Hyperglycemia-induced Intracellular Depletion of Ascorbic Acid in Human Mononuclear Leukocytes

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SUMMARY

It has recently been reported that glucose and its analogues inhibit in vitro ascorbic acid transport across the cell membrane of polymorphonuclear leukocytes and fibroblasts. We have studied the effect of in vivo hyperglycemia on the intracellular ascorbic acid level of mononuclear leukocytes in normal and diabetic human subjects. Administration of an intravenous glucose load resulted in a prompt decrease of mononuclear leukocyte ascorbic acid level in the normal subjects. The rate of its decline correlated closely with the rate of change of plasma glucose. Among the NIDDM subjects in the fasting state, the plasma glucose was high and the leukocyte ascorbic acid level was low when compared with that of the normal subjects. The decrease in the leukocyte ascorbic acid level during disposition of the i.v. glucose load was not statistically significant in the diabetics. The hyperglycemia-induced intracellular depletion of ascorbic acid could be clinically important and requires further evaluation. DIABETES *32*:1078–1081, November 1983.

Ascorbic acid (AA) levels in various tissues of diabetic animals are decreased.[1] In diabetic patients, alterations in plasma levels and turnover of AA have been reported but not explained.[2,3] Recently, glucose has been reported to competitively inhibit in vitro AA transport across the membrane of human lymphocytes.[4] Others have described similar effects of glucose analogues on the in vitro uptake of dehydroascorbic acid by human polymorphonuclear leukocytes and fibroblasts.[5] In this study we have evaluated the effect of hyperglycemia in vivo on the intracellular AA concentration of human mononuclear leukocytes (MNL) in both normal and diabetic subjects.

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MATERIALS AND METHODS

HUMAN SUBJECTS

All subjects provided signed informed consent. Nine healthy male volunteers (aged 20–84 yr) were studied after 12-h overnight fast. Five non-insulin-dependent diabetics (aged 42–76 yr) with fasting plasma glucose ranging from 145 to 309 mg/dl were studied after 12-h overnight fast. Two were diet controlled and three were treated with oral hypoglycemic agents. All subjects were instructed to withhold all their medications on the morning of the study. Otherwise no changes were made in their usual dietary habits or medications.

EXPERIMENTAL PROTOCOL

After a baseline blood sample was obtained from the opposite hand vein, 20 g glucose in 50% solution was given as a bolus through an indwelling butterfly needle in the antecubital vein. Subsequent blood samples were drawn at 3, 6, 10, 14, 22, 30, and 60 min from a vein in the opposite hand kept in a heating box maintained at 70°C to arterialize the venous blood.[6] The blood was anticoagulated with EDTA and stored in ice until separated into plasma and cellular fractions.

PLASMA AND MNL PREPARATIONS

Plasma and cells were separated by centrifuging 4–5 ml of EDTA-anticoagulated blood. Part of the plasma was frozen at −20°C for subsequent determinations of insulin and glucose. The remainder of each plasma specimen and the cellular fraction was prepared on the day of sample collection for AA determination as previously described.[7] The MNL fraction obtained is essentially pure, with <5 RBC/100 MNL contamination. Platelets are essentially absent.

CHEMICAL ANALYSES

Glucose. Plasma glucose was measured by the ferricyanide method using a Technicon SMA 12-60 autoanalyzer.

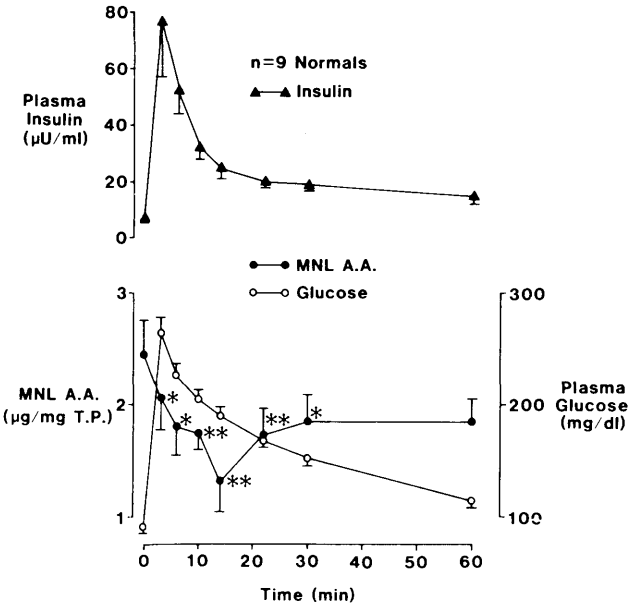

FIGURE 1. The effect of 20 g i.v. glucose pulse on MNL AA, plasma glucose, and insulin levels (mean ± SEM) in nine normals (*P < 0.05, **P < 0.01 AA before versus after glucose pulse).

Insulin. Plasma insulin was determined by a modified double antibody method.[8]

Protein. Total protein (TP) content of the cell protein precipitate was determined by the biuret procedure.[9]

Ascorbic acid. Both plasma (mg/dl) and MNL (μg/mg TP) AA levels were determined by HPLC.[7] The intraassay coefficient of variation (CV) of this method is 2.4%. The interassay CV is 4.3%.

RESULTS

Normal subjects. The basal MNL AA in nine normal subjects ranged from 1.42 to 4.50 μg/mg TP with a mean ± SEM of 2.44 ± 0.33 μg/mg TP. After 20 g glucose i.v. pulse (Figure 1) as the plasma glucose rose from 90 mg/dl to 263 mg/dl in 3 min with a corresponding insulin peak of 77 ± 20 μU/ml, the mean MNL AA level dropped significantly to 2.06 ± 0.29 μg/mg TP ($P = 0.02$, paired Student's t test). The intracellular AA level continued to drop and reached a nadir of 1.33 ± 0.27 (51% of basal, $P = 0.001$) at 14 min, representing a decrement of 1.21 ± 0.19 μg/mg TP. At this time the plasma glucose level had decreased to 191 mg/dl. After this point the intracellular AA level gradually recovered, but remained at 78% of baseline at 60 min, which was not statistically significant at the $P < 0.05$ level (actual $P = 0.065$).

The expected acute insulin response was observed (Figure 1) in the normal subjects. The rate of AA change between 0 and 14 min correlated positively with the rate of glucose disappearance as shown in Figure 2 ($r = 0.675$, $P < 0.05$).

No significant changes in plasma AA concentration were observed during the intravenous glucose tolerance test (IVGTT) in four normal subjects, including the interval from baseline (1.25 ± 0.17, mean ± SEM) to 14 min (1.22 ± 0.18 mg/dl) (P = NS), during which prominent changes in plasma glucose and insulin, as well as intracellular AA content, took place.

Diabetic subjects. The basal MNL AA levels in five diabetic subjects ranged from 0.95 to 2.2 with mean ± SEM of 1.44 ± 0.22 μg/mg TP, significantly lower than in the nine normal subjects ($t = 2.59$, $P < 0.05$). The mean fasting plasma glucose (215 ± 29 mg/dl) was much higher in the diabetics (Figure 3). During the IVGTT, the mean plasma glucose levels increased further to a peak of 359 ± 34 mg/dl at 3 min and remained above 250 mg/dl during the 60-min study period. The mean MNL AA levels during IVGTT showed a slight trend of decrease and reached a nadir at 60 min (0.97 ± 0.13 μg/mg TP, paired $t = 2.23$, $P > 0.05$). The decrement in mean MNL ascorbate at 60 min after glucose infusion did not reach statistical significance and the change from baseline represented only 0.47 ± 0.21 μg/mg TP. In each of the five diabetics, however, the MNL AA level at 60 min fell below the baseline level (individual data not shown). The insulin responses were markedly blunted in the diabetic subjects (Figure 3).

The basal plasma AA level in four diabetic subjects ranged from 0.12 to 0.86 mg/dl, with mean ± SEM of 0.46 ± 0.15 mg/dl, which was lower than that of the four normals ($P < 0.01$). As in the normal subjects, no significant change occurred during the IVGTT.

DISCUSSION

Although AA is a widely distributed intracellular component of animal tissues,[10] its physiologic significance is incompletely understood. Scurvy is characterized by abnormal collagen formation, associated with vascular fragility and faulty wound healing. The mechanism linking scorbutic diet with impaired collagen synthesis is not totally clear, although prolyl hydrolase, the enzyme that catalyzes the conversion of proline to hydroxyproline during collagen synthesis, requires AA.[11] The role of AA in other tissues remains somewhat speculative, although its ability to act as a strong reducing substance suggests it may function to maintain the redox potential at certain intracellular loci, and perhaps serves to protect subcellular components from potential injury from free radicals. In 1973, Mann proposed a possible role for

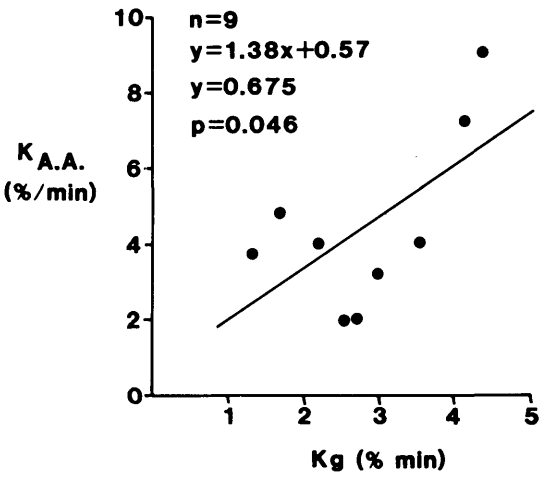

FIGURE 2. The correlation between rate of changes in MNL AA level (K_{AA}) between 0 and 14 min and plasma glucose levels (Kg) between 3 and 14 min in nine normals.

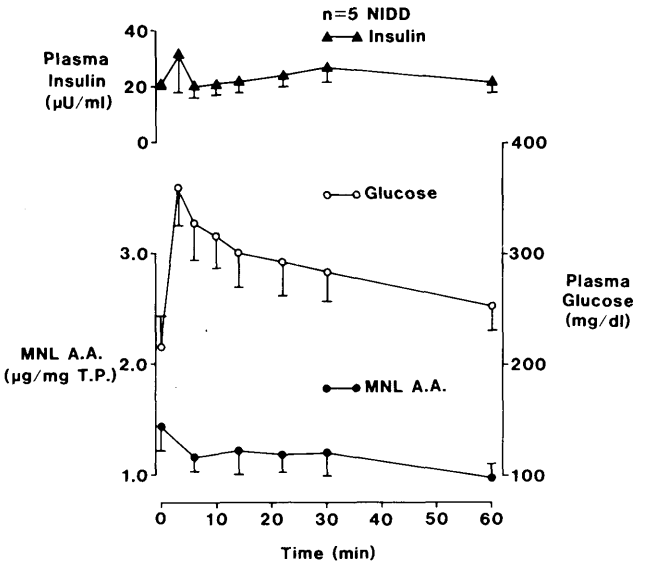

FIGURE 3. The effect of 20 g i.v. glucose pulse on MNL AA, plasma glucose, and insulin (mean ± SEM) levels in five non-insulin-dependent diabetics (NIDD).

AA in the pathogenesis of diabetic angiopathy. He speculated that hyperglycemia might compromise the transport of ascorbate into cells, with glucose and dehydroascorbate sharing the same transport mechanism.[12] Noting the structural similarities between vitamin C and glucose, Mann subsequently observed that physiologic levels of D-glucose impair the transport of DHA in human erythrocytes.[13]

Recently Bigley et al.[5] reported that the nonmetabolized glucose analogues 3-O-methylglucose and 2-deoxyglucose competitively inhibit the in vitro uptake by human neutrophils of dehydroascorbic acid (DHA), the nonpolar oxidized form of ascorbic acid. DHA uptake by cultured human skin fibroblasts was also competitively inhibited by 2-deoxyglucose. Previous studies by these authors demonstrated that human neutrophils and cultured fibroblasts accumulate AA by prompt intracellular reduction of DHA to ascorbate after uptake from the incubation media.[14] Furthermore, the uptake of both DHA and 2-deoxyglucose inhibitable by cytochalasin B, which apparently blocks the transmembrane carrier induced by insulin and responsible for facilitated glucose uptake,[15] was enhanced in cultured fibroblasts after exposure to insulin.[5] These observations led the authors to conclude that facilitated transport of DHA is mediated by the common glucose transport system in human neutrophils and fibroblasts. Similarly, the AA uptake by human MNL in vitro was found recently by Davis et al. to be competitively inhibited by glucose at a concentration of 250 mg/dl.[4]

Our study demonstrates not only that depletion of AA occurs in vivo in human MNL during the disposition of an intravenously administered glucose load, but also that this is a prompt effect. The basal MNL AA levels in NIDDM were significantly lower than that of normal subjects. Perhaps of more importance, among the diabetics a markedly diminished depletion of intracellular ascorbate was observed during the IVGTT and no recovery of intracellular AA was observed within 1 h. In contrast among the normal subjects, the nadir of MNL AA was reached at 14 min and was highly statistically significant ($P < 0.001$). At 60 min the mean intracellular AA had recovered toward the baseline level, and was, in fact, not statistically different from the initial level.

Although the mean MNL AA level did not change significantly during the IVGTT in the diabetic group, the nadir of mean MNL AA among the normals was comparable to this unchanged level; similarly the maximal mean plasma glucose achieved in the nondiabetics (263 mg/dl) is comparable to the elevated mean glucose levels throughout the IVGTT among the diabetics. The mean fasting plasma glucose level was 215 mg/dl in the diabetic group, with all subsequent mean glucose levels after the glucose pulse having exceeded 250 mg/dl. These observations are compatible with the possibility that adequate repletion or maintenance of optimal intracellular AA levels in human MNL may not be achievable with ambient plasma glucose levels above 215 mg/dl, or perhaps some undetermined lower level. Evans et al.[16] reported that the intracellular level of AA is highest in MNL among various human blood cell types, representing a concentration factor above plasma levels of 3800-fold. Granulocytes and platelets concentrated AA to a lesser extent, with erythrocyte intracellular AA concentration found to be identical to plasma levels. Our finding of decreased AA levels in human MNL in vivo in the presence of high glucose implies that the ability to maintain this concentration gradient may be significantly impaired in hyperglycemic states.

The data of Bigley et al.[5] and Davis et al.[4] suggest that our observation of in vivo depletion of the MNL AA level during IVGTT is most likely secondary to competitive inhibition of DHA or AA transport by glucose. The correlation we observed between glucose disappearance (3–14 min) and change of intracellular AA level (Figure 2) would support the notion that plasma glucose is an important factor in inducing intracellular depletion of AA. In their in vitro studies, Bigley et al. showed a proportional enhancement of cytochalasin B-inhibitable fibroblast uptake of DHA and 2-deoxyglucose after preincubation with insulin, but no apparent insulin effect on neutrophil uptake.[5] Though we consider it unlikely, we cannot exclude an effect of insulin or rule out the possibility of increased MNL consumption or increased exit of AA induced by glucose in vivo. It is not known whether these alterations may affect the immune functions of these cells.

ACKNOWLEDGMENT

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