Effect of Endogenous Insulin on Human Amino Acid Metabolism

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SUMMARY

This study describes the effect of endogenous insulin on free plasma amino acids. The secretion of insulin was provoked by means of a standard glucose tolerance test.

Four healthy males responded by a drop in the levels of all twenty-two plasma amino acids, which were measured. In four insulin-dependent diabetics the glucose tolerance experiment effected only slight decreases in nineteen of twenty-two amino acids. Ornithine and histidine decreased significantly, whereas phenylalanine increased.

The over-all result indicates that endogenous insulin reduces the levels of the majority, if not all, free plasma amino acids. DIABETES *15*:5-8, January, 1966.

The effect of insulin on the concentration of free plasma amino acids was described more than three decades ago.[1] It has been demonstrated repeatedly that the injection of exogenous insulin depresses not only the levels of sugar but also those of the free amino acids in the human blood.[1-3] Lotspeich studied the effect of injected exogenous insulin on the plasma concentration of ten "essential" amino acids in dogs and noted that insulin depressed the plasma levels of all of them. He noted a correlation between the drop of each amino acid with its proportional representation in skeletal muscle and suggested that insulin might promote the synthesis of protein from circulating amino acids.[4]

The absence of insulin was reported to be associated with elevated levels of free plasma amino acids. Insulin therapy reduced them to normal levels.[5] These and other reports, cited below, present considerable variations in the extent of the changes as well as in the methods employed. Since hypoglycemia due to administration of exogenous insulin also results in release of growth hormone, epinephrine and cortisol, it seemed worthwhile to study the stimulus provided by a glucose load. The standard glucose tolerance test provides a physiological stimulus for the release of endogenous insulin without provoking the release of other factors, which might influence the response to insulin. Insulin-dependent diabetics served as controls. These patients presumably will not respond to a glucose load with a release of endogenous insulin.

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MATERIALS AND METHODS

A standard three-hour glucose tolerance test, providing one single dose of 100 gm. of glucose, dissolved in 200 ml. of lemon-flavored water, was selected to provoke the release of endogenous insulin. Blood samples were obtained prior to the glucose load, after thirty minutes, and at one, two and three hours. Plasma sugars were measured by the method of Hoffman[6] as adapted to the AutoAnalyzer (Technicon). Four young or middle-aged healthy males and four patients with known insulin-dependent diabetes mellitus were subjected to the test. All subjects had fasted for at least twelve hours prior to the procedure. The healthy male employees were on a diet of their choosing; the diabetic patients were receiving 200 gm. or more of carbohydrates in their daily diet. The diabetic individuals had received their usual dose of insulin the day prior to the test, but no insulin was administered on the day of the experiment, until after completion of the test. None of these patients was ketotic during the study. A radioimmunoassay method was used for the estimation of plasma insulin.[7]

Free amino acids of the blood plasma were determined by ion exchange chromatography on columns of Amberlite resins, using an automatic amino acid analyzer (Spinco, Model 120B), as described by Spackman, Stein and Moore.[8] The free plasma amino acids are reported as μmoles per 100 ml. of plasma. Heparinized blood specimens were obtained and deproteinized by precipitation with picric acid.[9]

RESULTS

Plasma insulin levels were assayed in two of the normal individuals and indicate that the return of the blood sugar toward normal levels closely follows the

maximal concentration of plasma insulin.

All of the measured amino acids decreased, as can be seen in figure 1 which shows the mean decrease of the concentration of twenty-two amino acids after the glucose load, as compared to the fasting state. In the healthy individuals, the maximal drop occurred after two hours.

Table 1 represents a statistical evaluation of the effect of the glucose tolerance test on the free amino acids of the blood plasma in normal individuals. Blood sugar levels, plasma insulin assays and measurements of free plasma amino acids were determined prior to glucose load and in all plasma samples, which were obtained thereafter. Changes of the free plasma amino acids followed a consistent pattern throughout the experiment. The pertinent changes could be demonstrated best by comparison of the fasting state with the time of return of blood sugars to normal or near-normal levels (two hours).

Based on a comparison of paired variates, fourteen of the twenty-two amino acids dropped significantly. The changes in the remaining eight did not reach statistical significance; however, a larger sample might well have provided a basis for their mathematical significance.

Table 2 relates the response of insulin-dependent diabetics to the glucose tolerance test. It compares the plasma amino acids of the fasting individual with those taken two hours after the glucose load. Plasma insulin assays were not obtained in this group of patients, since they had been treated for some time with insulin, and the presence of insulin-binding antibody in their sera interfered with the insulin immunoassay as usually performed. The plasma amino acid concentrations have been found to be higher in diabetics than in normal individuals.[5] In our group of diabetics they were essen-

TABLE 1

The changes of free plasma amino acids in four normal males during a glucose tolerance test. The fasting state is compared to the period two hours after the glucose load. The values are given as micromoles per 100 ml. of plasma.

Amino acid	Fasting mean	Mean after return of blood sugar to normal*	As paired variates T	P
Taurine	7.14	5.16	1.28	<0.3
Aspart. acid	0.73	0.35	2.41	0.1
Threonine	14.32	10.68	3.26	0.05
Serine	10.88	8.12	3.74	0.02
Asparagine/glutamine	36.75	24.82	1.60	0.20
Proline	18.52	14.88	3.74	0.02
Glutamic acid	15.80	8.00	1.49	0.20
Citrulline	3.42	1.31	5.40	0.01
Glycine	25.49	22.16	2.51	0.10
Alanine	39.59	36.94	1.70	0.20
α-amino-n-butyric acid	3.00	1.82	3.27	0.05
Valine	28.61	18.31	4.63	0.01
Cystine/2	24.12	17.13	2.08	0.20
Methionine	2.31	1.27	2.84	0.05
Isoleucine	8.46	3.62	5.50	0.01
Leucine	14.35	6.96	5.14	0.01
Tyrosine	6.22	3.85	4.18	0.02
Phenylalanine	5.51	3.80	3.41	0.02
Ornithine	5.39	4.53	1.71	0.20
Lysine	17.89	15.00	3.43	0.05
Histidine	7.78	6.42	2.41	0.10
Arginine	7.43	5.23	3.06	0.05

*All free plasma amino acids decreased, fourteen to a significant degree.

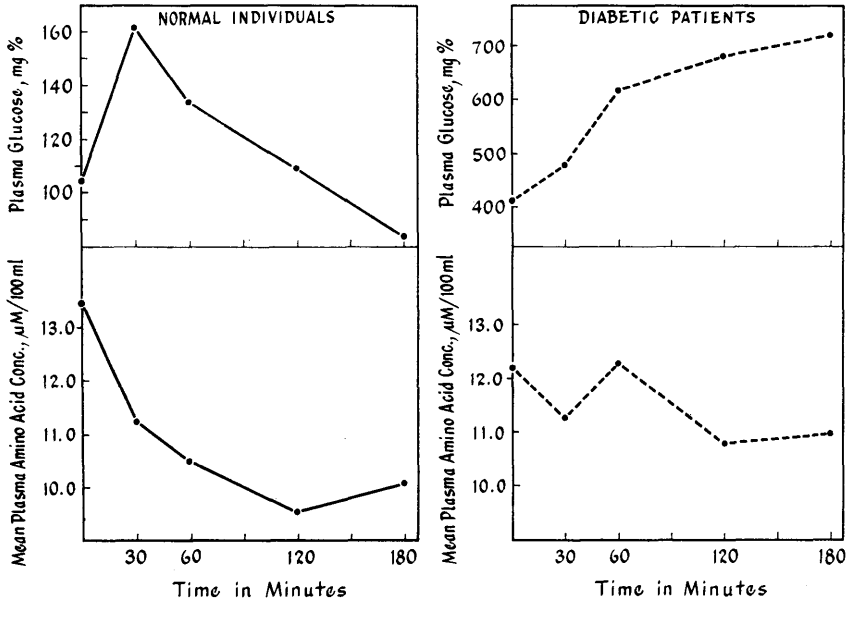

FIG. 1. The upper compartments of the figure show a comparison of plasma glucose levels between normal males and insulin-dependent diabetic patients during a standard glucose tolerance test. Note the difference in scale between the two groups. The lower compartments show a comparison of the mean plasma amino acids of the two groups. There was a consistent and progressive drop in all free amino acids in the groups of normal males from the time of the glucose load to the return of blood sugar levels to normal (two hours). The group of diabetics showed a small but inconsistent drop. Table 2 reveals that this decrease was not significant.

TABLE 2

Free plasma amino acids in four male insulin-dependent diabetics during a glucose tolerance test. The fasting state is compared to the period two hours after the glucose load. The values are given as micromoles per 100 ml. of plasma.

Amino acid	Fasting mean amino acid levels	Mean amino acid levels after two hours*	As paired variates T	P
Taurine	5.63	5.53	0.69	<0.60
Aspartic acid	0.50	0.41	1.48	0.30
Threonine	11.10	10.19	1.14	0.30
Serine	10.35	9.83	0.64	0.60
Asparagine/glutamine	34.56	20.21	1.97	0.20
Proline	16.53	14.32	2.56	0.10
Glutamic acid	12.87	11.82	0.34	0.80
Citrulline	3.02	2.34	1.20	0.20
Glycine	23.57	21.52	2.26	0.10
Alanine	29.20	29.42	0.35	0.80
α-amino-n-butyric acid	2.94	2.85	2.69	0.10
Valine	27.81	29.21	1.35	0.30
Cystine/2	8.06	5.76	1.02	0.40
Methionine	1.95	1.64	1.62	0.10
Isoleucine	10.77	11.87	0.69	0.60
Leucine	18.53	18.72	0.16	0.80
Tyrosine	5.39	5.28	0.50	0.70
Phenylalanine	5.15	5.38	2.84	0.05
Ornithine	7.22	6.03	2.73	0.05
Lysine	21.35	17.62	0.53	0.70
Histidine	6.23	4.36	2.80	0.05
Arginine	3.59	1.21	1.16	0.40

*Only two amino acids (ornithine and histidine) decreased significantly. Five actually increased, one (phenylalanine) to a significant degree.

tially the same as in normal controls. However, the diabetic subjects in this study were under diabetic control and had received insulin twenty-four hours prior to the test. It is not likely that they were totally insulin depleted. Only two amino acids, ornithine and histidine, decreased significantly after two hours, whereas phenylalanine actually increased to a significant degree.

DISCUSSION

The preceding data did not indicate a material difference between the free plasma amino acids of normal persons and diabetics in the fasting state. Glucose tolerance experiments raised the blood sugar levels in diabetics as well as in normal individuals, but the increase of blood sugar per se had little effect on the levels of free plasma amino acids of the insulin-dependent diabetic group. A slight drop in the diabetic group may have been due to an expansion of the extracellular fluid compartment, concomitant with high blood sugar levels. Perhaps the rather impressive hyperglycemia also was associated with a diminished reabsorption of amino acids by the renal tubules.[10] The insulin response of four normal individuals effected a decrease of all measured amino acids. This decrease was statistically significant in fourteen of twenty-two amino acids. Three of the fourteen (citrulline, tyrosine and arginine) dropped below the lower limits of the normal range. Had a larger number of individuals been studied, the decrease in the remaining eight might also have reached statistical significance.

Decreases of free plasma amino acids following exogenous or endogenous insulin were also observed by Lotspeich,[4] Luck,[1] Farr,[2] Harris and Harris,[3] Crofford and associates,[11] and Floyd and his group.[12] All of these investigators found decreases of free plasma amino acids. The reports vary in regard to the extent of change and amino acids involved. These variables may be an expression of the use of different experimental methods as much as of the relatively small experimental samples. Although not all studies documented decreases of the same number of amino acids, all of them were contained in our group of fourteen amino acids, which showed the most significant decrease as consequence of endogenous insulin effect. Hernandez and Coulson[13] noted a similar effect on amino acids, but exogenous insulin did not seem to affect valine, leucine and isoleucine. This is in contrast to the findings of most other investigators. It must be pointed out that these experiments were conducted in alligators and rats. At this juncture, it would be reasonable to overlook the minor disagreements between the previously mentioned studies, and to emphasize the outstanding fact that each study noted a drop of some of the free plasma amino acids in response to the insulin effect. The diabetics in this study, who were incapable of an endogenous insulin response, presented a strikingly different picture. Nineteen free plasma amino acids remained essentially unchanged throughout the three-hour experiment. Table 2 compares the fasting levels with those two hours after ingestion of the glucose load. Ornithine and histidine decreased to a degree of some significance, whereas phenylalanine increased significantly. Previous experimental data on cortisol administration[14] indicate that cortisol exerts an effect on amino-acid metabolism which is opposite to that of insulin. This agrees with the observations of Eichhorn and associates,[15] who found that administration of cortisol to rats reduced the insulin effect on amino acid incorporation into cells.

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The Fine Structure of the Early Atherosclerotic Lesion

Since the first induction of experimental atherosclerosis in rabbits, the relevance of these lesions to human atherosclerosis has been challenged. For example, the lesions induced in the rabbit aorta show the greatest intensity centripetally, they are more uniform, and are predominantly made up of foam cells, in contrast to most human lesions (G. L. Duff, Arch. Path. 20:81, 259, 1935; Nutrition Reviews 20:207, 1962). On light microscopy, they most nearly resemble the "fatty streak" lesions seen in the aortas of children. Because the latter seem to occur with greater frequency in infancy than later on in childhood they are thought to be reversible and, the argument goes further, probably have little to do with the atherosclerotic lesions seen in later life (W. Ophuls, in *Arteriosclerosis,* E. V. Cowdry, Editor, MacMillan, New York, 1933). Other authors have argued that, while the fatty streak is reversible it may at times progress to a point of no return when the lipid containing cells die, discharge amorphous lipid material into the extracellular space, and provoke a fibrous tissue reaction in the subintima (H. C. McGill and J. C. Geer, in *Evolution of the Atherosclerotic Plaque,* R. J. Jones, Editor, p. 65, University of Chicago Press, Chicago, 1964).

With application of the electron microscope, new interest has centered on identification of the earliest changes that take place at the cellular level in the development of incipient atherosclerosis. Out of such observations has come one view that the earliest change in the human subintima is the development of lipid inclusions in cells which have all the cytological characteristics (muscle fibrils, dense bodies, and a normal complement of organelles) of smooth muscle cells. Transitional forms are then seen between this earliest of involved cells and the foam cell, which is packed with lipid-containing vacuoles, so that certain pathologists believe that the foam cells of the human lesion probably originate from smooth muscle cells (McGill and Geer, loc. cit.).

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