Alterations in Immunoreactive Proinsulin and Insulin Clearance Induced by Weight Loss in NIDDM

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Subjects with overt non-insulin-dependent diabetes mellitus (NIDDM) were studied in comparison to obese nondiabetic control subjects and patients with subclinical diabetes. Pancreatic insulin secretion rates were measured by deconvolution of peripheral C-peptide over a 24-h period while subjects consumed an isocaloric mixed diet. Subjects were then placed on caloric restriction for at least 6 weeks, during which time body weight fell by at least 10%. Refeeding with solid mixed meals was then resumed for at least 2 weeks until isocaloric intake was attained, and then the meal profiles were repeated. Before weight loss, insulin, C-peptide, and insulin secretion rates were significantly higher in subjects with subclinical diabetes than in the other two groups. Proinsulin concentrations were significantly greater in the two diabetic groups than in control subjects, but, when expressed as a percentage of the total insulin immunoreactivity, the differences were significant only in the group with overt diabetes. Weight loss because of hypocaloric feeding resulted in a significant increase in the rate of clearance of endogenously secreted insulin but did not affect the clearance of C-peptide. In obese subjects and those with subclinical diabetes, weight loss was associated with a reduction in insulin secretion rates, presumably as a result of improvements in insulin sensitivity. In patients with overt diabetes and hyperglycemia, weight loss improved β-cell responsiveness to glucose and was associated with an increase in insulin clearance and a reduction in proinsulin immunoreactivity. As a result of changes in insulin clearance and the contribution of proinsulin to total insulin immunoreactivity, measurement of total insulin-like immunoreactivity alone may provide misleading information in comparing β-cell function before and after weight loss in patients with insulin resistance, glucose intolerance, and diabetes. *Diabetes* 43:871–877, 1994

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IRI, immunoreactive insulin; NIDDM, non-insulin-dependent diabetes mellitus; IGT, impaired glucose tolerance; ISR, insulin secretion rate; OGTT, oral glucose tolerance test; BMI, body mass index; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; K_1, rate at which C-peptide passes from the central to the peripheral compartment; K_2, rate at which C-peptide passes back from the peripheral to the central compartment; K_3, rate constant for metabolism of C-peptide from central compartment; V_d, volume of distribution; ANOVA, analysis of variance.

Under normal circumstances, proinsulin-like immunoreactivity constitutes ≤15% of total immunoreactive insulin (IRI) measured in the peripheral circulation (1–3). In non-insulin-dependent diabetes mellitus (NIDDM) subjects, a disproportionate elevation of proinsulin has been described, and proinsulin-like immunoreactivity makes up a greater proportion of serum IRI (4–9). A study of Pima Indians by Saad et al. (10) has suggested that the degree of elevation in proinsulin is related to the severity of hyperglycemia. The extent to which proinsulin is increased in patients with impaired glucose tolerance (IGT) has varied in different studies, and both normal and increased levels have been reported. Limited information is available on the response of proinsulin to different forms of diabetic therapy (11). Cohen et al. (12) determined that proinsulin concentrations were greater in patients who had been treated with insulin than in patients treated with sulfonylureas. However, in that study, because proinsulin levels were not measured before and after treatment and because the results in nondiabetic control subjects were not presented, it is not possible to determine if proinsulin levels were normalized by insulin therapy. The effect of weight loss from hypocaloric feeding on proinsulin responses to mixed meals in patients with untreated NIDDM is not known. This study tested the hypothesis that the effects of weight loss on proinsulin-like immunoreactivity, insulin immunoreactivity, and insulin secretion rates (ISRs) vary with the degree of glucose intolerance.

RESEARCH DESIGN AND METHODS

Studies were performed in 9 individuals with NIDDM, and data were compared with 8 obese nondiabetic control individuals and 10 individuals with subclinical diabetes. The family history for NIDDM was positive in six of nine subjects with subclinical diabetes (one not available) and three of seven with overt diabetes (two not available). None of the subjects were receiving concomitant therapy of any kind during these studies. All medications, including diabetic therapy, were discontinued at least 2 weeks before the studies. Eight subjects with overt NIDDM had previously been treated with oral hypoglycemic agents; one was newly diagnosed and had not yet received treatment; and none had been treated with insulin. All patients and control subjects underwent an oral glucose tolerance test (OGTT) and were classified as belonging to the control or overt NIDDM groups according to National Diabetes Data Group criteria. Normal glucose tolerance was documented in all the control subjects, and none had a family history of NIDDM. Subclinical diabetes was defined as the presence of a fasting plasma glucose level of <7.8 mM but plasma glucose levels during the OGTT that met the criteria for diabetes (n = 9) or a nondiagnostic result (i.e., criteria between IGT and diabetes with glucose values between 7.8 and 11.1 mM between 0 and 2 h and at the 2-h sampling time; n = 1). The metabolic and clinical characteristics of the study groups are detailed in

TABLE 1
Metabolic and clinical characteristics

	Control subjects	Subclinical NIDDM subjects	Overt NIDDM subjects
n	8	10	9
Age (years)	55 ± 4	52 ± 4	55 ± 2
Weight (kg)			
pre	98.9 ± 5.4	116.8 ± 9.7	105.5 ± 6.6
post	79.7 ± 3.6*	98.7 ± 9.3*	87.9 ± 4.3*
change	19.2 ± 3.4	18.1 ± 2.0	17.7 ± 3.0
BMI (kg/m^2)			
pre	34.6 ± 1.4	39.1 ± 2.4	36.7 ± 3.1
post	28.0 ± 0.8*	33.0 ± 2.3*	30.5 ± 2.0*
change	6.7 ± 1.2	6.1 ± 0.7	6.2 ± 1.2
HbA_{1c} (%)			
pre	5.9 ± 0.3	7.8 ± 0.4	12.3 ± 1.1†
post	—	6.4 ± 0.4*	8.4 ± 0.3‡
change	—	−1.4 ± 0.3	−3.8 ± 1.1

*$P < 0.05$ pre- vs. post-weight loss; †$P < 0.05$ overt vs. subclinical and control subjects; ‡$P < 0.05$ overt vs. subclinical subjects only.

Table 1. Differences in age, change in weight, and change in body mass index (BMI) were not statistically significant between groups.

Pre–weight-loss studies. These studies were performed while patients were consuming normal diets. All subjects participated in at least two separate studies: *1*) Measurement of C-peptide kinetics: C-peptide kinetics were measured as previously described in detail (13); and *2*) 24-h meal profile: each subject consumed three standardized solid mixed meals over 24 h. The meal plan was isocaloric (~30 kcal/kg), and each meal comprised 55% carbohydrate, 30% fat, and 15% protein. Twenty percent of total calories were served at breakfast, 40% at lunch, and 40% at dinner. Meals were consumed within a 40-min period starting at 0900, 1300, and 1700. Plasma insulin, C-peptide, proinsulin, and glucose were measured every 60 min from 0800 through 2100 and then every 2 h until final samples were drawn at 0700 and 0800 the next day for a sampling period of 24 h.

Post–weight-loss studies. All subjects underwent a weight-reducing diet of ~600 calories/day. Subjects dieted for at least 6 weeks to attain a weight loss of a minimum of 10% of body weight. The diet consisted of a powdered formula comprising 55% carbohydrate, 42% protein, and 3% milk fat (supplied by the Medibase Weight Management Program, Monterey, CA) reconstituted in 240 ml of water and supplemented with 180 cal in the form of fruit and vegetables. The subjects were advised to limit exercise to pre-diet activity. After completion of the diet phase, subjects were then refed solid mixed meals for at least 2 weeks under the supervision of a registered dietitian. The subjects were weighed twice weekly and then met with the dietitian to titrate calories to isocaloric intake by increments of 300–500 cal until weight maintenance was attained. The C-peptide kinetic study and the meal-profile study were then repeated with the use of the same protocol described above. At the time of the second study, a significant reduction in both weight and BMI had been achieved as a result of the hypocaloric diet (Table 1).

Sample collection and assay methods. Blood samples for insulin measurements were allowed to clot at room temperature, and the serum was stored at −20°C until assayed. Samples for C-peptide were drawn into tubes at 4°C containing 500 Kallikrein inhibitor units/ml Trasylol and 1.2 mg/ml EDTA. The plasma was immediately separated and stored frozen until assayed. Serum insulin was assayed by a double-antibody technique (14). This assay has a lower limit of sensitivity of 20 pM, and the average intra-assay coefficient of variation (CV) is 8%. Plasma C-peptide immunoreactivity was measured as previously described (15). The lower limit of sensitivity of the assay is 0.02 pmol/ml, and the intra-assay CV averages 6%. Plasma glucose was measured with a YSI glucose analyzer (model 23A; Yellow Springs Instruments, Yellow Springs, OH). The intra-assay CV of this method is <3%.

Proinsulin-like immunoreactivity was measured with the use of an enzyme-linked immunosorbent assay (ELISA) that does not demonstrate cross-reactivity with insulin and C-peptide (3). The two major proinsulin conversion products (des-31,32 and des-64,65 proinsulin) cross-react 100% in the assay in relation to proinsulin. Thus, the proinsulin concentration measured in this assay reflects the combined concentrations of proinsulin and its conversion intermediates. Biosynthetic human proinsulin is used as the assay standard. The intra-assay CV for the proinsulin ELISA was between 9 and 13%, and the minimal detectable concentration of proinsulin was 1.25 pM.

TABLE 2
C-peptide kinetic parameters

	Control subjects	Subclinical NIDDM subjects	Overt NIDDM subjects
n	8	10	9
Pre–weight-loss			
k_1 (min^{-1})	0.060 ± 0.008	0.104 ± 0.003	0.076 ± 0.014
k_2 (min^{-1})	0.046 ± 0.004	0.069 ± 0.008	0.061 ± 0.006
k_3 (min^{-1})	0.062 ± 0.005	0.080 ± 0.013	0.065 ± 0.005
V_d (l/m^2)	2.36 ± 0.26	2.20 ± 0.23	2.26 ± 0.14
MCR (l · m^{-2} · min^{-1})	0.141 ± 0.012	0.151 ± 0.006	0.144 ± 0.009
Post-weight loss			
k_1 (min^{-1})	0.063 ± 0.004	0.093 ± 0.011	0.096 ± 0.018
k_2 (min^{-1})	0.043 ± 0.001	0.060 ± 0.005	0.061 ± 0.005
k_3 (min^{-1})	0.051 ± 0.003	0.069 ± 0.004	0.067 ± 0.007
Vd (l/m^2)	2.51 ± 0.17	2.17 ± 0.17	2.15 ± 0.15
MCR (l · m^{-2} · min^{-1})	0.128 ± 0.011	0.148 ± 0.014	0.138 ± 0.007

Data analysis.

ISRs. Individual C-peptide kinetic parameters were derived by a two-compartmental analysis of the individual decay curves, as previously described (13). This approach assumes that C-peptide distributes into a central compartment, from which sampling occurs, and a peripheral extravascular compartment. The central compartment consists of the plasma space and tissues in rapid equilibration with plasma. Fractional rate constants describe the rate at which C-peptide passes from the central to the peripheral compartment (K_1) and back again (K_2). According to this model, C-peptide is irreversibly metabolized from the central compartment, with K_3 being the rate constant that describes this process. The C-peptide volume of distribution (V_d) is the fourth parameter of the model.

Fasting and average meal responses. To facilitate comparisons between groups and within groups before and after weight loss, summary measures were derived from each meal profile. These were the average fasting levels of insulin, C-peptide, glucose, proinsulin, and ISR derived as the mean of the two measurements obtained after an overnight fast before ingestion of the breakfast meal. Average levels over the entire 24-h sampling were calculated as the mean of all measurements. The proinsulin concentration also was expressed as a percentage of total IRI and as a fraction of the simultaneously measured ISR.

Insulin clearance rates. The rate of clearance of endogenously secreted insulin was calculated according to the formula: metabolic clearance rate (ml · m^{-2} · min^{-1}) = ISR (pmol · m^{-2} · min^{-1})/peripheral insulin concentration (pmol/ml). Average values over the 24 h were used in this calculation.

Statistical analysis. Data are expressed as means ± SE. To determine if there were statistically significant differences between groups before and after weight loss in each of the parameters measured, two-way repeated measures analysis of variance (ANOVA) was used. Where statistically significant group effects were detected, the significance of individual group differences was determined using one-way ANOVA and post hoc tests with Tukey's adjustment for multiple comparisons. Proinsulin concentrations were not normally distributed, but the distribution was skewed because of the extremely high values in some subjects, particularly in the two diabetic groups. Log transformations of the proinsulin concentrations were therefore used in the statistical analyses. Fractional rate constants for C-peptide clearance were compared by multivariate ANOVA. Significant differences were assessed at the 5% level. Analyses were performed with the use of the Statistical Analysis System (SAS version 6 for personal computers, SAS Institute, Carey, NC).

RESULTS

C-peptide kinetic parameters pre- and post-weight loss. C-peptide kinetic parameters derived by analysis of the individual C-peptide decay curves before and after weight loss are listed in Table 2. Between-group differences in the parameters were not statistically significant, nor were the differences pre- and post-weight loss.

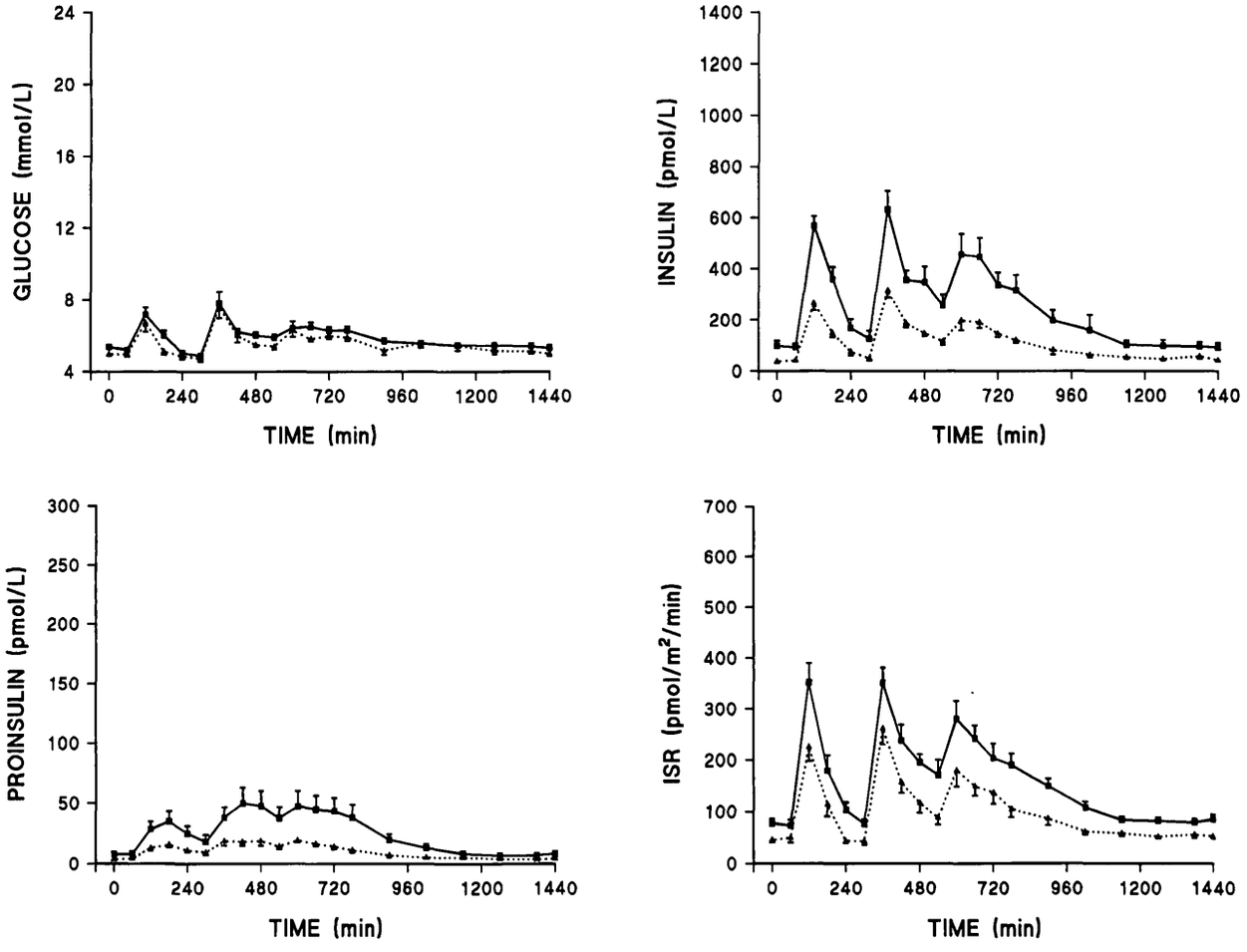

FIG. 1. Twenty-four hour profiles of glucose, insulin, proinsulin, and ISR in nondiabetic control subjects before (■) and after (▲) weight loss. Meals were consumed at 60, 300, and 540 min into the 24-h sampling period. These times corresponded to times of 0900, 1300, and 1700, respectively.

Fasting and average 24-h values of glucose, insulin, C-peptide, proinsulin, insulin, and insulin secretion before weight loss. Basal and 24-h levels of glucose, insulin, C-peptide, proinsulin, and ISR before weight loss are shown for each of the three groups in Figs. 1–3 and in Tables 3 and 4. One-way ANOVA revealed significant group differences in all the variables listed in Table 3 (i.e., glucose, C-peptide, insulin, proinsulin, ISR, and proinsulin expressed as a percentage of total IRI). Significant between-group differences as judged by post hoc testing using Tukey's adjustment for multiple comparisons are shown in Table 3. As expected, glucose concentrations were elevated in the patients with overt diabetes, and the differences between these subjects and those in the other groups were greatest over the 24-h sampling. However, the patients with subclinical diabetes demonstrated the greatest degree of β-cell hypersecretion, as evidenced by insulin and C-peptide levels and ISRs that were significantly greater than in the other two groups both under basal conditions and during the meal sampling. Insulin, C-peptide, and ISRs were not significantly different in the overtly diabetic and control subjects. Proinsulin concentrations in the two diabetic groups were significantly greater than those in the control group. However, when viewed in relation to the level of IRI, although both groups showed a tendency for an increase in the relative proportion of proinsulin secreted, the differences reached statistical significance only in the patients with overt diabetes. Values in the patients with subclinical diabetes were intermediate, between the values in the control subjects and those in subjects with overt diabetes.

Effects of weight loss on fasting and average 24-h values of glucose, insulin, C-peptide, proinsulin, insulin, and ISR. In the control subjects, weight loss induced a significant reduction in fasting values of insulin ($P < 0.002$), C-peptide ($P < 0.0001$), and ISR ($P < 0.006$), but proinsulin levels and proinsulin/insulin percentage did not change significantly. In the patients with subclinical diabetes, significant reductions in glucose ($P < 0.006$), insulin ($P < 0.0009$), C-peptide ($P < 0.01$), ISR ($P < 0.015$), and proinsulin ($P < 0.003$) occurred as a result of weight loss, but the percentage of contribution of proinsulin to total IRI did not change significantly. The patients with overt diabetes demonstrated significant reductions in fasting levels of glucose ($P < 0.0001$) and proinsulin ($P < 0.02$), but the percentage of contribution of proinsulin to total IRI, C-peptide levels, and ISR did not change significantly after weight loss. When the average 24-h values were considered, a similar overall pattern of change was seen as a result of weight loss, with some variations. In the control subjects, the reduction in average 24-h proinsulin values was significant ($P < 0.034$). In the patients with overt and subclinical diabetes, proinsulin levels fell to a greater extent than did insulin, and the percent contribution of proinsulin to total IRI was lower as result of the weight-loss program ($P < 0.04$ in both groups).

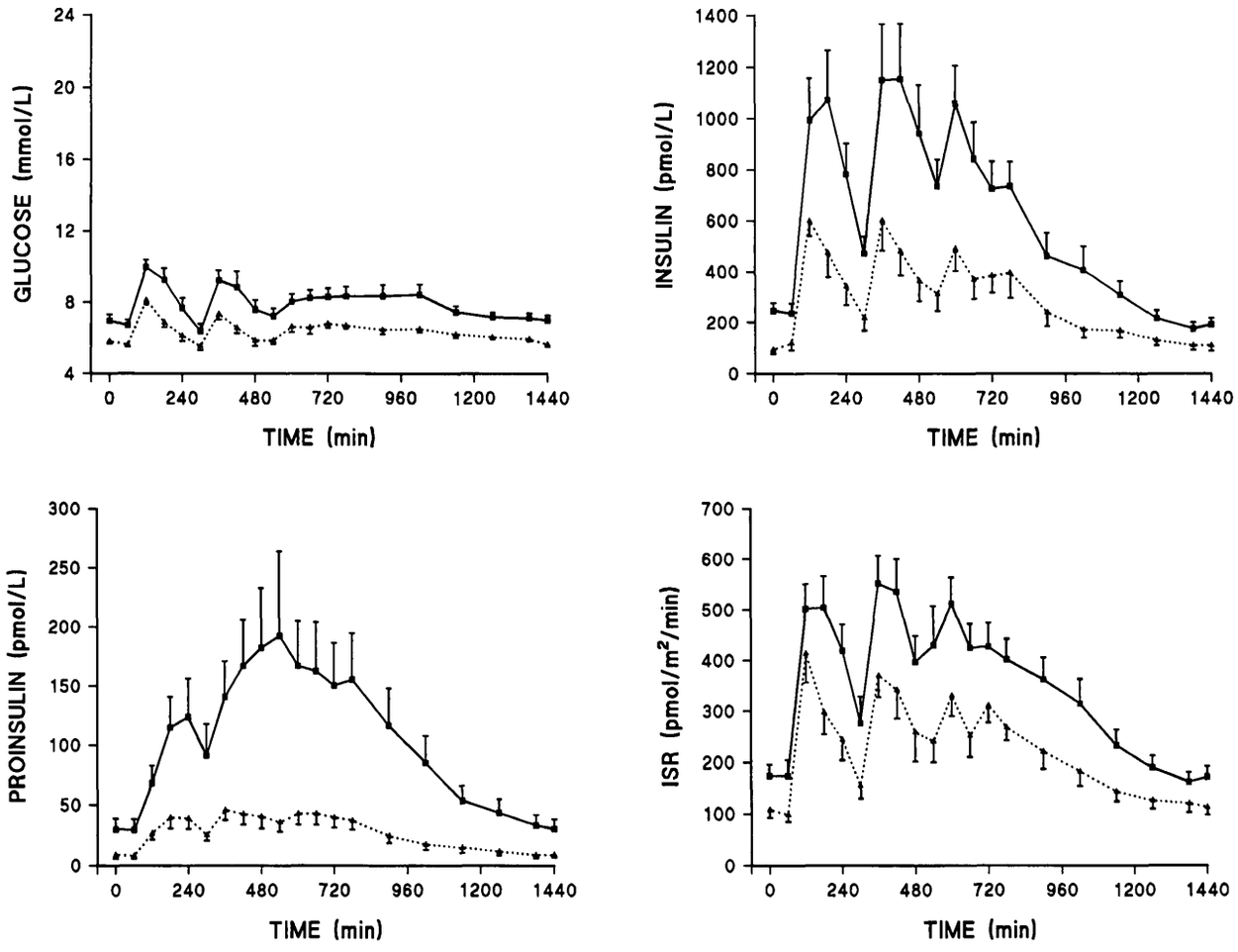

FIG. 2. Twenty-four hour profiles of glucose, insulin, proinsulin, and ISR in subjects with subclinical diabetes before (■) and after (▲) weight loss. Meals were consumed at 60, 300, and 540 min into the 24-h sampling period. These times corresponded to times of 0900, 1300, and 1700, respectively.

Changes in insulin clearance. Endogenous insulin clearance rates are shown in Fig. 4. The group differences in insulin clearance rates were not statistically significant either before or after weight loss. However, significant changes in insulin clearance rates occurred as a result of weight loss (Fig. 4). In the control subjects (695 ± 69 vs. 897 ± 75 $ml \cdot m^{-2} \cdot min^{-1}$, $P < 0.05$) and patients with overt diabetes (604 ± 46 vs. 919 ± 66 $ml \cdot m^{-2} \cdot min^{-1}$, $P < 0.0031$), insulin clearance increased significantly with weight loss. The same trend was evident in the patients with subclinical diabetes, but the increase in clearance missed statistical significance (627 ± 66 vs. 806 ± 54 $ml \cdot m^{-2} \cdot min^{-1}$, $P < 0.0528$).

DISCUSSION

This study was undertaken to define the effects of weight loss on immunoreactive proinsulin concentrations, 24-h ISRs, and insulin clearance rates in subjects with varying degrees of glucose intolerance from untreated overt NIDDM to subjects with normal fasting but elevated plasma glucose levels after glucose ingestion. The results were compared with those obtained in a group of nondiabetic control subjects. A comparison of the responses during ingestion of a weight maintenance diet over a 24-h period before and after weight loss enabled the relative effects of improved peripheral insulin sensitivity and reduced β-cell glucotoxicity to be evaluated.

In the control subjects and those with subclinical diabetes, a significant reduction was noted in both basal and 24-h ISRs as a result of the weight-reducing diet. Changes were particularly dramatic in the patients with subclinical diabetes. The latter group demonstrated the greatest degree of hyperinsulinemia initially, presumably due, in part, to the retained ability of these patients to increase insulin secretion in response to mild hyperglycemia. The basal (173.5 ± 25.9 $pmol \cdot m^{-2} \cdot min^{-1}$) and 24-h ISRs (515.2 ± 55.9 $nmol \cdot m^{-2} \cdot 24\ h^{-1}$) in this group before weight loss are particularly remarkable when viewed in relation to values that we have reported previously in normal-weight control subjects (50.9 ± 4.8 $pmol \cdot m^{-2} \cdot min^{-1}$ and 145.8 ± 8.8 $nmol \cdot m^{-2} \cdot 24\ h^{-1}$; 16). However, a likely additional contributory factor was the tendency for the subjects in this group to be the most obese and, therefore, the most insulin resistant of the three study groups, even though the differences in weight were not statistically significant. Hyperinsulinemic (120 $mU \cdot m^{-2} \cdot min^{-1}$) isoglycemic clamps were performed in seven obese control subjects and eight patients with subclinical diabetes. The steady-state glucose disposal rates tended to be higher in the obese control subjects than in the subjects with subclinical diabetes (7.73 ± 0.72 vs. 5.48 ± 0.80 $mg \cdot kg^{-1} \cdot min^{-1}$, $P = 0.06$), and although the differences did not reach statistical significance, the subjects with subclinical diabetes tended to be more resistant than the control subjects. In the subclinical diabetes group, ISRs post–weight loss fell to

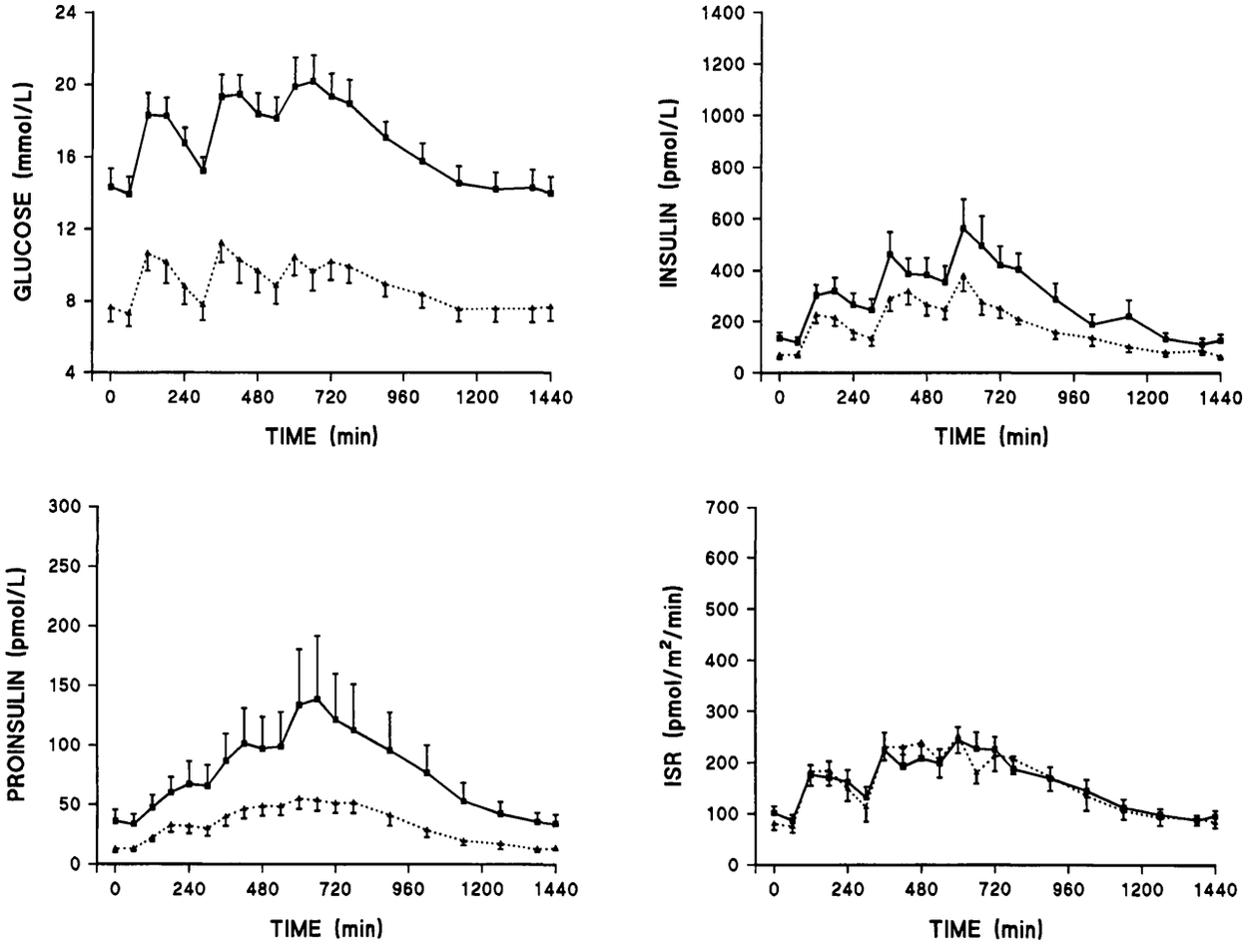

FIG. 3. Twenty-four hour profiles of glucose, insulin, proinsulin, and ISR in subjects with overt diabetes before (■) and after (▲) weight loss. Meals were consumed at 60, 300, and 540 min into the 24-h sampling period. These times corresponded to times of 0900, 1300 and 1700, respectively.

values that represented only 44% of the pre–weight-loss level under basal conditions and only 36% of the pre–weight-loss value when the average 24-h insulin secretion rate was considered. In the obese, nondiabetic control subjects who were less hyperinsulinemic at the outset, the fall in insulin secretion induced by the weight-reducing diet represented ~33% of the pre–weight-loss value. In the patients with overt NIDDM, average ISRs did not change as a result of weight loss, despite the ~50% fall in plasma glucose.

TABLE 3
Average values pre-weight loss

	Control subjects	Subclinical NIDDM subjects	Overt NIDDM subjects
n	8	10	9
Basal values			
Glucose (mM)	5.3 ± 0.1	6.8 ± 0.3*	14.2 ± 1.0†
C-peptide (pmol/ml)	0.5 ± 0.1	1.1 ± 0.1‡	0.7 ± 0.1*
Insulin (pM)	95.4 ± 17.5	241.7 ± 34.7‡	126.9 ± 21.2*
Proinsulin (pM)	7.9 ± 2.3	29.8 ± 9.1‡	35.0 ± 9.0*
ISR (pmol · m^{-2} · min^{-1})	74.8 ± 9.2	173.5 ± 25.9‡	93.7 ± 11.8*
Proinsulin/insulin (%)	8.6 ± 2.3	11.4 ± 2.8*	28.0 ± 5.1†
Average 24-h values			
Glucose (mM)	5.9 ± 0.1	7.9 ± 0.4‡	17 ± 1*
C-peptide (pmol/ml)	1.2 ± 0.1	2.3 ± 0.2‡	1.2 ± 0.2*
Insulin (pM)	264.5 ± 34.5	645.3 ± 92.8‡	295.9 ± 47.3*
Proinsulin (pM)	26.8 ± 6.7	107 ± 26‡	76.9 ± 22.7†
ISR (pmol · m^{-2} · min^{-1})	165.9 ± 11.7	357.8 ± 38.8‡	162.3 ± 18.3*
Proinsulin/insulin (%)	11.0 ± 2.5	18.1 ± 4.6	27.6 ± 4.2†

*$P < 0.05$, subclinical vs. overt NIDDM subjects; †$P < 0.05$, control vs. overt NIDDM subjects; ‡$P < 0.05$, control vs. subclinical NIDDM subjects.

We believe that the reason for the dichotomous effects on insulin, ISRs, and proinsulin levels in the two groups relates to the fact that weight loss may result in a reduction in insulin resistance (which will lower insulin secretion) and blood glucose (which, by reducing glucose toxicity, will tend to improve insulin responsiveness). In overt diabetes, the reduction in glucose toxicity is the predominant effect, and the net result is no change in ISR despite lower glucose levels. In the subjects with subclinical diabetes because hyperglycemia is modest, relatively little glucose toxicity occurs, and the predominant effect is a fall in insulin secretion resulting from a reduction in insulin resistance.

These data also demonstrate that weight loss that leads to an improvement in insulin sensitivity also causes significant increases in rates of insulin clearance. In the patients with overt diabetes, insulin clearance increased by ~50% after weight loss. In the other two groups, the increase in clearance was ~30%, although the differences did not reach statistical significance in the subjects with subclinical diabetes. Receptor-bound insulin is thought to represent the substrate for insulin degradation (16), and the increase in insulin clearance after weight loss presumably relates to the

TABLE 4
Average values post-weight loss

	Control subjects	Subclinical NIDDM subjects	Overt NIDDM subjects
n	8	10	9
Basal values			
Glucose (mM)	5.0 ± 0.1	5.7 ± 0.1*	7.5 ± 0.8†
C-peptide (pmol/ml)	0.4 ± 0.03	0.8 ± 0.1‡	0.6 ± 0.1
Insulin (pM)	41.2 ± 4.8	106.1 ± 20.2‡	69.4 ± 12.5
Proinsulin (pM)	4.1 ± 0.9	8.6 ± 2.8	13.1 ± 2.5†
ISR (pmol · m^{-2} · min^{-1})	47.0 ± 6.8	103 ± 13.7‡	77.4 ± 12.2
Proinsulin/insulin (%)	12.0 ± 3.7	9.6 ± 3.6	22.0 ± 5.1*
Average 24-h values			
Glucose (mM)	5.6 ± 0.1	6.4 ± 0.1*	9.0 ± 0.9†
C-peptide (pmol/ml)	0.8 ± 0.1	1.7 ± 0.1‡	1.1 ± 0.1*
Insulin (pM)	118.6 ± 5.8	308.5 ± 50.3‡	186.4 ± 27.0
Proinsulin (pM)	10.6 ± 1.3	28.0 ± 5.5‡	33.7 ± 5.5†
ISR (pmol · m^{-2} · min^{-1})	103.9 ± 8.2	229.5 ± 26.7‡	161.1 ± 22.5
Proinsulin/insulin (%)	11.3 ± 1.5	9.6 ± 1.7*	20.8 ± 2.5†

*$P < 0.05$, subclinical vs. overt NIDDM subjects; †$P < 0.05$, control vs. overt NIDDM subjects; ‡$P < 0.05$, control vs. subclinical NIDDM subjects.

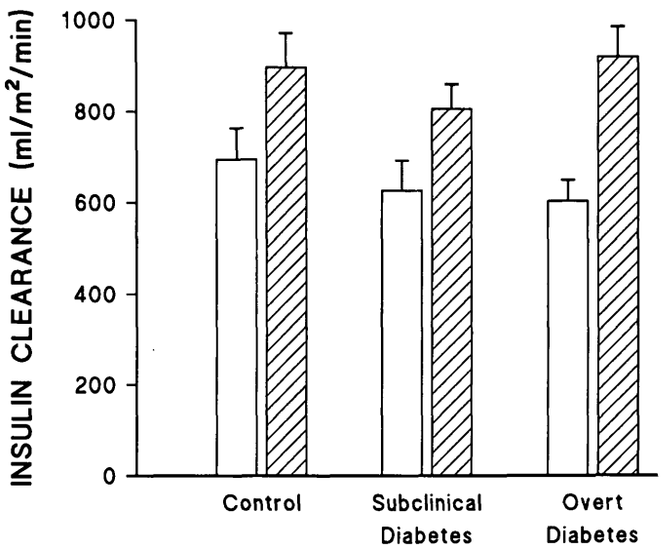

FIG. 4. Insulin clearance rates in control subjects and subjects with subclinical and overt diabetes before (□) and after (▨) weight loss.

associated increase in insulin-receptor binding and insulin sensitivity. The increase in insulin clearance after weight loss resulted in an even greater fall in peripheral insulin levels than in the ISR.

Proinsulin concentrations were elevated in subjects both with subclinical and with overt diabetes compared with the nondiabetic control subjects, and although these levels fell significantly in response to weight loss in all three groups, values still remained elevated in the group with overt diabetes. When calculated in relation to total IRI, the percentage of proinsulin was increased significantly in the patients with overt diabetes under basal conditions (28.0 ± 5.1 vs. 8.6 ± 2.3%) and over the 24-h sampling interval (27.6 ± 4.2 vs. 11.0 ± 2.5%) when compared with the nondiabetic control subjects. Values in the patients with subclinical diabetes were intermediate between the nondiabetic control subjects and patients with overt diabetes, but the differences did not reach statistical significance. Thus, proinsulin may indeed contribute to the hyperinsulinemia of NIDDM in a way that could change with changes in physiological status. However, the hyperinsulinemia in this study appeared to be due primarily to hypersecretion of insulin and not to an increase in proinsulin.

The availability of quantitative pancreatic insulin production rates based on individual C-peptide kinetic parameters allowed us to estimate the relative production rates of insulin and proinsulin. Unfortunately, we were unable to measure proinsulin clearance rates individually in our subjects because proinsulin is not generally available for use in humans. However, with the use of a proinsulin clearance rate of 3.3 ml · kg^{-1} · min^{-1}, the average value reported by Revers (18), we estimate that over the 24-h period, proinsulin production rates represented 5.2 ± 1.3% of the production rates of insulin in the control subjects, with corresponding values of 10.7 ± 2.2 and 15.0 ± 2.7% in the subjects with subclinical and overt diabetes, respectively. These estimates are approximations of the differences in the production rates of insulin and proinsulin because they do not take into account possible group differences in proinsulin clearance. However, this calculation does demonstrate that the expression of the serum proinsulin concentration as a percentage of the immunoreactive serum insulin certainly overestimates the relative production rate of proinsulin, because insulin is cleared at approximately four times the rate of proinsulin.

This study also highlights two pitfalls in the interpretation of insulin concentrations before and after weight loss. The first relates to the alterations in proinsulin. Virtually all the insulin assays currently in use demonstrate a high degree of cross-reactivity with proinsulin, and alterations in proinsulin levels induced by weight loss may alter the relative contribution of proinsulin to total insulin immunoreactivity. This problem can be overcome by the application of a specific insulin assay that does not cross-react with proinsulin or its conversion intermediates (8,18,19). However, such an approach would not avoid the effects of alterations in insulin clearance on serum IRI. Estimates of β-cell function based on C-peptide measurements should satisfactorily address these problems. Proinsulin and its conversion intermediates demonstrate <10% cross-reactivity in the C-peptide assay used in this study. Because levels of plasma C-peptide immunoreactivity are normally so much higher than levels of proinsulin immunoreactivity, the contribution of proinsulin-related peptides to immunoreactive C-peptide is negligible, even in overt diabetes that is associated with a selective increase in the concentrations of proinsulin and the proinsulin intermediates. Thus, in addition to being unaffected by alterations in proinsulin, C-peptide clearance does not change after weight loss; estimates of β-cell function based on C-peptide levels, therefore, will provide valid estimates of insulin secretory capacity.

In summary, weight loss as a result of hypocaloric feeding results in significant alterations in ISRs, proinsulin, and insulin clearance rates. The specific alterations depend on the extent of the glucose intolerance and whether the predominant effect of weight loss is a reduction in insulin resistance with a resultant fall in insulin secretion and an increase in insulin clearance or a reduction in glucose that is associated with enhanced β-cell responsiveness by reducing the effects of glucotoxicity.

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