Insulin Sensitivity of Forearm Tissues in Prediabetic Man

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ABSTRACT In genetic prediabetic subjects (the glucose tolerant offspring of two diabetic parents or the identical twin of a known diabetic) serum insulin concentrations after glucose administration are subnormal. Maintenance of glucose tolerance in this setting is apparently paradoxical, suggesting increased tissue insulin sensitivity. Accordingly, forearm tissue insulin sensitivity in nine genetic prediabetic males was compared with that of seven males without familial diabetes. Diabetes was excluded in all subjects by preliminary oral glucose tolerance testing.

On the preliminary 3 h oral glucose tolerance test (OGTT) the sum of increments in blood glucose above fasting was greater in prediabetic than in control subjects. Conversely, the sum of increments in serum insulin was subnormal for the first 2 h. The insulin index (the sum of increments in insulin divided by the sum of increments in glucose) was significantly lower in prediabetics throughout the test. High physiologic levels of insulin were produced in the forearm by intrabrachial arterial insulin infusion (100 μU/kg per min for 26 min). Balances of glucose and amino acids across forearm muscle became more positive, as did balances of glucose and free fatty acids across adipose tissue plus skin. There were no differences in response between prediabetic and normal subjects.

Hence, the insulin sensitivity of peripheral tissues is normal in genetic prediabetes. Increased tissue insulin sensitivity is not essential to explain coexisting euglycemia and insulinopenia in prediabetes because blood glucose values on the OGTT are, in fact, elevated although still within the range considered normal.

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INTRODUCTION

A diminution in the pancreatic beta cell response to insulinogenic stimuli characterizes diabetes mellitus. Although in absolute terms high serum immunoreactive insulin (IRI)[1] concentrations have been observed in diabetics after glucose loading (2), it is clear that these can be explained either by excessive increases in blood glucose, or by coexisting obesity (3–5). Thus, in nonobese diabetics, the rise in serum IRI is consistently subnormal when consideration is given the glycemic level achieved. Similarly, reduced beta cell sensitivity to amino acids (6), glucagon (7), and tolbutamide (8) has also been reported.

Recent studies have extended these observations on beta cell function to an earlier euglycemic, or prediabetic, phase of the disease. Among genetic prediabetics, that is the glucose tolerant offspring of diabetic parents (9–11), or the normoglycemic identical twins of diabetics (12, 13), IRI concentrations after glucose administration are low, as in overt diabetes. Similar reductions in IRI release after oral glucose have been observed in a genetically more heterogeneous population, consisting of subjects having a single first degree relative with diabetes (14). While in overt diabetes abnormally high blood sugar is quite understandable in the context of diminished IRI levels, normal glucose tolerance in association with hypoinsulinemia, as seen in prediabetes, is

[1] *Abbreviations used in this paper:* AAN, alpha amino nitrogen; FFA, free fatty acids; IRI, immunoreactive insulin; OGTT, oral glucose tolerance test.

apparently paradoxical and suggests that the sensitivity of certain tissues to insulin may be increased. To test this hypothesis the insulin sensitivity of skeletal muscle and of subcutaneous adipose tissue plus skin of the forearm has been examined in a group of genetic prediabetic subjects.

METHODS

Subjects. Nine prediabetic males were studied, eight of whom were offspring of two diabetic parents, and one who was the identical twin (established by blood grouping) of a known diabetic. Males were used exclusively since their more muscular limbs afforded easier access to the several veins required for studies of forearm metabolism *in situ*. The subjects were otherwise selected randomly from among a panel of prediabetics undergoing prospective studies at the Elliott P. Joslin Research Laboratory solely on the basis of willingness to participate and the absence of obesity. Their mean age was 36±1.6 (SEM) yr (range 27–45) and mean weight was 103±2.2% of ideal (range 93–114).[2] Seven male volunteers with family histories negative for diabetes served as controls. Their mean age was 32±3.9 yr (range 22–49) and mean weight was 105±3.1% of ideal (range 95–118). Differences in age and weight between the two subject groups were not statistically significant. None of the subjects had any complicating illness, nor was any receiving medications.

Experimental protocols. An oral glucose tolerance test (OGTT) was performed in 15 of the 16 subjects following 3 days of a high carbohydrate diet (250–300 g/day), and after an overnight fast. A solution containing 100 g of glucose (Dextol, Scientific Products Co., Evanston, Ill.) was consumed from 0 to 4 min, and blood samples were subsequently drawn at 15, 30, 45, 60, 90, 120, and 180 min for determination of glucose and IRI. One prediabetic was unavailable for an OGTT, but had a normal cortisone-primed oral glucose tolerance test (2 h blood glucose 102 mg/100 ml) thereby documenting the absence of chemical diabetes. All OGTT's were normal according to the criteria used by this laboratory and published previously (15).

Studies of forearm tissue insulin sensitivity were carried out after an overnight fast in all 16 subjects as described in a previous publication (16). Briefly, the brachial artery was entered in the antecubital fossa with a double lumen needle. Evans blue dye for measuring forearm blood (and plasma) flow was infused through one lumen, and through the other arterial blood was sampled intermittantly to measure metabolite and IRI concentrations. Venous blood was collected simultaneous with arterial blood from two sites, a deep forearm vein draining predominantly muscle, and a superficial vein draining subcutaneous adipose tissue and skin. Three metabolic sets, each consisting of an arterial, a deep, and a superficial venous blood sample were collected at approximately 15-min intervals during a control period. Collections were then made at 26, 45, 60, and 90 min after beginning a brachial intra-arterial insulin infusion (100 μU/kg per min for 26 min).[3] Insulin infused at this rate yields concentrations of IRI in the high physiologic range within the forearm. A sphygmomanometer cuff about the wrist was inflated above arterial pressure for 5 min before and during each blood collection and during the insulin infusion to exclude the hand from study. Muscle metabolism of glucose and amino acids was estimated from arterio-deep venous concentration differences (A-DV). Similarly, the metabolism of glucose and free fatty acids by subcutaneous adipose tissue plus skin was estimated from arterio-superficial venous concentration differences (A-SV). A forearm blood (and plasma) flow measurement accompanied each set of blood samples. Flow measurements were considered valid only when the concentrations of Evans blue in deep and superficial venous plasma were no more than 20% apart (17). Metabolite arteriovenous concentration differences observed in normals and prediabetics were compared both before and after insulin infusion by use of Student's *t* test (18).

Analyses. Portions of blood from each collection during the OGTT were delivered into oxalate-fluoride tubes, and whole blood glucose determined in triplicate by Hoffman's ferricyanide method (19) using a Technicon AutoAnalyzer (Technicon Corp., Tarrytown, N. Y.). The remainder of each sample was permitted to clot overnight, serum was separated, and the IRI concentration determined in duplicate by a modification (20) of the double antibody technique (21). In the studies of forearm metabolism blood was collected in heparinized syringes for determination of metabolite concentrations. Glucose concentration in whole blood was measured using a portion of each sample as described above. Plasma, separated by immediate centrifugation at 4°C, was analyzed in duplicate for free fatty acids (FFA) (22) and amino acids as alpha amino nitrogen (AAN) (23). Serum from nonheparinized blood, collected in separate syringes, was used to measure IRI concentration.

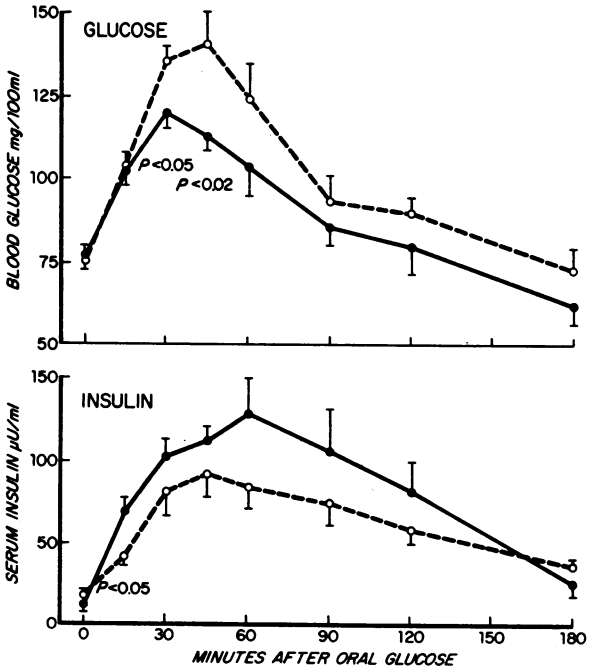

FIGURE 1 Blood glucose and serum insulin concentrations during a 100 g oral glucose tolerance test in seven normal (●——●) and eight prediabetic (○- - -○) subjects. Means ±SEM are shown.

[2] From the Metropolitan Life Insurance Tables, 1959.

[3] Glucagon-free crystalline zinc insulin, lot C226 6B, was kindly provided by Dr. W. R. Kirtley, Eli Lilly & Co., Indianapolis, Ind.

TABLE I

Blood Glucose and Serum Insulin Response during a 100 g Oral Glucose Tolerance Test in Seven Normal and Eight Prediabetic Subjects

	Cumulative sum of increments						Serum insulin/ blood glucose		
	Blood glucose			Serum insulin					
	1 h	2 h	3 h	1 h	2 h	3 h	1 h	2 h	3 h
	mg/100 ml			*μU/ml*					
Normals	133* ±17.8	151 ±20.4	152 ±20.5	370 ±38.0	530 ±79.4	548 ±86.7	3.07 ±0.55	3.68 ±0.58	3.77 ±0.61
Prediabetics	202 ±22.7	234 ±32.0	240 ±34.3	231 ±35.3	329 ±48.1	348 ±44.5	1.32 ±0.28	1.69 ±0.35	1.75 ±0.34
P‡	<0.05	<0.05	<0.05	<0.02	<0.05	<0.1	<0.02	<0.02	<0.02

* Mean ± SEM.

‡ Significance of difference between normals and prediabetics.

RESULTS

Oral glucose tolerance test (Fig. 1, Table I). The mean blood glucose curve was higher in prediabetics throughout the OGTT as shown in Fig. 1. Differences between groups were statistically significant at 30 min ($P < 0.05$) and 45 min ($P < 0.02$). Serum IRI levels, on the other hand, were generally lower during the first 120 min of the test. Significant differences between the groups occurred, however, at 15 min only ($P < 0.05$). The sum of increments in glucose above fasting was higher in prediabetics than normals throughout all 3 h of the OGTT (Table I). Conversely, the sum of increments in IRI was significantly lower for the prediabetic group during the first 2 h. When the increments in insulin were divided by the increments in glucose, values were consistently lower for prediabetics than normals. Seltzer, Allen, Herron, and Brennan have used the area above fasting under the glucose and insulin response curves to calculate an insulinogenic index which is abnormally low in mild diabetes (3). In the present study the insulinogenic index for prediabetics over the 3 h of the OGTT was 2.45±0.64 (SEM), significantly below that of 5.63±0.99 for the normal subjects ($P < 0.02$). Thus, prediabetics were insulinopenic in the context of their slightly elevated blood glucose concentrations after oral glucose. Glucose tolerance curves were normal in all subjects according to the standard criteria used by this laboratory (15).

Base-line forearm tissue metabolism (Table II). In 7 normals and 9 prediabetics the metabolism of deep (muscle) and superficial (subcutaneous adipose tissue

TABLE II

*Forearm Blood Flow and Arteriovenous Concentration Differences of Glucose, FFA, and AAN Across Muscle and Subcutaneous Adipose Tissue plus Skin under Basal Postabsorptive Conditions**

	Normals (7)	Prediabetics (9)	P‡
Blood flow (ml/min per 100 ml forearm)	3.5±0.57	3.9±0.74	NS
Muscle			
A-DV glucose (mg/100 ml)	3.0±0.66	3.7±0.43	NS
A-DV AAN (mmol/liter)	−0.51±12.6	−0.32±7.3	NS
Adipose tissue plus skin			
A-SV glucose (mg/100 ml)	4.1±0.62	4.4±0.44	NS
A-SV FFA (μeq/liter)	−72±19.0	−122±25.1	NS

* Data shown are means ±SEM derived from the averages of three determinations in each subject.

‡ Significance of difference between normals and prediabetics.

TABLE III

Effect of Brachial Intra-arterial Insulin Infusion on Forearm Blood Flow and Serum Insulin Concentration in Seven Normal and Nine Prediabetic Subjects

Time after insulin started	Blood flow		Deep venous insulin	
	Normals	Prediabetics*	Normals	Prediabetics
min	*ml/min per 100 ml forearm*		*μU/ml*	
0‡	3.5±0.57 (SEM)	3.9±0.74	11±1.2	10±1.7
26	4.2±0.85	5.1±0.71	169±24.4	172±12.3
45	4.0±0.68	4.6±0.48	23±3.0	34±3.9
60	3.8±0.75	4.4±0.49	13±2.8	17±1.6
90	3.2±0.52	4.1±0.50		

* Maldistribution of Evans blue between deep and superficial venous blood invalidated all flow measurements after the control period in one prediabetic.

‡ 0 time values represent the mean of three control determinations in each subject.

plus skin) forearm tissues was examined under basal postabsorptive conditions, prior to brachial intraarterial insulin infusion. Arterial concentrations of glucose, FFA, and AAN were the same in both subject groups. Resting forearm flow was slightly, but not significantly, greater among prediabetics (Table II). In normals, muscle extracted glucose and released AAN as previously reported (16). Adipose tissue plus skin also consumed glucose, and free fatty acids were released. In regard to the resting metabolism of both deep and superficial tissues, there were no significant differences between normals and prediabetics.

Response of forearm tissues to intra-arterial insulin (Tables III, IV, Figs. 2, 3). Table III shows the effect of insulin infusion at a rate of 100 μU/kg per min on forearm blood flow and deep venous IRI concentration. Flow at each time interval tended to be higher in prediabetics than in normals, though differences were not statistically significant. Deep venous IRI peaked at 169±24.4 (SEM) μU/ml in normals just before termination of the infusion at 26 min, and fell thereafter. The IRI concentration in prediabetics rose to a comparable value of 172±12.3 μU/ml, indicating that in both subject groups forearm tissues were exposed to high but physiologic insulin concentrations. Arterial IRI proximal to the point of infusion reflects recirculating insulin and did not change (not shown), since infused insulin was diluted in the systemic venous pool upon leaving the forearm.

A-DV for glucose became more positive and for AAN less negative (i.e. more positive) after insulin as shown in Fig. 2. These changes in A-DV tended to be somewhat smaller in prediabetics than in normals; but, at no time period were differences in the change in A-DV between subject groups statistically significant. The curves illustrated in Fig. 2, however, do suggest that a method of data analysis which summates differences between subject groups at each time interval might actually reveal muscle insulin resistance in prediabetics. This, however, is not the case. When the absolute values for glucose uptake and AAN release by deep tissues are calculated as the product of flow and A-DV, account is taken of the somewhat higher blood flow observed in prediabetics. The insulin response of muscle in the two groups is then revealed to be the same. With regard to glucose this point is illustrated by the data given for individual subjects in Table IV. It applies equally to AAN release. Clearly, there was no increase in sensitivity of muscle to insulin among prediabetics.

As shown in Fig. 3 insulin increased glucose extraction by subcutaneous adipose tissue plus skin. Antilipolysis was also evident as negative basal values for FFA A-SV became more positive after insulin. For both glucose and FFA, the changes in A-SV in the two groups were superimposable. Since that portion of forearm flow supplying subcutaneous adipose tissue plus skin is small and variable, estimates of superficial flow based on measurement of total forearm flow are subject to large error. For this reason calculations of absolute metabolite uptake or output by these tissues customarily are not attempted in experiments of this type (24).

DISCUSSION

The relative importance of peripheral tissues as opposed to the liver in disposing of an oral glucose load has not been defined with precision in intact man. From studies of glucose uptake by forearm tissues after glucose ingestion it is known that muscle and adipose tissue play a significant if not preeminent role (25, 26). Consequently, increased insulin sensitivity of peripheral tissues could potentially explain the paradoxical findings of normal glucose tolerance despite insulinopenia previously reported in genetic prediabetic subjects after oral (9, 10, 13) and intravenous glucose (11, 12). In the present

TABLE IV

Effect of Insulin on Arteriovenous Concentration Difference of Glucose across Muscle and on Glucose Uptake by Deep Forearm Tissues in Seven Normals and Nine Prediabetics

Minutes after start of insulin*:	A-DV glucose					Glucose uptake‡				
	0	26	45	60	90	0	26	45	60	90
	mg/100 ml					*mg/min per 100 ml forearm*				
Normals										
H. G.	4.3	18.5	18.7	16.1	18.1	0.10	0.44	0.49	0.39	0.43
W. B.	0.9	27.0	30.8	24.5	17.1	0.04	1.37	1.43	1.02	0.54
G. W.	3.2	26.9	29.2	21.1	10.3	0.11	1.13	1.17	0.80	0.33
J. D.§	5.9	30.3	35.4	24.4		0.17	1.41	1.88		
R. R.	3.1	7.7	7.4	9.2	6.3	0.14	0.60	0.46	0.61	0.33
W. Bk.	1.2	14.1	20.5	12.2	4.4	0.03	0.41	0.52	0.34	0.11
J. W.	2.2	29.2	18.2	19.9	3.9	0.07	0.71	0.42	0.61	0.10
Mean	3.0	22.0	22.9	18.2	10.0	0.09	0.87	0.91	0.63	0.31
±SEM	0.66	3.27	3.60	2.24	2.57	0.019	0.163	0.220	0.104	0.070
Prediabetics										
J. Wi.	2.9	8.1	3.6	4.3	1.3	0.15	0.51	0.20	0.23	0.08
D. H.	4.8	2.8	5.9	3.6	6.3	0.04	0.05	0.11	0.07	0.11
R. D.	6.2	15.6	9.2	9.7	17.9	0.19	1.22	0.54	0.43	0.84
H. W.	4.2	19.7	31.0	22.0	10.8	0.12	0.67	1.13	1.16	0.29
R. S.	2.0	27.0	23.3	18.2	10.0	0.17	1.89	1.24	1.10	0.36
J. S.	2.9	35.1	33.9	28.5	13.4	0.10	1.95	1.49	1.40	0.48
F. C.	3.7	23.5	21.6	15.4	6.2	0.08	0.89	1.22	0.43	0.28
P. B.	2.5	24.2	14.8	13.6	6.5	0.09	1.27	0.70	0.62	0.36
W. F.‖	4.1	4.2	4.2	5.2	5.8					
Mean	3.7	17.8	16.4	13.4	8.7	0.11	1.04	0.83	0.68	0.35
±SEM	0.43	3.67	3.86	2.86	1.64	0.015	0.227	0.183	0.171	0.085
P¶	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

* 0 time values are the mean of three preinfusion determinations. Insulin was infused from 0 to 26 min.

‡ Glucose uptake is the product of flow and glucose A-DV.

§ Blood flow could not be measured accurately in J. D. at the 60 min period because differences in Evans blue concentration between deep and superficial venous plasma exceeded 20%. The concentration of glucose in deep venous blood at 90 min could not be measured for technical reasons.

‖ Blood flows after the control period could not be measured in W. F. because of Evans blue maldistribution between deep and superficial venous blood. The peak deep venous IRI concentration in this subject was 145 μU/ml at 26 min.

¶ Significance of the difference between normals and prediabetics.

investigation the response of forearm muscle and adipose tissue to insulin in prediabetic subjects has been compared to that of age and weight matched volunteers without familial diabetes. Muscle and adipose tissue were exposed to insulin concentrations in the high physiologic range, and the increase in glucose translocation for both tissue types was found to be precisely the same in the two subject groups. Moreover, the anabolic effect of insulin on muscle amino acid balance and its antilipolytic action on adipose tissue were also comparable. Hence, no abnormality in peripheral tissue insulin sensitivity could be identified in prediabetic subjects.

These prediabetic subjects were, nonetheless, typical of those reported previously in that diminished insulin release after glucose ingestion could be demonstrated. Since blood glucose values were somewhat higher in prediabetic subjects than in normals, IRI concentrations are not strictly comparable. Yet, despite the greater glycemic stimulus, the sum of increments in IRI was below normal in the prediabetic group during the first 2 h of the OGTT. The insulin index (the sum of increments in insulin divided by the sum of increments in glucose) corrects for these differences in blood glucose concentration and was subnormal throughout the entire 3 h OGTT.

In the absence of altered peripheral tissue insulin sensitivity, increased sensitivity of the liver could ex-

plain coexisting insulinopenia and euglycemia after glucose administration. Perley and Kipnis, in studies of normal man, have estimated that as much as 69% of a 100 g oral glucose load may be initially extracted by the liver (5); and insulin is known to facilitate hepatic glucose uptake (27). The work of Felig and Wahren suggests further that the liver may be of particular importance in blood glucose regulation in a setting of relative insulin lack. They showed that when the beta cell is stimulated by infusion of small amounts of glucose, the normal rise in portal venous insulin can be sufficient to block hepatic glucose output, thereby tending to lower blood glucose concentration, at a time when the change in systemic insulin concentration is too small to influence peripheral tissue glucose uptake (28). These workers have recently extended their studies of hepatic insulin sensitivity to genetic prediabetes, and report that suppression of hepatic glucose output after glucose infusion is greater than normal (29).

It must be emphasized, however, that in the present study blood glucose concentrations during the OGTT were significantly greater in prediabetics than normals. Differences in blood glucose concentration between subject groups were necessarily small since normal glucose tolerance was a prerequisite for inclusion in the study.[4] Thus, the paradox of euglycemia and insulinopenia after glucose ingestion in prediabetics is more apparent than real. A subtle deterioration of glucose tolerance appears to accompany the diminished insulin response, yet the glucose profile is still "normal" by currently accepted criteria. Support for this interpretation is lent by the results of oral glucose tolerance testing in a series of 24 apparently normal identical twins of known diabetics (13). Although none of the subjects was overtly diabetic, several demonstrated mild glucose intolerance, and significant hyperglycemia throughout the entire OGTT characterized the entire group when compared with normal subjects. The mean blood glucose curve was also elevated in the genetic prediabetic population studied by Ricketts, Cherry, and Kirsteins (30). In this investigation insulin levels were not subnormal; however, one-third of the prediabetics were obese (body weight exceeded ideal by more than 20%) hampering a direct comparison of this series to those in which the obese were excluded. In other investigations encompassing smaller groups, significant differences between normal and prediabetic subjects in the distribution of blood glucose values on the OGTT, or K value of glucose disappearance

[4] The upper limit of normal on the OGTT at each time interval was set at two standard deviations above the mean value obtained in a group of 57 normal males aged 15–35 yr without familial diabetes (15). Individual blood glucose values in our prediabetic subjects consequently always fell within the normal distribution of values for subjects without familial diabetes.

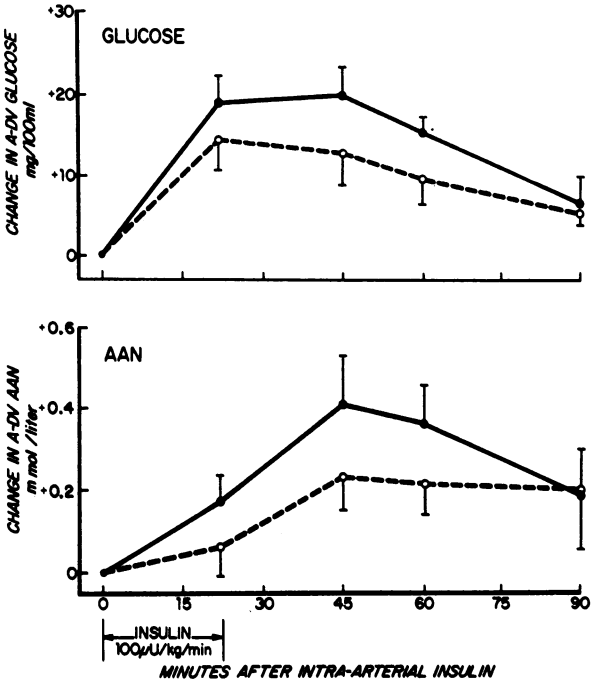

FIGURE 2 Changes from control in glucose and AAN balance across muscle after intra-arterial insulin in seven normals (●——●) and nine prediabetics (○- - -○). Means ±SEM are shown.

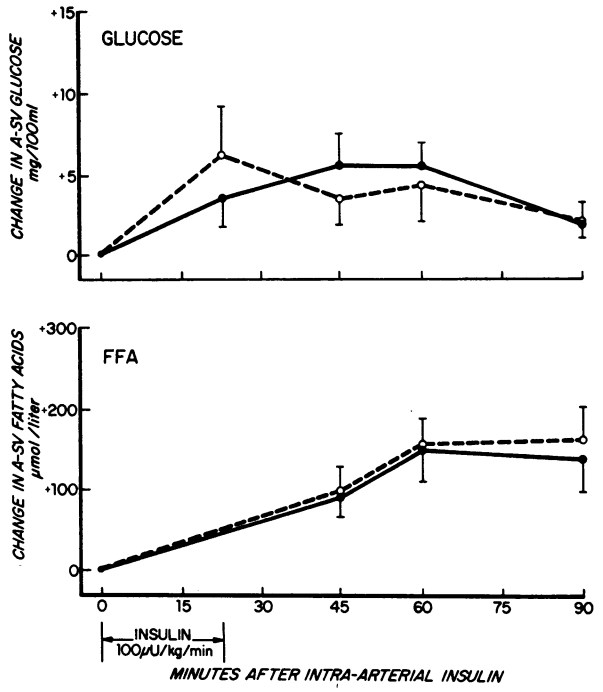

FIGURE 3 Changes from control in glucose and FFA balance across adipose tissue plus skin after intra-arterial insulin in seven normals (●——●) and nine prediabetics (○- - -○). Means ±SEM are shown.

on the intravenous glucose tolerance test, have also been observed indicating mild glucose intolerance in prediabetes (9, 12, 31). In still other studies, although differences were not significant, mean blood glucose curves in genetic prediabetics were above those of controls (10, 11, 32). Even in the recent investigations of hepatic insulin sensitivity, the prediabetic subjects had higher blood glucose values after glucose infusion than did the normals (29). Finally, K rates of glucose disappearance on the intravenous glucose tolerance test are significantly below average (though not in the diabetic range) among subjects whose insulin response to intravenous glucose is reduced, independent of familial diabetes (33). Cerasi and Luft have termed these subjects "prediabetic" exclusively on this functional basis.

In conclusion, it is clear that insulin sensitivity of muscle and adipose tissue is not altered in genetic prediabetes. Furthermore, there is no need to postulate increased insulin sensitivity of any tissue in view of the mild impairment of glucose tolerance typically seen.

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