

# PLASMA DEHYDROASCORBIC ACID LEVELS IN IRANIAN SUBJECTS WITH DIABETES MELLITUS

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## ABSTRACT

Several reports concerning high plasma dehydroascorbic acid (DHAsA) levels in diabetics have been published and from these reports, suggestions that monitoring of DHAsA levels in those persons with a predisposition to diabetes mellitus would be of value. However, conflicting reports have also appeared which do not confirm high levels of DHAsA in diabetic subjects when compared to controls. Because of these conflicting results, this investigation using Iranian diabetic subjects was undertaken to ascertain whether or not periodic monitoring of DHAsA levels would be of value as an indicator of prediabetic conditions. Our results do not confirm high levels of DHAsA in diabetics but because of the many theories concerning the mechanism of action and the metabolism of DHAsA, previous findings cannot be disregarded.

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## INTRODUCTION

L-ascorbic acid, commonly known as vitamin C, is an internal ester of hexonic acid. The participation of L-ascorbic acid in collagen biosynthesis in the hydroxylation of peptide-bound proline to hydroxyproline and lysine to hydroxylysine by prolyl and lysyl hydroxylase is its most important biochemical function.

The levels of ascorbic acid (AsA) and its metabolite dehydroascorbic acid (DHAsA) have been the topic of much discussion in recent years. The double bond between carbons two and three in the five-membered lactone ring in AsA is readily oxidized and it is this oxidation at the enediol linkage which produces DHAsA. Reports indicating low levels of plasma AsA in human subjects have led to the concern that some diabetics may be in a chronic state of vitamin C deficiency.<sup>1-4</sup> Therefore it has been recommended that diabetic patients should be given AsA supplementation to maintain nutritional status. In contrast to this low AsA level reported in diabetics, high concentra-

tions of plasma DHAsA levels in diabetics in comparison with control groups have also been reported.

Chatterjee, et.al.<sup>5</sup> postulated that a metabolic defect leading to the accumulation of DHAsA in the blood may be a cause of diabetes mellitus and insulin or antidiabetic drugs do not correct the metabolic defect, although it is well known that such treatment temporarily lowers the blood glucose level.

In contrast to the studies previously mentioned, an investigation with British diabetic subjects did not confirm high DHAsA levels compared to controls.<sup>6</sup>

It has been suggested that in persons having a hereditary predisposition to diabetes, DHAsA levels may be used as a marker for early detection of the disease, leading to easier management and thereby possibly preventing mild to frank diabetes.<sup>3</sup> However, because of the apparent contradictions of previous studies concerning blood DHAsA levels in diabetic subjects, this investigation was undertaken in an effort to determine if periodic analysis of blood DHAsA would indeed be of value for the detection of prediabetic conditions.

## Plasma Dehydroascorbic Acid In Diabetes Mellitus

Table I. General Specifications of Diabetic Patients and Controls

	# of Subjects	Age Range (yrs)	Sex	Blood Glucose Level (mg/100 ml)	Treatment
Type I <sup>a</sup>	11	21-64	M-5 F-6	91-380	Insulin Oral <sup>c</sup>
Type II <sup>a</sup>	31	26-80	M-13	95-315	Insulin Oral <sup>c</sup>
Controls <sup>b</sup>	17	18-60	M-7 F-10	55-15	

<sup>a</sup>Confirmed diabetic patients from The Center for Endocrinology and Metabolic Diseases in Tehran. Patients received no vitamin therapy and had no history of other complications or co-existing disease of other systems

<sup>b</sup>No Family history of diabetes, were receiving no vitamin therapy and had no history of organic disease.

<sup>c</sup>Oral therapy includes chlorpropamide and sulfonylureas.

### PATIENTS AND METHODS

42 male and female subjects with confirmed diabetes mellitus types I and II were chosen from the Center for Endocrinology and Metabolic Diseases in Tehran. All patients were receiving either insulin or oral therapy which included chlorpropamide and sulfonylureas. None of the patients were receiving vitamin C or any other drug therapy and had no history of other complications or coexisting disease of other systems. A 24-hour history of food intake was recorded before blood samples were taken. The 17 control subjects had no family history of diabetes mellitus and were receiving no vitamin C supplementation.

Plasma ascorbic acid for both patients and controls was measured using the dye 2,6-dichlorophenolindophenol.<sup>7</sup> Total vitamin C was determined by the 2,4-dinitrophenyl-hydrazine method after oxidation of ascorbic acid by copper to form dehydroascorbic acid.<sup>8</sup> Dehydroascorbic acid was calculated as total vitamin C minus ascorbic acid. The amount of diketogulonic acid was considered insignificant and, as in other reports, was disregarded. Fasting blood glucose levels were measured in all samples spectrophotometrically with o-toluidine.

### RESULTS

Table I gives the general description of diabetic patients and controls including the range of blood glucose levels. Table II shows the concentration of vitamin C components in diabetic and control subjects. Here the percent of DHAsA of total vitamin C is given. Total AsA in both male and female diabetics is comparatively higher than male and female controls. The percent of DHAsA in female diabetics is 1.75 times higher than in male diabetics and 3.5 times higher than non-diabetic female controls. The AsA levels of diabetic subjects are slightly higher than control levels.

Table III lists male and female diabetic subjects of different ages in order to show individual values of vitamin C components and blood glucose levels.

### DISCUSSION

The results presented in this communication are compared with three other reports in Table IV.<sup>2,3,6</sup> In Studies 1 and 2 the percent of DHAsA is high in diabetic subjects, 59% and 63%, respectively, and both report no detectable DHAsA in controls. Compared to Studies 1 and 2, Study 3 reports a low percentage (13%) of DHAsA for diabetic patients and a higher percentage (13%) of DHAsA for control subjects. In the present study the percent of DHAsA in diabetic patients was found to be only slightly higher (18%) than Study 3 but considerably lower than Studies 1 and 2. The percent of DHAsA in controls is 7%.

In our opinion these variations in results cannot be attributed to factors such as differences in type or severity of diabetes or blood glucose levels. Also, since basically the same analytical techniques were employed in all studies, it is doubtful that this factor could

Table II. Plasma Levels of Vitamin C Components in Diabetic Patients and Controls

	# of Subjects	Concentration of Vitamin C Components <sup>a</sup> (mg/100 ml ± S.D.)			% of DHAsA of Total
		AsA	DHAsA	Total	
Diabetics					
Male	18	0.89±.45	0.12±.12	1.00±.47	12%
Female	24	0.89±.44	0.25±.24	1.17±.48	21%
Controls					
Male	7	0.82±.32	0.07±.07	0.87±.35	8%
Female	10	0.76±.19	0.05±.04	0.81±.18	6%

<sup>a</sup>Mean values

Table III. Plasma Ascorbic Acid and Dehydroascorbic Acid Levels in Diabetic Patients of Different Ages

Subject	Age (yrs)	Sex	concentration of Vitamin C Components (mg/100ml)		Blood Glucose Level (mg/100 ml)
			AsA	DHAsA	
1	21	M	0.75	0.45	380
2	26	F	0.57	0.03	224
3	35	M	0.85	0.05	96
4	38	F	0.58	0.08	144
5	40	F	1.12	0.13	147
6	47	F	0.59	0.16	150
7	50	F	1.65	0.05	195
8	54	M	0.31	0.09	140
9	64	F	1.97	0.33	305
10	80	M	0.67	0.03	115

account for these differences.

Even though our results did not confirm the presence of high levels of plasma DHAsA in diabetic subjects, the theoretical aspects of this molecule in the pathogenesis of diabetes mellitus and the mechanism of action of DHAsA must be taken into consideration.

Because DHAsA and alloxan have similar chemical structures, it has been suggested that high doses of DHAsA may be an alloxan-type diabetogenic. In an investigation by Patterson,<sup>9</sup> large doses of DHAsA injected into experimental animals caused degranulation of the  $\beta$  cells of the islets of Langerhans, accompanied by hyperglycemia. However, in contrast, Domke and Weis,<sup>10</sup> using purified crystalline DHAsA in animal experiments did not confirm this diabetogenic effect. They have suggested that impurities in the injection of DHAsA may have been responsible for the diabetogenic effect observed by Patterson.

One suggestion concerning the role of DHAsA in the production of hyperglycemia is that DHAsA reacts with sulphur amino acids which are essential for the synthesis of insulin.<sup>5</sup> Another theory which has been advanced is that DHAsA mimics the destructive effect of alloxan on the permeability of the islet tissue.<sup>11</sup>

An additional hypothesis states that in diabetes, the accumulation of DHAsA may be due to an impairment of the sequential reaction which takes place in erythrocytes.<sup>11</sup> In normal blood, DHAsA is reduced back to AsA in erythrocytes. The reduction of DHAsA is dependent on the presence of reduced glutathion and activation of glutathion reductase and glucose-6-P-dehydrogenase. The level of reduced glutathion in erythrocytes is regulated by NADPH-dependent glutathion reductase, while the NADPH availability is controlled by the activity of glucose-6-P-dehydrogenase. However, the level of reduced glutathion and the activity of glucose-6-P-dehydrogenase of diabetic and normal subjects have been similar in some studies and different in others. This indicates that some unknown factors may possibly play a part in the

accumulation of DHAsA in the red cells of diabetics.

In the pathogenesis of diabetes there is speculation that hyperglycemia might compromise the transport of AsA into the cell, with glucose and DHAsA showing the same transport mechanism. Due to structural similarities between AsA and glucose, it was observed that the physiological levels of D-glucose impair the transport of DHAsA in human erythrocytes.<sup>12,13</sup> A similar observation was also described in human neutrophils and fibroblasts in that transport of DHAsA is mediated by the common glucose transport system.<sup>14</sup>

Our findings show the proportion of DHAsA to be low in both diabetic subjects and controls. Even though our results did not confirm higher levels of plasma

Table IV. Comparative Value of Vitamin C Components in Plasma of Various Studies in Diabetic Patients and Controls

Study	# of Subjects <sup>c</sup>	Concentration of Vitamin C Components (mg/100 ml) <sup>f</sup>			% of DHAsA of total
		AsA	DHAsA	Total	
1 <sup>a</sup>					
Diabetics	37	0.15	0.22	0.37	59%
Controls	37	0.38	0	0.38	0
2 <sup>b</sup>					
Diabetics	6	0.09	0.15	0.24	63%
Controls	6	0.31	0	0.31	0
3 <sup>c</sup>					
Diabetics	17	0.55	0.09	0.65	13%
Controls	12	0.81	0.12	0.95	13%
4 <sup>d</sup>					
Diabetics	42	0.89	0.19	1.08	18%
Controls	17	0.79	0.06	0.85	7%

<sup>a</sup>Chatterjee and Banerjee, 1979

<sup>b</sup>Banerjee, 1982

<sup>c</sup>Newell, Habibzadeh, Bishop and Schorah, 1984

<sup>d</sup>This study

<sup>e</sup>Includes both male and female subjects

<sup>f</sup>Mean values

## Plasma Dehydroascorbic Acid In Diabetes Mellitus

DHAsA in diabetics, previous findings cannot be disregarded and the possibility for use of DHAsA levels as an indicator of prediabetic conditions should still be considered. Therefore, to explain these dissimilarities, we believe further investigation is necessary on the exact nature of dehydroascorbic acid and its metabolism in which variations may exist possibly because of differences in ethnic backgrounds.

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