Oral Glucose-stimulated Insulin Release in Nondiabetic Twin Siblings of Diabetic Twins

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SUMMARY

A total of twenty-five monozygotic and dizygotic glucose-tolerant twin siblings of diabetic patients were compared with age-, sex-, and weight-matched groups of glucose-tolerant offspring of two diabetic parents and normal control subjects with regard to glucose, insulin, free fatty acid and growth hormone responses during a five hour oral glucose tolerance test. Few differences in mean levels of glucose, insulin or free fatty acids were found, the trend being a higher insulin level in the twin study groups. Although the numbers of subjects were small, significantly higher insulin-glucose relationships were found in monozygotic females compared to normals and in dizygotic females compared to offspring and to normals. The fasting plasma free fatty acid level was highest in the twins among each of the four subgroups, possibly indicating a blunting of the antilipolytic effect of the basal insulin level in the twins.

A "paradoxic" type of growth hormone response was seen in both the twin and offspring groups compared to the respective normal groups.

It appears that alterations in insulin-glucose relationships and growth hormone response exist in potential diabetic groups that cannot be accounted for by small but important differences in age, weight, or glucose tolerance. DIABETES *23*:684-92, August, 1974.

There is no recognized genetic marker for diabetes mellitus. Furthermore, no specific test has been universally accepted as a reliable predictor of the eventual development of the disease. If diabetes mellitus is a hereditary disorder, and there is evidence from family and twin studies that it is, then nondiabetic monozygotic twin siblings of diabetics are more likely to be genetically prediabetic than any other relative. Regardless of the genetic composition of the disorder (single gene, multifactorial), a monozygotic twin sibling has the same genes as his diabetic twin sibling and is therefore equally prone to purely genetically determined factors with any disease differences being explained on the basis of nongenetic effects.[1,2] The other frequently studied "prediabetic" population, the offspring of two diabetic parents (PD), is as likely to carry the "diabetic" genes as the monozygotic twin sibling of a diabetic parent if diabetes is determined by a single recessive gene, and only if both parents are homozygotic for the same gene. If more than one gene can determine diabetes, and if one parent has diabetes due to one gene and the other due to another gene, then there would be little assurance that their offspring might consistently develop diabetes on a genetic basis. Dizygotic twins are genetically similar to ordinary siblings. Therefore, they could share some of the same genes but the likelihood of diabetes mellitus would be similar to a nontwin sibling of a diabetic.[3] The disease rate in dizygotic twins is usually compared to that in monozygotic twins to determine environmental contribution.

A nondiabetic, monozygotic twin sibling of a diabetic parent has a normal blood glucose response during an oral glucose tolerance test, but might show an abnormal serum insulin, serum growth hormone, plasma free fatty acid response, or a disturbed serum insulin-blood glucose relationship that could indicate the nature of the inherited diabetic defect before the diabetes is able to be diagnosed.

Earlier analysis of this twin series has shown that 32 per cent of the monozygotic and none of the dizygotic twins were concordant for overt diabetes.[2] However, an additional 14 per cent of the monozygotic twins, and 35 per cent of the dizygotic twins were concor-

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dant for chemical diabetes.[2] This increased concordance for chemical diabetes in dizygotic twin siblings of diabetics is of importance with regard to the genetic or nongenetic factors contributing to the etiology of diabetes. If diabetes is a disorder determined solely on a genetic basis, one would not expect chemical diabetes to occur with as much frequency in the dizygotic twin siblings. Therefore chemical diabetes may either not be inevitably related to the disease spectrum of diabetes mellitus, or diabetes may have a strong environmental component.

Pike et al. studied twenty-four apparently healthy identical twin siblings of diabetics and found an "inappropriately" low serum insulin response to an oral glucose load (50 gm. glucose).[4] However, one half of the individuals in these studies showed levels of blood glucose above the "normal" range. Cerasi and Luft infused glucose into monozygotic twins, one of whom had diabetes, and noted a similarity in glucose and insulin curves for both members of the twin pair.[5] In addition, a strikingly abnormal reduction of the early insulin response to the glucose infusion was noted.

To evaluate the magnitude and interrelationships among blood glucose (BG), serum insulin (IRI), serum growth hormone (GH), and plasma free fatty acid (FFA) levels, which might predict the eventual development of diabetes, these substances were measured during an oral glucose tolerance test in monozygotic (MZ) and dizygotic (DZ) twin siblings of diabetic patients; offspring of two diabetic parents individually matched to monozygotic and dizygotic twins by age, sex and weight; and similarly matched normal controls.

MATERIALS AND METHODS

A series of 245 diabetic twins and triplets belonging to 242 sets was systematically identified from the records of the Joslin Clinic (figure 1).[2] There were 104 sets in which both siblings were alive. Forty-eight of these (table 1) sets [(ninety-five siblings, forty-three monozygotic (there was one triplet set which equals two twin sets) and fifty-two dizygotic)] were studied at the Elliott P. Joslin Research Laboratory.[2] A five hour oral glucose tolerance test (OGTT) was included in the study if either twin sibling claimed to be nondiabetic at the time of examination.

Among the forty-three twin siblings tested with a five hour OGTT were twenty-five siblings (twelve monozygotic: seven female and five male; thirteen dizygotic: five female and eight male) who were between the ages of ten and fifty years, nonobese (not more

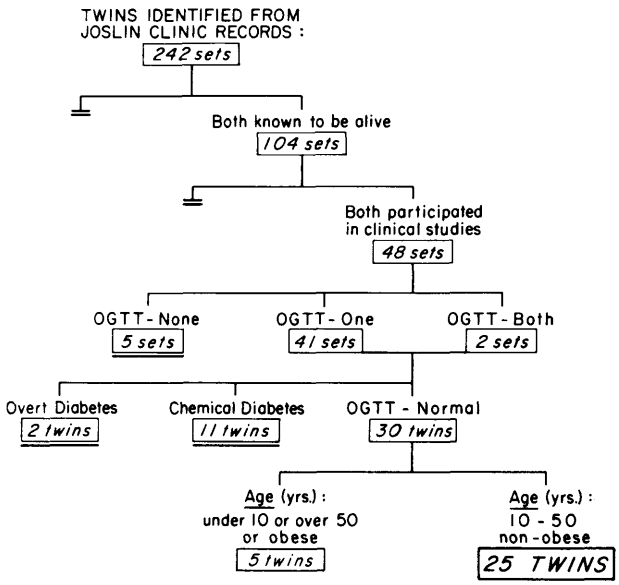

FIG. 1. Description of source of subject groups.

than 20 per cent in excess of average body weight—Metropolitan Life Insurance Tables, 1959), and glucose tolerant (BG response to the OGTT within the limits described previously as normal).[2] These twins were grouped by sex and zygosity and were matched by sex, age, and weight to normal controls drawn from a larger pool of individuals with no family history of diabetes and to glucose-tolerant offspring of two diabetic parents (prediabetics), one twin matched to two controls or offspring in each group (table 2).[6] No subject was taking steroids, thiazides, or oral contraceptive agents.

Twin siblings whose BG responses to the OGTT were abnormal (fasting greater than 100 mg. per 100 ml., one hour greater than 160 mg. per 100 ml., and two hours greater than 110 mg. per 100 ml.) were designated *chemical diabetics* (table 1).

TABLE 1

Distribution of twin siblings by sex, zygosity and carbohydrate tolerance

	Sex	Total	Overt diabetics	Chemical diabetics	Normal OGTT
Monozygotic	MM	10	4	1	5
	FF	12	3	1	8
Dizygotic	MM	8	0	2	6
	FM	5	0	1	4
	FF	7	0	4	3
	MF	6	0	2	4
Totals		48	7	11	30

TABLE 2

Mean age and per cent average weight of groups

	Males			Females		
	N	Age, Mean ± S.E.M.	% Av. Wt. Mean ± S.E.M.	N	Age, Mean ± S.E.M.	% Av. Wt. Mean ± S.E.M.
Monozygotic	5	21.4 ± 4.6	95.0 ± 1.0	7	29.7 ± 3.4	102.7 ± 5.5
Offspring	10	20.5 ± 2.6	96.5 ± 2.8	14	30.4 ± 2.3	101.1 ± 2.4
Normals	10	21.0 ± 2.7	96.1 ± 1.6	14	29.1 ± 2.2	96.4 ± 1.5
Dizygotic	8	25.5 ± 4.2	100.4 ± 4.8	5	36.0 ± 4.0	100.8 ± 9.2
Offspring	16	25.2 ± 2.6	101.3 ± 2.0	10	38.1 ± 2.3	101.1 ± 3.2
Normals	16	25.0 ± 2.2	102.9 ± 2.4	10	34.0 ± 2.6	94.6 ± 2.3

Zygosity was determined by blood group analysis [ABO, Rh, P, Kelly, Lewis, MNS, Lutheran, Duffy, Kidd, miscellaneous (Wr^a Vel, Yt^a), four serum factors (haptoglobin, transferrin, Gm, Inv)]*, and phenylthiocarbamide testing.

Overt diabetes was classified as outlined by the Committee on Professional Education, American Diabetes Association, or by the requirement of a daily insulin dose greater than 20 units to maintain blood sugar control; or by having evidence of ketoacidosis.[7]

Each subject consumed a 300 gm. carbohydrate diet for three days before the OGTT. A 100 gm. dose of glucose was administered orally to all subjects weighing over 100 pounds. For those under 100 pounds, the dose was calculated on the basis of one gram per pound of body weight. Blood samples were drawn in the fasting state and at 15, 30, 45, 60, 90, 120, 180, 240, and 300 minutes after glucose loading. BG was determined by the ferricyanide method of Hoffman as modified for the AutoAnalyzer.[8] Simultaneous blood samples were obtained for determination of serum IRI, GH, and plasma FFA.[9-11] All subjects were studied as outpatients. The regression coefficients represent over-all coefficients adjusted for random sampling and are applicable to heterogeneous data.[12] This enables us to estimate the amount of change that occurs in the dependent variable for each unit of change in the independent variable.[11] Comparisons of regression coefficients were made using Student's *t*-test.[13]

RESULTS

The groups were subdivided by sex, and an initial comparison within each group showed a number of significant differences of BG, IRI and/or FFA. In the total twin group from which the current subjects were selected there was a greater concordance for overt diabetes in the older (age 40 and greater) monozygotic twins than in the younger monozygotic and dizygotic groups; also, there was an increasing concordance for chemical diabetes with increasing age among the dizygotic twins.[2] Therefore, since twin siblings with abnormal glucose tolerance tests were not included in this study the highest risk cases were excluded. It is possible that the "prediabetic" (and perhaps even the normal groups) had a larger number of potential diabetics yet to be discovered than the twin group.

Males

There was a lower fasting mean BG in MZ than normals, and also a higher mean IRI (and IRI/BG ratio) compared with the offspring at 120 minutes. However, mean FFA levels were higher in the MZ compared to the normals at thirty and forty-five minutes and to the offspring at F to forty-five minutes. Serum GH was higher in MZ than offspring and normals at ninety minutes (figures 2 and 3).

Conversely, DZ twins showed a lower serum IRI at F (and lower IRI/BG ratio at F and fifteen minutes) than the offspring. However, DZ (like MZ) also showed a higher mean FFA compared with both normals at F and 240 minutes, and offspring at fasting. The DZ exhibited a marked "paradoxical" GH response during the first sixty minutes, significantly higher than normals at fifteen to sixty and 120 minutes, and offspring at sixty minutes (figures 4 and 5).

Females

The only BG, IRI and FFA difference noted in MZ females was more insulin per unit of glycemic stimulus than normals as indicated by a higher regression coefficient (table 3). Despite the wide variation (and therefore lack of detectable significance) in mean levels of GH in MZ, there appears to be an increase comparable to that of DZ males during the first sixty minutes. Whereas both normal and MZ show similar

*These analyses were performed at the Blood Grouping Laboratory of the Childrens Hospital Medical Center, Boston, Massachusetts.

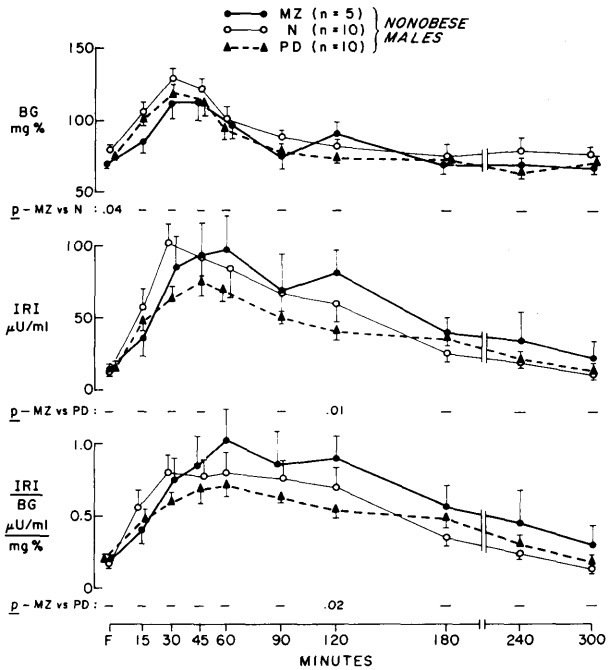

FIG. 2. Comparison of mean levels (± S.E.M.) of blood glucose, serum insulin and insulin/glucose ratios during oral glucose tolerance tests between nonobese male monozygotic twins, normals, and prediabetics.

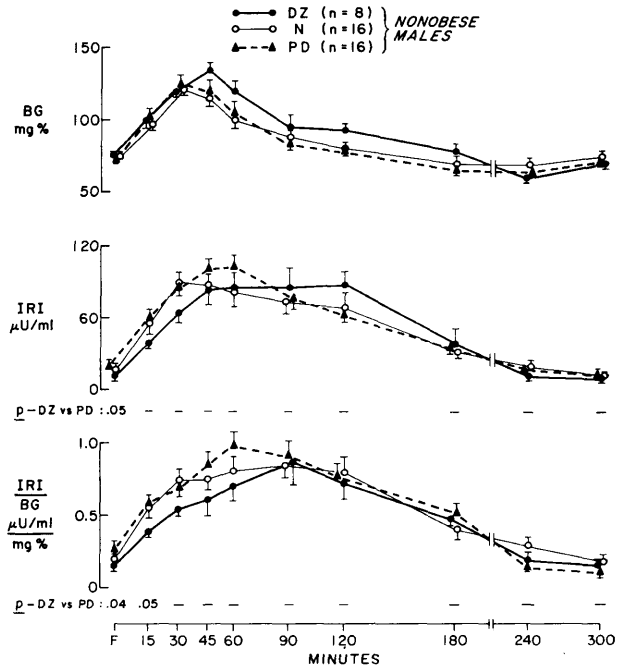

FIG. 4. Comparison of mean levels (± S.E.M.) of blood glucose, serum insulin and insulin/glucose ratio during oral glucose tolerance tests between nonobese male dizygotic twins, normals and prediabetics.

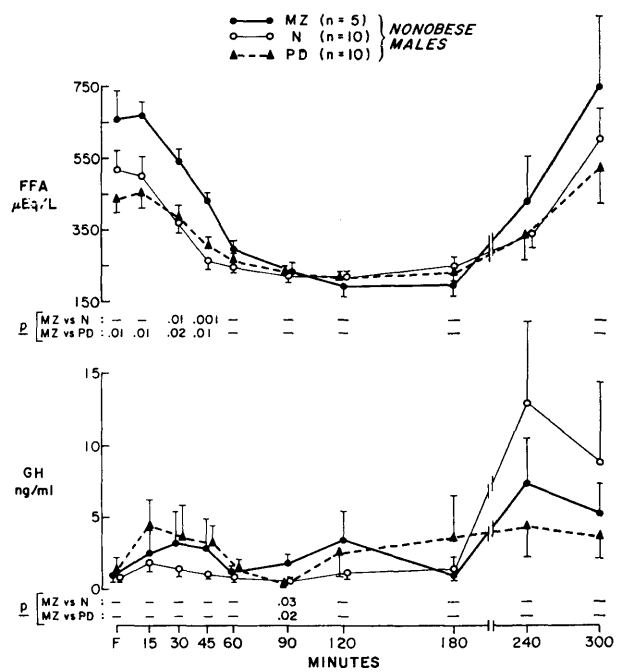

FIG. 3. Comparison of mean levels (± S.E.M.) of plasma free fatty acids and serum growth hormone during oral glucose tolerance tests between nonobese male monozygotic twins, normals and prediabetics.

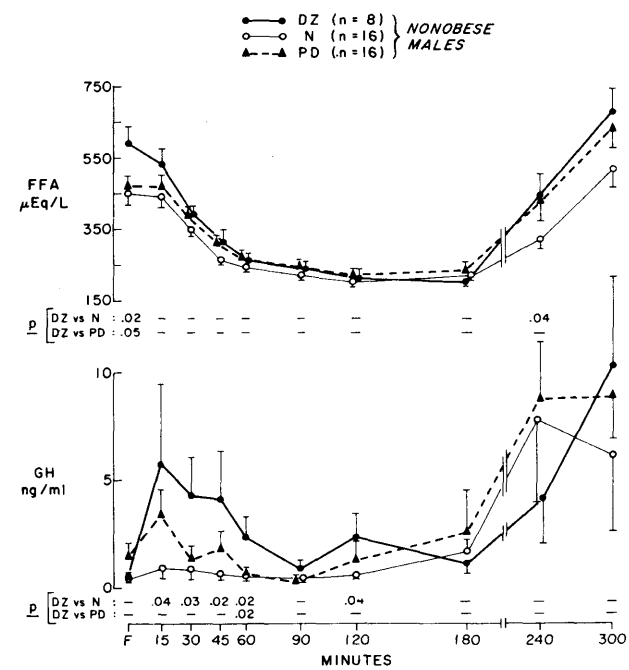

FIG. 5. Comparison of mean levels (± S.E.M.) of plasma free fatty acids and serum growth hormone during oral glucose tolerance tests between nonobese male dizygotic twins, normals and prediabetics.

TABLE 3

Comparison of serum insulin-blood glucose relationships (adjusted to random sampling)

Group (n)	All males	All females
	Regression Coefficients (± Standard Deviation) μU./ml./mg. per 100 ml.	
Monozygotic (5)	1.157 ± .238	(7) 1.236 ± .266
Offspring (10)	0.813 ± .086	(14) 1.102 ± .103
Normals (10)	1.046 ± .150	(14) 0.648 ± .114
Dizygotic (8)	1.101 ± .133	(5) 1.555 ± .210
Offspring (16)	0.971 ± .082	(10) 0.683 ± .105
Normals (16)	1.188 ± .115	(10) 0.660 ± .169

Females, Monozygotic > normals $p < .02$
Females, Dizygotic > Offspring $p < .001$
Females, Dizygotic > Normals $p < .01$

mean levels of fasting GH, the decline is much slower in the MZ twins (figures 6 and 7).

The DZ females showed an elevated mean BG compared with normals at sixty minutes, and offspring at 180 minutes. A greater insulin response was apparent as demonstrated by increased mean IRI (and IRI/BG ratio) at 180 minutes compared with offspring, and at 240 minutes compared with normals. Likewise, the

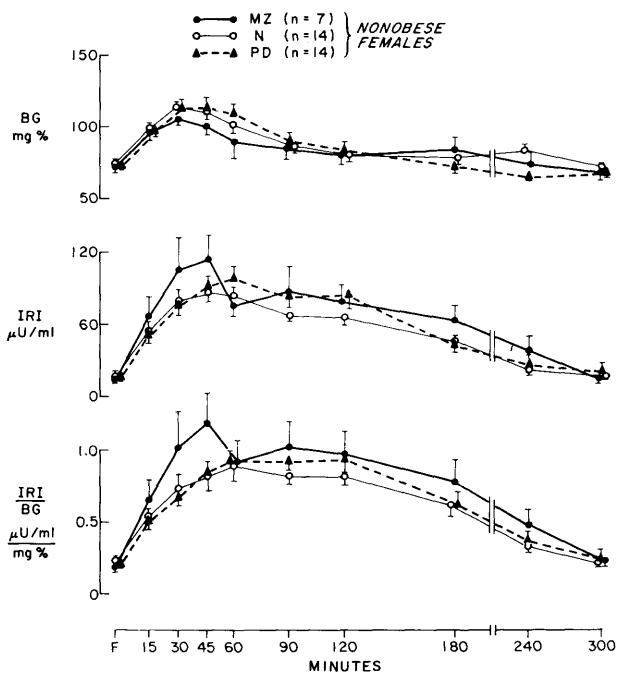

FIG. 6. Comparison of mean levels (± S.E.M.) of blood glucose, serum insulin and insulin/glucose ratio during oral glucose tolerance tests between nonobese female monozygotic twins, normals and prediabetics.

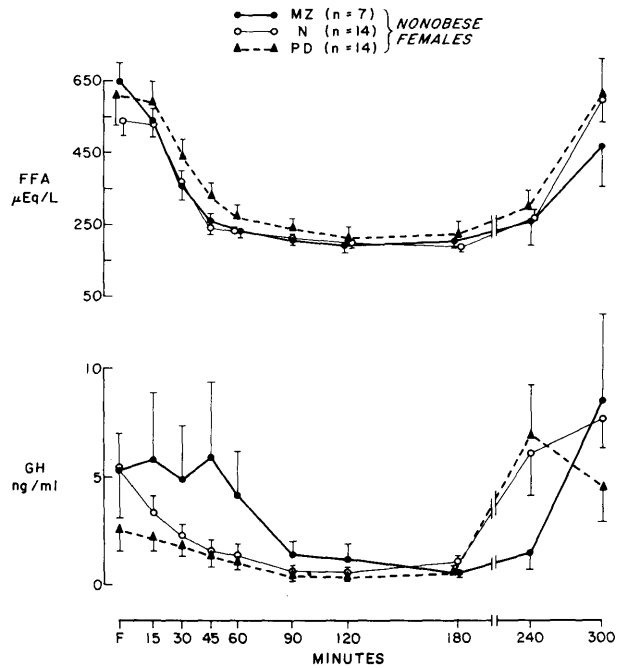

FIG. 7. Comparison of mean levels (± S.E.M.) of plasma free fatty acids and serum growth hormone during oral glucose tolerance tests between nonobese female monozygotic twins, normals and prediabetics.

regression coefficient (units of insulin per unit of glucose) was increased in comparison with normals and offspring (table 3). Also, unlike the other twin groups, the DZ showed no rebound of FFA, and therefore lower mean values at 240 and 300 minutes compared with normals (figures 8 and 9).

Influence of Age and Weight

Several investigators have reported an age and weight effect on glucose and insulin levels during glucose tolerance testing.[14-18]

Despite a systematic selection of subjects to control for these factors, some small differences between groups remained (table 2). To determine if effects of per cent ideal weight or age were responsible for between-group differences in insulin-glucose dynamics, correlations were assessed between these two factors and BG, IRI, IRI/BG, GH and FFA, as well as the glucose and insulin responses characterized by the area under the respective curves for the zero to sixty- and zero to 300-minute time interval, the ratio between the respective insulin and glucose areas and the regression coefficient.

Males

In MZ males the IRI was positively correlated with

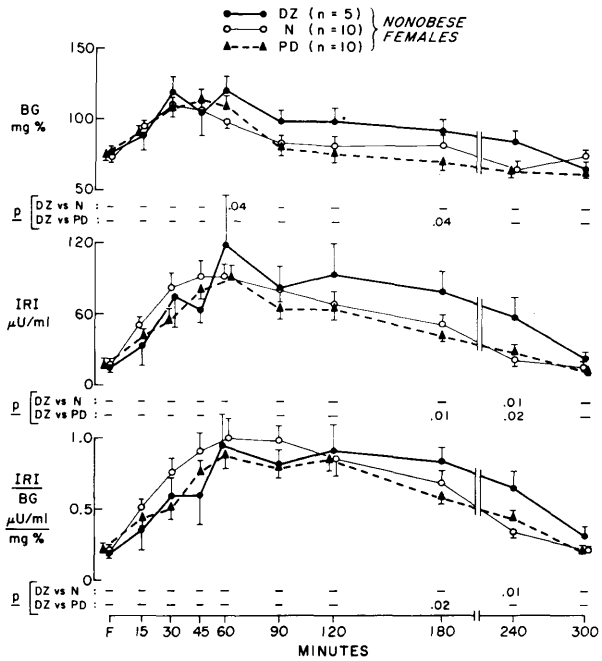

FIG. 8. Comparison of mean levels (± S.E.M.) of blood glucose, serum insulin and insulin/glucose ratio during oral glucose tolerance tests between nonobese female dizygotic twins, normals and prediabetics.

age, and the MZ males were older than offspring, in whom the IRI is negatively correlated with per cent ideal weight, and who weigh more than the MZ. Thus, these could represent confounding variables accounting for the increased insulin response in the MZ compared with offspring. Adjusting for both age and per cent ideal weight by analysis of covariance, the IRI difference between the MZ and the offspring persisted.

Despite the negative correlation of age with GH in the normal comparison group for MZ, since the normals were slightly younger, the significance of the greater GH in the MZ compared with normals and offspring was sustained.

In offspring matched to MZ, mean levels of FFA were positively correlated with per cent ideal weight. Since offspring have a higher per cent ideal weight than MZ, the increase in FFA of the MZ appears to be sustained.

In DZ, per cent ideal weight was positively correlated with IRI. Since DZ were slightly lighter than the comparison offspring group, this could be a confounding variable accounting for the lower fasting insulin noted among the DZ. After adjusting by analysis of covariance for both age and per cent ideal weight, the difference in fasting IRI between DZ and prediabetics persisted.

However, in the offspring group compared with the DZ, GH was negatively correlated with age. Since the offspring were not older, the increase in mean GH levels in DZ compared with offspring was sustained.

Females

In MZ females, the insulin response was negatively correlated with weight. Since the MZ were heavier than normals, weight could not account for the greater insulin response noted in the regression coefficient in this comparison. Furthermore, whereas age was also negatively correlated with insulin for the MZ and offspring groups, the MZ females were not significantly younger (they were older) than the other groups, sustaining the observation of a greater amount of insulin noted in the MZ female group.

Among the DZ females, a greater insulin response was noted in comparison to offspring and normals. In both DZ and in normals, however, weight was positively correlated with insulin. Since the DZ were heavier than the normals, this could account for the greater amount of insulin. After adjusting by analysis of covariance for both age and per cent ideal weight, the IRI difference between the DZ and normals per-

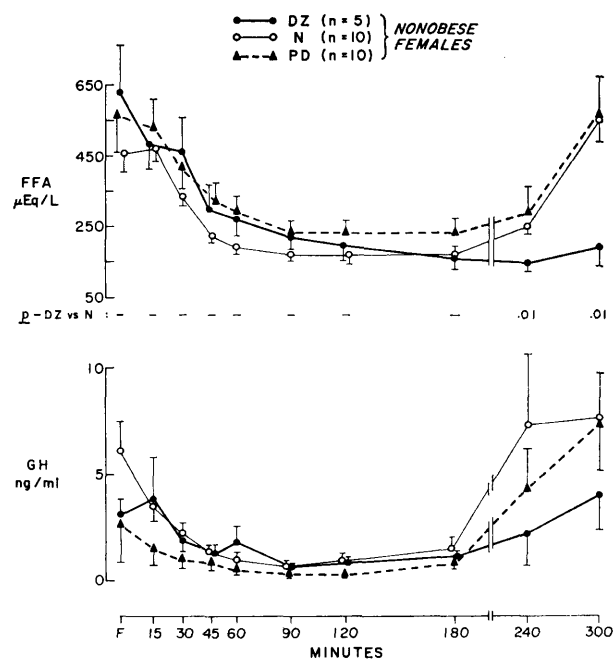

FIG. 9. Comparison of mean levels (± S.E.M.) of plasma free fatty acids and serum growth hormone during oral glucose tolerance tests between nonobese female dizygotic twins, normals and prediabetics.

sisted. The DZ were not heavier than the offspring, and here the greater insulin response in DZ compared with offspring was sustained.

The DZ females were the only group which showed a smaller FFA response compared with the normals. Since FFA was positively correlated with weight, if this group were lighter, it could account for the lack of response; however, it was heavier, therefore this decreased response was sustained. This could be a complementary response to the later and stronger insulin responses.

DISCUSSION

In assessing the cause and development of diabetes mellitus, it is still controversial as to whether absolute or relative hypoinsulinemia or hyperinsulinemia precedes and/or accompanies carbohydrate intolerance.

If one presumes a decreased insulin response, then a defect in the beta cell is hypothesized. On the other hand, an increase in measurable insulin would be consistent with a decreased tissue sensitivity, a circulating insulin antagonist, an abnormal quality of the measurable insulin, or a hyper-response of the beta cell. Neel, as part of his "thrifty genotype" hypothesis, has proposed that the basic defect in diabetes is a larger than normal availability of insulin at some stage of the food ingestion cycle.[19]

In searching for factors predictive of future glucose intolerance, the study of subjects able to maintain blood glucose levels in an expected "normal" fashion would seem to be preferable to subjects who can no longer regulate blood glucose within "normal" levels. Therefore, "prediabetics" should be able to provide more information than decompensated "mild diabetics."

In earlier studies of prediabetic subjects, Siperstein, Unger and Madison noted that serum insulin levels did not differ significantly from those of fifteen normal control subjects.[20] Reaven and Farquhar concluded from continuous glucose infusion studies that patients with diabetes did not have lower steady state plasma insulin concentrations.[22] Ricketts, Cherry, and Kirsteins reported higher insulin levels in "prediabetics" after an oral glucose load, and Yalow and Berson and also Hales et al. have described higher serum insulin levels in diabetic patients.[22-24] However, Colwell and Lein, Daweke et al., Simpson et al., and Soeldner et al. have presented evidence that prediabetic subjects have lower insulin responses than do normal persons. Colwell and Lein re-examined the data of other investigators who reported higher insulin levels in diabetic patients, and they showed that the serum insulin relationship to blood glucose was reduced.[11,12,14,19-27] Perley and Kipnis, and Seltzer et al. described a decreased relative insulin response to intravenous and oral glucose loads in early mild diabetes mellitus.[28,29]

Furthermore, Pyke and Taylor, and Cerasi and Luft in examining nondiabetic monozygotic twin siblings of diabetic patients reported a decreased insulin response to glucose in potentially diabetic twins.[5,30,31] A more recent study of twenty-four monozygotic twins with a diabetic twin mate revealed significantly higher glucose levels and lower insulin levels following oral glucose.[4] This twin group was heterologous in that it included obese and nonobese subjects from eight to fifty-eight years of age and about one half of the group showed an abnormal glucose response. However, in the study made by Taylor et al. of first-degree relatives of diabetic patients, two types of insulin responses were noted.[30] In most cases, the serum insulin levels were low or normal despite mildly elevated blood sugar levels. A similar number of individuals with symptoms of reactive hypoglycemia during an abnormal glucose tolerance test revealed a rise in insulin from normal fasting levels to higher levels before returning to normal. Reaven and Farquhar reported that absolute levels of plasma insulin produced by glucose infusions were extremely variable from patient to patient and were relatively independent of the degree of hyperglycemia.[21]

In this study, it has been possible to examine glucose-tolerant monozygotic and dizygotic twin siblings of overt diabetic patients, glucose-tolerant offspring of two diabetic parents and normal controls matched for sex, age and weight. An overview of the analyses comparing the mean levels of blood glucose in the groups showed only a few statistically significant differences. Therefore, glucose homeostasis appears very similar in all study groups. Also, the mean levels of insulin at all time intervals examined showed only the rare significant difference with the trend being a greater insulin level in the twin study groups. The analyses of the IRI/BG ratios showed marked similarity among the twin study groups. In addition, other insulin-glucose relationships were examined. These included (data not shown) the ratio of the insulin area above baseline divided by the glucose area above baseline for both the zero- to sixty-minute time interval or the zero- to 300-minute time interval. Comparisons of these parameters also showed no significant differences. There were, however, a number of significant differences among the groups when re-

gression coefficients of serum insulin upon blood glucose were examined. A significantly elevated regression coefficient was found in the monozygotic twin females compared to the normal controls and in the dizygotic twin females compared to their matched prediabetics. In addition, the dizygotic twin females had a significantly greater regression coefficient than the normal controls. This elevation in the insulin-glucose relationship of glucose-tolerant twins differs from the results of Colwell and Lein in the glucose-tolerant offspring of two diabetic parents and in mildly diabetic patients and also differs from earlier studies from this laboratory in two groups of glucose-tolerant offspring of two diabetic parents.[11,14,25] These earlier studies utilized highly selected groups of normal and prediabetic patients which were matched by weight and sex and were subdivided into decile age groups. There were insufficient monozygotic and dizygotic twins in the current study to warrant subdivision into decile age groups.

The comparisons that withstand age and weight influences appear to be the increased insulin response in the female MZ and DZ groups (later in the DZ) and the higher fatty acid level in the twins. Since the fasting serum insulin levels were generally identical in at least three of the four groups, one might speculate that there is a blunting of the antilipolytic effect of the basal insulin level in the twins.

There was a wide variation of serum growth hormone levels throughout the test in each of the four groups. However, it is noteworthy that a "paradoxic" type of growth hormone response was seen in both the twin and prediabetic groups compared to the respective normals. Some suggestion of at least a sluggish suppression of growth hormone was noted in the monozygotic twin females. These "paradoxic" responses appear to be a frequently found marker during the course of oral glucose tolerance tests in potential diabetic groups.[32-34]

The lack of rebound FFA noted in the DZ females might reflect the prolonged effect of a larger and larger insulin response as compared with other groups.

An important consideration in the comparison of prediabetic groups to twin groups has been the greater assurance that overt diabetes will develop in the twins rather than in the offspring. Recent studies from this laboratory have suggested that a large proportion of the offspring of two diabetic parents exhibit only sporadically abnormal glucose tolerance tests and the prevalence of overt diabetes is slightly less than 9 per cent.[6] Although the offspring group previously analyzed is not entirely comparable to the monozygotic twin group, it has been shown that 32 per cent of the monozygotic twins already exhibit overt diabetes. Thus, qualitative differences in the degree of expression of the diabetic trait between the two "potential" diabetic groups may have an important, although as yet unclear, bearing upon the serum insulin-blood glucose relationship. It is somewhat appealing to speculate that the prediabetic offspring groups represent a population with nearly compensated glucose homeostasis in whom there is a thrifty insulin economy. This group will show little if any progression beyond the chemical diabetic state. In contrast, the twins may either represent a group with a relatively inefficient insulin economy who are more likely progressing to an overt diabetic state with eventual partial to complete exhaustion of insulin secretory reserves or represent the remnants of a very high risk group who will not develop diabetes. These patterns of insulin efficiency and inefficiency may represent different genetic constellations or progressive stages in the development of a single complex genetic syndrome. On the other hand, it is conceivable that these twins may never develop diabetes as their twin mate acquired diabetes from some environmental influence.[35]

Two courses of action could supply further vital information that might elucidate these interpretations and speculations. There may be other metabolic-hormonal factors (viz. synalbumin insulin antagonists and proinsulin) which might clarify these concepts. Secondly, further longitudinal studies will be critical for a definitive picture of the natural history of diabetes in these groups, and these studies are in progress in this laboratory.

Another consideration is that the twin sample available for these comparisons, although systematically collected, is biased. The glucose-tolerant twin of one who has had diabetes for many years may be inherently different from those twin siblings who will become glucose intolerant soon after their diabetic twin siblings. The concept that diabetes can result from many causes, and all twin siblings of diabetic patients may not be considered comparable to each other, could be reason for the lack of clarity in the results of this study. A larger series of twins might be just as proportionately misrepresentative.

Since there is no recognized earlier or more specific predictor of diabetes than the glucose tolerance test, the solution to this problem will have to await better methodology or the identification of other hormones, substrates, or insulin antagonists.

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