjects in Wadena who were taking insulin,[13] a group not reported in the present study because of the interference in insulin measurement caused by endogenous insulin antibodies. C-peptide levels in these subjects, although distinctly measurable, were approximately 50% or less of those in non–insulin-treated subjects. We suggest that these subjects resemble those with low insulin levels reported by Temple et al[8] in their initial publication. The matter of assay specificity raised by Temple et al is not trivial, since it has been well documented that proinsulin levels are disproportionately high in established type II diabetes.[18-23]

Our data, together with those presented in three recent publications, appear to resolve the question at least in part. Rhys Williams et al,[24] studying seven adults found to have IGT when screened from the register of a general practice, found specifically measured insulin levels in IGT subjects equal to but not higher than levels in controls. Davies et al,[25] studying 51 white subjects with IGT from a community diabetes screening program, found specific insulin levels to be normal in the fasting state, low at 30 minutes postglucose, and high at 120 minutes. Both reports showed disproportionately high proinsulin levels, as in related previous studies of diabetic subjects.[8,9] The differences from our results, we believe, may be attributed to the use of a mixed meal in our study (probably a brisker stimulus than glucose to insulin secretion,[26]), to a difference in timing of the postmeal sample, and to differences in the definition of IGT between the two diabetes classification schemes, World Health Organization and NDDG (the NDDG definition of IGT requires higher plasma glucose levels).

Closer to our methodology is the report by Reaven et al[15] of studies in 42 men and women in California, which used NDDG criteria to define IGT and mixed meals as the stimulus to insulin secretion. They found, as did we, that specifically measured insulin levels during fasting or postmeal were "in general .... highest in patients with IGT, intermediate in patients with NIDDM, and lowest in subjects with NGT."

The increasing proportion of positive family history for diabetes with increasing glucose intolerance is compatible with the expression of an inherited factor contributing to insulin resistance,[27] especially for the IGT group, which does not differ in mean age from the NGT group.

In summary, we suggest that when population-based, random samples of northern European-American subjects with mild degrees of glucose intolerance are included in cross-sectional studies of serum insulin, normal or increased mean levels will be found. On the other hand, under hospital or clinical conditions, where symptomatic diabetes is ordinarily encountered, normal or low mean levels are more likely to be found. Our answer to the question posed by our title is, "No, serum insulin levels are not low in IGT or asymptomatic type II diabetes, although in clinically established diabetes the ability to overproduce insulin may eventually be lost."

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