Normal Insulin Sensitivity of the Islets of Langerhans in Obese Subjects with Resistance to Its Glucoregulatory Actions

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SUMMARY

The ability of varying levels of circulating insulin to suppress α- and β-cell secretion was assessed by plasma glucagon and C-peptide measurement in 6 obese and 6 nonobese subjects maintained in a euglycemic state, with an insulin concentration elevated by 10, 20, or 100 μU/ml above basal levels by a primed-continuous infusion of insulin. The 10-μU/ml increase did not suppress C-peptide levels significantly in either group. However, incremental increases in plasma insulin of approximately 20 and 100 μU/ml above basal suppressed plasma C-peptide by 0.27 ± 0.14 and 0.53 ± 0.07 pmol/ml, respectively, in the obese subjects (14% and 31% of the basal values of 2.20 ± 0.18 and 2.19 ± 0.26 pmol/ml, respectively) and by 0.16 ± 0.06 and 0.17 ± 0.06 pmol/ml in the nonobese subjects (20% and 25% the basal values of 0.74 ± 0.11 and 0.78 ± 0.11 pmol/ml, respectively). Plasma glucagon levels were suppressed to a similar degree in each group in a dose-related manner during both the 20-μU/ml and 100-μU/ml clamps. We were unable to identify an increment of insulin that suppressed C-peptide and/or glucagon in one group but not in another. These data demonstrate inhibition of α- and β-cell secretion by insulin within its physiologic range in both nonobese and obese man, and exclude insulin resistance of α- and β-cells in obese individuals. However, despite their much higher insulin levels before and during the insulin infusions, obese subjects had C-peptide levels that, at all times, were 3–3.5-fold higher than those of the nonobese subjects. This is consistent with a greater number and/or a higher secretory activity of β-cells in the obese subjects. **DIABETES *33*:305–310, April 1984.**

This work was presented in part at the 17th Annual Meeting of the European Association for the Study of Diabetes, Amsterdam, The Netherlands, September 15–18, 1981.

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Received for publication 9 November 1982 and in revised form 26 August 1983.

It has been recognized for many years that obesity is associated with hyperinsulinemia and peripheral insulin resistance.[1–3] Since insulin is known to inhibit its own secretion,[4–6] resistance of the β-cell to the inhibitory effects of circulating insulin could be a cause for the hyperinsulinemia. We have previously demonstrated, in both obese and nonobese subjects, suppression of C-peptide levels when insulin is increased by 100 μU/ml under euglycemic conditions.[6] The present study was designed to extend this work by determining the insulin-dose relationships of both C-peptide and glucagon levels to determine if differences in sensitivity of α- and β-cells to insulin could be identified.

MATERIALS AND METHODS

Twelve male, Pima Indian[7] volunteers were admitted to the Phoenix Clinical Research Section, NIADDK, NIH metabolic ward. They were in good health as judged by history, physical examination, and laboratory tests, and gave informed consent for subsequent studies. All subjects were placed on a weight-maintenance diet for at least 3 days (45% carbohydrate, 35% fat, and 20% protein). After this period of dietary stabilization, each subject received 100 g of glucose (Koladex, Custom Laboratories, Baltimore, Maryland), and only subjects with normal glucose tolerance (with normal fasting and 2-h plasma glucose level <140 mg/dl) were included in the study. The clinical characteristics of the volunteers are summarized in Table 1.

Euglycemic hyperinsulinemic clamp. Three euglycemic clamps were performed on each subject on separate days (in random order), with insulin infusion rates designed to achieve plasma concentrations of insulin of (1) 10, (2) 20, and (3) 100 μU/ml above basal. Antecubital venous blood sampling was used throughout all 36 experiments. A second antecubital catheter was used for infusion of insulin and glu-

TABLE 1
Characteristics of subjects

			BMI*	Age (yr)	M values† 1‡	2	3
Nonobese subjects		1	22.7	19	0.99	2.74	5.05
		2	23.2	19	1.31	2.36	5.89
		3	24.0	21	0.81	1.05	5.16
		4	26.9	27	0.41	1.90	
		5	28.4	19	0.39	1.19	6.17
		6	29.0	24	0.74	0.78	2.51
	Mean		25.7	21.5	0.78	1.67	4.96
	SEM		1.1	1.4	0.14	0.32	0.65
Obese subjects		7	38.2	23	0.07	0.77	1.63
		8	40.3	19	0.20	0.48	1.30
		9	51.7	29	0.32	0.37	1.18
		10	52.7	25	0.12	0.55	1.43
		11	52.8	23	0.51	0.55	1.22
		12	57.3	20	0.36	0.68	1.61
	Mean		48.8	23.2	0.33	0.57	1.40
	SEM		3.1	1.5	0.06	0.06	0.08
					$P < 0.02$	$P < 0.02$	$P < 0.01$

*BMI = Body mass index (kg/m^2).
†M value in mg/kg/min; P values indicate comparison of M values obtained for nonobese and obese groups at each infusion rate.
‡1,2,3 = Insulin infusion rates of 4, 10, and 40 mU/m^2/min, respectively.

cose. After an overnight fast, venous blood was drawn for determination of fasting plasma glucose and hormone concentrations. A square wave of hyperinsulinemia was produced by a primed-continuous insulin infusion, which was modified as described previously[6] to produce the desired insulin levels. The insulin infusion rate was changed at 2-min intervals for the first 10 min until the final necessary infusion rate was reached (4, 10, and 40 mU/min/m^2 for the 10-, 20-, and 100-μU/ml clamps, respectively) for the next 80 min. Purified, single-component insulin (Eli Lilly and Company, Indianapolis, Indiana) was used. Euglycemia was maintained by the clamp technique[8] throughout the 150 min of the study. Samples for plasma glucose were obtained every 2 min for 10 min, and then every 5 min during the insulin infusion. After termination of the insulin infusion, samples were obtained every 2.5 min for 15 min, and then every 5 min for the duration of the study. Blood samples for hormone analysis were taken during 15 min throughout the whole study. All the data for the 100-μU/ml clamp for one nonobese subject were excluded from the analyses, because a steady-state glucose infusion rate was not achieved during the insulin infusion. In addition, samples were not obtained for measurement of plasma glucagon in one nonobese subject for the 20-μU/ml clamp.

Chemical analysis. Plasma glucose levels were measured immediately after sampling by the glucose-oxidase method (Beckman Glucose Analyzer, Beckman Instruments, Inc., Fullerton, California). Plasma insulin (IRI) levels were measured by radioimmunoassay using dextran-coated charcoal to separate bound and free hormone.[9] For the radioimmunoassay of C-peptide (CPR) with the ethanol separation technique,[10] an antiserum generously supplied by Dr. Arthur Rubenstein (University of Chicago, Chicago, Illinois) was used. Plasma glucagon (IRG) was measured using 30K antibody and dextran-coated charcoal for the separation of bound and free hormone.[11]

Statistics. Comparison within studies and differences between them were evaluated using Student's paired and nonpaired *t* test, respectively. Results are expressed as mean ± SEM.

RESULTS

Plasma glucose levels. The mean fasting plasma glucose levels on three different experimental days did not differ significantly, either within or between groups [nonobese: (1) 97 ± 2 mg/dl, (2) 95 ± 3 mg/dl, (3) 90 ± 3 mg/dl; obese: (1) 98 ± 1 mg/dl, (2) 98 ± 3 mg/dl, (3) 96 ± 3 mg/dl]. Plasma glucose concentration was maintained at the fasting level for the study period at each of the three levels of hyperinsulinemia. Coefficients of variations of glycemia during the experiment were less than 5.3%. Similarly, mean plasma glucose level during the study, computed for each subject and expressed as percent of basal level, varied only minimally from 0 to 2% of the fasting value. There was evidence of resistance to the glucoregulatory actions of insulin as demonstrated by decreased M values in the obese group (Table 1).

Plasma insulin levels. The mean fasting plasma insulin levels on three different days were very similar within each group, but were significantly greater in the obese subjects, who exhibited levels approximately three times those of the nonobese subjects (Table 2). A square wave of hyperinsulinemia was achieved in each subject, and increases of about 10, 20, and 100 μU/ml above basal insulin concentrations were obtained in both groups. With the termination of the insulin infusion at 90 min, plasma insulin levels decreased rapidly, approaching the fasting insulin values within 15 min.

C-peptide levels. An increase in plasma insulin of 10 μU/ml did not suppress C-peptide levels in either group (Figure 1 and Table 2). Increases in plasma insulin of approximately 20 and 100 μU/ml above basal concentrations suppressed

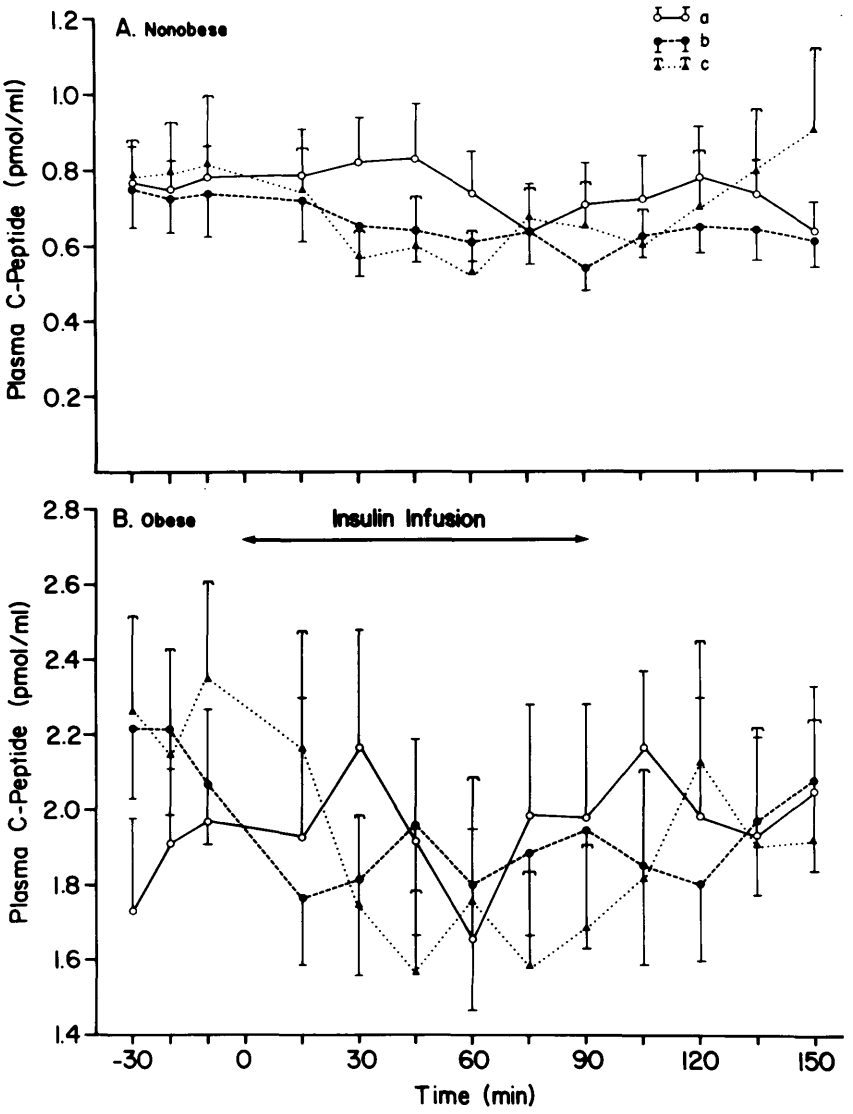

FIGURE 1. (A) Changes in plasma C-peptide in nonobese subjects during the hyperinsulinemic euglycemic clamps; C-peptide in pmol/ml. ○—○, 10 μU/ml; ●-----●, 20 μU/ml; and ▲ · · · · ▲, 100 μU/ml. (B) Changes in plasma C-peptide in obese subjects during hyperinsulinemic euglycemic clamps; C-peptide in pmol/ml. ○—○, 10 μU/ml; ●-----●, 20 μU/ml; and ▲ · · · · ▲, 100 μU/ml.

plasma C-peptide by 0.16 ± 0.06 and 0.17 ± 0.06 pmol/ml, respectively, in the nonobese group and by 0.27 ± 0.14 and 0.53 ± 0.07 pmol/ml in the obese Indians. The decline of plasma C-peptide was clearly evident by 20 min, and maximum suppression was reached between 60 and 90 min. The absolute decrement of C-peptide in the 100-μU/ml clamp was significantly greater in the obese subjects than in the nonobese subjects ($P < 0.005$), but the percent decline of C-peptide levels in the two groups was similar (Figure 2) at all three insulin levels.

Plasma glucagon levels. The 10-μU/ml increment in insulin was associated with a slight, but not significant, decrease of plasma IRG in both groups. At the 20-μU/ml and 100-μU/ml increments, a significant suppression of plasma IRG

TABLE 2
Plasma IRI and CPR before and during 60–90 and 120–150 min of the experiment

Study type		Nonobese			Obese		
		F†	60–90 min	120–150 min	F	60–90 min	120–150 min
1*	IRI	13 ± 2	19 ± 3	12 ± 2	41 ± 6	54 ± 7	48 ± 7
	CPR‡	0.77 ± 0.09	0.70 ± 0.10	0.68 ± 0.09	1.91 ± 0.27	1.94 ± 0.26	1.99 ± 0.28
2	IRI	12 ± 2	30 ± 3	14 ± 3	44 ± 4	63 ± 3	39 ± 4
	CPR	0.74 ± 0.11	0.58 ± 0.06	0.65 ± 0.07	2.20 ± 0.18	1.92 ± 0.27	1.95 ± 0.20
3	IRI	12 ± 3	101 ± 12	21 ± 4	51 ± 11	144 ± 8	45 ± 3
	CPR	0.78 ± 0.12	0.61 ± 0.10	0.76 ± 0.15	2.19 ± 0.26	1.66 ± 0.24	2.03 ± 0.26

*See legend for Table 1.
†Fasting plasma insulin (all IRI data in μU/ml).
‡All C-peptide data in pmol/ml.

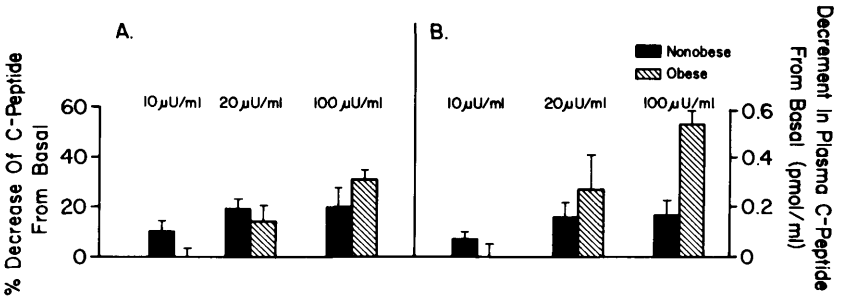

FIGURE 2. Mean percentage decrease of C-peptide at 60–90 min from basal concentration (left) and mean 60–90 min absolute decrease of C-peptide (pmol/ml) from basal during euglycemic hyperinsulinemic clamps (right).

was observed in both groups (Figure 3, A and B). The magnitude of the decrease in plasma IRG was related to the increase of circulating insulin level. Thus, the decrease in plasma glucagon below basal during the 20-μU/ml clamp was greater than during the 10-μU/ml clamp in the obese subjects ($P < 0.01$) and nonobese subjects ($P < 0.0002$) when analyzed by paired *t* test. Similarly, the suppression of plasma glucagon during the 100-μU/ml clamp was significantly greater than during the 10-μU/ml clamp in both nonobese ($P < 0.001$) and obese subjects ($P < 0.001$). However, there were no significant differences between the obese and nonobese subjects at each increment of plasma insulin (Figure 4).

Relationship between C-peptide suppression and glucoregulatory actions of insulin. There was a significant increase in M with each increase in insulin level. The M value for the 20-μU/ml clamp was significantly higher than for the 10-μU/ml clamp in the obese ($P < 0.05$) and nonobese ($P < 0.05$) subjects. The M value for the 100-μU/ml clamp was also significantly higher than for the 10-μU/ml clamp in

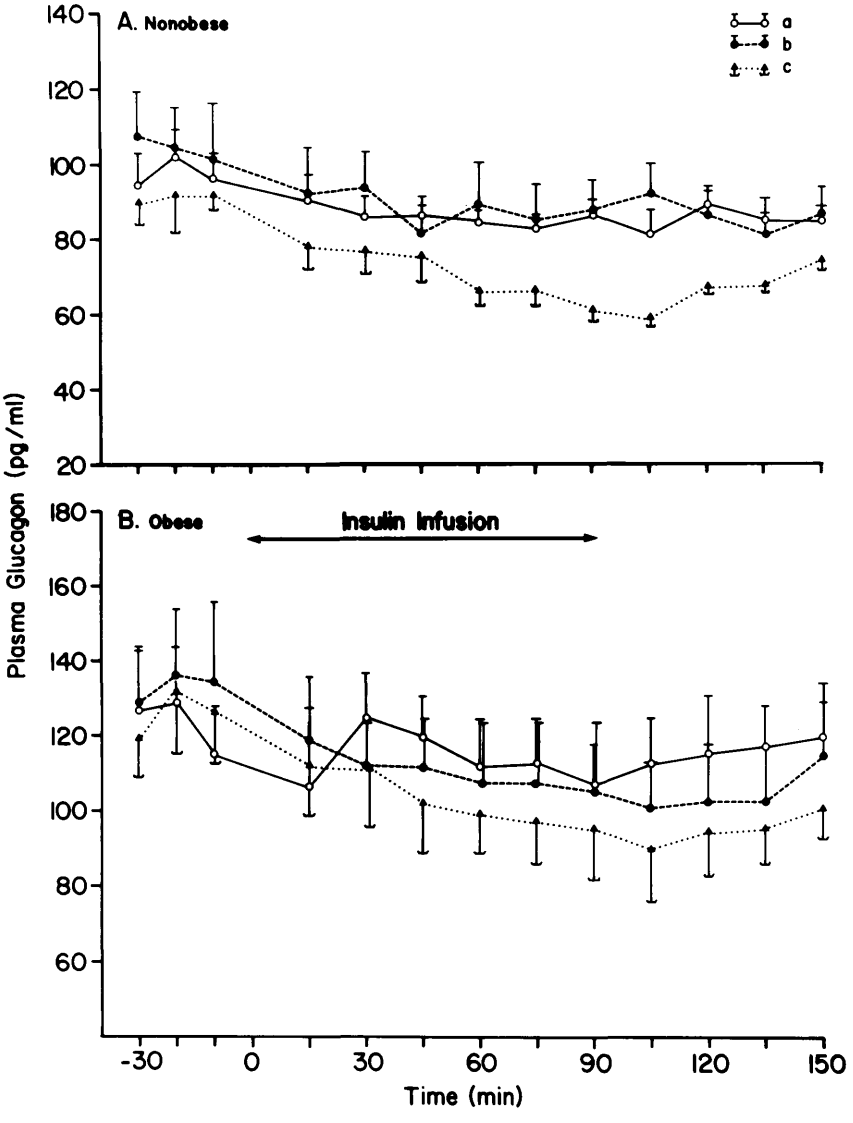

FIGURE 3. Changes in plasma glucagon in nonobese (A) and obese (B) subjects during hyperinsulinemic euglycemic clamp. ○——○, 10 μU/ml; ●- - - -●, 20 μU/ml; and ▲ · · · · ▲, 100 μU/ml.

both the obese ($P < 0.001$) and nonobese ($P < 0.005$) subjects.

The obese subjects were far less sensitive to the glucoregulatory action of insulin, with M values of only 30–50% of those of the lean subjects for all three clamps (Table 1). This discordance of insulin action between the sensitivity of the islets to its C-peptide suppression and an insensitivity to its effects on glucose metabolism is depicted in Figure 5.

DISCUSSION

This study demonstrates the suppressive effect of insulin on C-peptide levels. The smallest incremental change that resulted in a significant decline was 20 μU/ml in both groups. The absolute decrement of C-peptide was greater in the obese group. Yet in the obese subjects, with much higher plasma insulin levels than the nonobese subjects, at all times before and during the clamp and with C-peptide levels 3–3.5-fold higher than those of the nonobese group, there was a shift to the right of the insulin dose-response curve for C-peptide suppression. Despite the striking difference in the absolute levels of C-peptide, the percent of C-peptide suppression was similar in both obese and nonobese subjects.

The foregoing combination of findings in the obese group seems most consistent with an augmentation of functional insulin secretory mass.[12] The augmentation includes both the fraction of the secretory mass that is inhibited by the insulin increments induced by the clamp (reflected by the greater than normal absolute decrement of C-peptide) and the fraction of the secretory mass that is not inhibited by these clamp-induced increments in insulin (reflected by the higher-than-normal C-peptide levels at every insulin level). The similarity in the percent decline of C-peptide in each group suggests that the augmentation of both of these putative fractions of secretory mass was symmetrical. IRG levels were similar in both groups, and insulin dose-dependent suppression of plasma glucagon during the clamp was equal in both groups, excluding insulin resistance of α-cells in obese subjects.

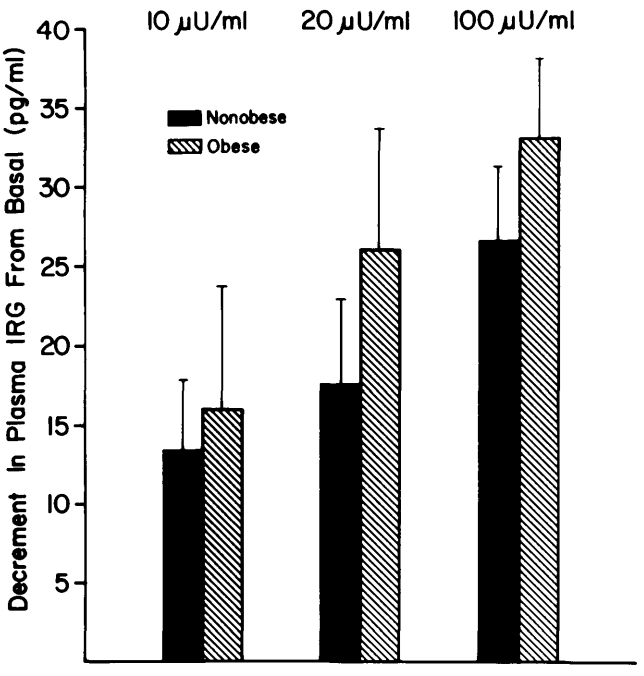

FIGURE 4. Mean decrease of plasma glucagon below basal during the steady-state period of hyperinsulinemic euglycemic clamps in nonobese (solid bars) and obese (hatched bars) subjects.

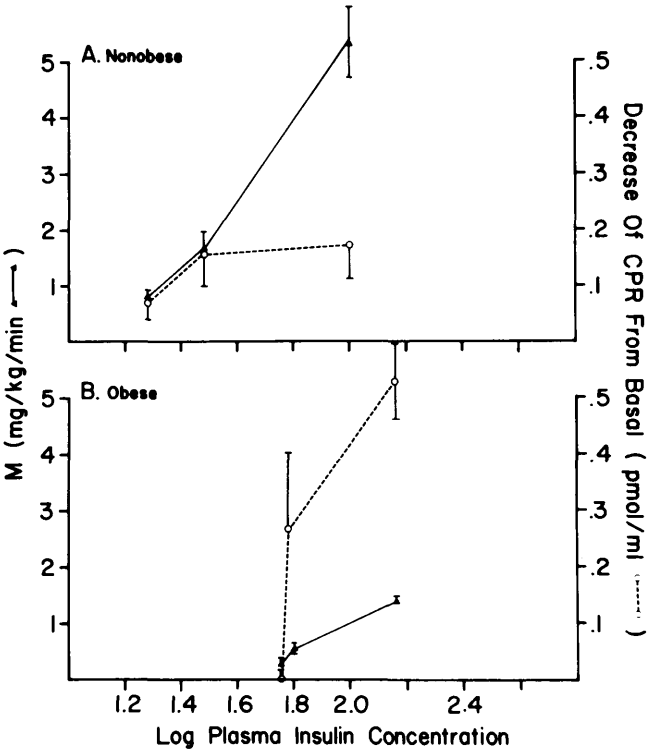

FIGURE 5. Insulin dose-dependent increases in insulin-induced glucose disposal (M) and decreases in plasma C-peptide (CPR) below basal for nonobese (A) and obese (B) subjects; M expressed as mg/kg/min (——▲——), and CPR as pmol/ml (------○------).

The results of the present study indicate that in obesity, there is no absolute insensitivity of α- or β-cells to insulin in increments within the physiologic range of this study and that the minimal effective dose is the same in each group. Conceivably, differences might exist in a supraphysiologic dose range that was not examined in these studies.

The high level of sensitivity of the islets to insulin in subjects with marked peripheral glucoregulatory insensitivity to insulin is noteworthy, suggesting that the effects of insulin on the islets might not be mediated through local effects of insulin upon glucose metabolism, or that such effects may not be impaired in obesity. Preservation of sensitivity to the antilipolytic actions of insulin has also been observed in these subjects,[13] raising the question that insensitivity to insulin in obesity may be limited to its glucoregulatory actions.

ACKNOWLEDGMENTS

We thank Ingeborg Harper, Victoria Mongillo, Ginger C. Johnson, and Pamela Thuillez for skillful technical assistance; the nursing staff of the fifth floor P.I.M.C. and the staff of the metabolic kitchen for help with the studies; and Dorothy Poleyma and Verna Kuwanhoyioma for secretarial assistance. This work was supported in part by NIH contract no. NO1-AM-6-2219. I.K. is on leave of absence from the Institute of Experimental Endocrinology, Center of Physio-

logical Sciences, Slovak Academy of Sciences, Bratislava, Czechoslovakia.

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