Medical Hypotheses (2004) **62**, 275–279

ELSEVIER

medical hypotheses

http://intl.elsevierhealth.com/journals/mehy

Ascorbic acid, glycation, glycohemoglobin and aging

Cheryl A. Krone[a,*], John T.A. Ely[b]

[a] *Applied Research Institute, P.O. Box 1969, Palmerston North, New Zealand*
[b] *Radiation Studies, Box 351310, University of Washington, Seattle, WA, USA*

Received 16 June 2003; accepted 19 October 2003

Summary The glycation of proteins alters both their structure and function. These changes have been linked to diabetic disorders and aging. The glycation of hemoglobin is also used as a diagnostic tool; the extent of glycation being a reflection of blood glucose averaged over a two to three month period. Accurate measures of average blood sugar (e.g., glycohemoglobin (GHb)) are important in clinical management of diabetes, pregnancy, cancer, etc. Ascorbic acid (AA) can react with proteins, including hemoglobin, and possibly interfere with GHb measurements. Past reports on the impact of AA on in vivo glycation have been equivocal. We studied GHb in subjects supplementing up to 20 g AA daily and found that for each 30 μmol/L increase in plasma AA, GHb was reduced by ~0.1. These results suggest that high AA intake can depress glycation, reduce GHb and lead to a clinically relevant underestimation of average blood sugar. Because AA is the most commonly consumed dietary supplement, awareness of an AA-associated bias in GHb is imperative. Of even broader significance is the possibility of AA-mediated inhibition of glycation in all proteins and the implications for aging. Moreover, AA could contribute through several other mechanisms to slowing of human aging (e.g., antioxidant properties, acceleration of pentose phosphate pathway, replacement of structural proteins).

Protein glycation and ascorbic acid

In 1987, Cerami et al. [1] summarized the interaction of glucose with protein and its association with human aging and diabetic disorders. Much additional research in the intervening years has expanded our knowledge of glycation and its effects on structure and function of various tissues (skeletal muscle, ocular lens, skin, nerves, arteries, etc.) and on individual proteins (e.g., collagen, elastin, crystallin, myosin, etc.) e.g., [2–5]. The changes seen in glycosylated structural proteins (e.g., increased collagen stiffness) are similar to changes observed in aging [1,6].

These non-enzymatic reactions of glucose with proteins have been studied since at least the 1930s by food chemists and in 1953 Hodge [7] proposed the general scheme of these "browning" reactions. Briefly, a sugar aldehyde or ketone combines with amino groups on proteins leading to a Shiff base and then rearrangement to a more stable Amadori product. Additional dehydration and rearrangements produce advance glycation endproducts (AGE) which are often brown in color and thought to be involved with crosslinking and other changes that cause protein dysfunction [2,6].

A similar reaction between amino acids or proteins and ascorbic acid (AA) that produces brown pigments has also long been studied in foods [8]. More recently, in vitro experiments primarily studying the effect of AA on processes involved in cataract formation have suggested a possible pro-oxidant property of AA due to ascorbylation of

* Corresponding author. Tel.: +64-6-353-1010; fax: +64-6-353-1012.
E-mail address: cakrone@u.washington.edu (C.A. Krone).

0306-9877/$ - see front matter
doi:10.1016/S0306-9877(03)00313-X

proteins. Yellow-brown colored materials and AGE have been identified and quantitated in these in vitro systems and compared to similar compounds occurring in human cataracts [3]. However, the preponderance of evidence currently available suggests that AA in vivo acts as an antioxidant toward lipids, DNA and proteins [9]. In fact, some animal and human studies have measured decreased protein oxidation with AA supplementation [9].

Glycation of hemoglobin as a model

Studying glycation of the protein hemoglobin can serve as a model for in vivo protein glycation in general. The glycation of hemoglobin is used as the basis for a common diagnostic test. Blood glycohemoglobin (GHb) concentration is an integrated measure of plasma glucose that is intended to represent glucose concentrations in blood averaged over a 2—3 month period. GHb is expressed as a percentage of total hemoglobin. Hyperglycemia is a major risk factor for cardiovascular disease, cancer, reproductive anomalies, complications of diabetes, etc. [10—15]. For example, susceptibility to neoplastic disease is increased [15,16] and response to cancer treatment is impaired by high blood glucose levels [17,18]. Strict glycemic control is important for avoiding many diabetes-associated disorders [14]. Therefore, accurate measures of blood glucose, especially average blood glucose (GHb) are essential.

Antioxidant nutrients, including AA, have long-term utility in attenuating the progression of diabetic complications, and diabetics are encouraged to have a high AA intake [14]. Ascorbic acid has been suggested to compete in vivo with glucose in the glycation of albumin, hemoglobin and other proteins [19—21]. If this were true, long-term supplementation of AA could be beneficial for decreasing the "aging" effects of glycation on proteins as well as explaining in part the benefits of AA in diabetics. Yet, interference of glycation by AA might also alter GHb concentration and produce "false" underestimates of the true average blood glucose.

Contradictory reports on AA's effect on hemoglobin glycation in animals and humans have appeared. Diabetic rats treated with AA in drinking water showed a 24% decrease in GHb, whereas, non-diabetic rats did not demonstrate this response [22]. Conversely, AA-treated non-diabetic mice exhibited a 23% reduction in GHb [23]. To further confuse the matter, Smith et al. [24] reported the completely opposite effect; rats receiving the largest dietary amount of AA had the highest GHb's and lowering the AA intake decreased GHb.

The picture is no clearer in human studies. Two trials designed to examine the influence of AA supplementation on GHb reported opposing results. In these studies, 0.75—1.5 g AA/d were administered for 3 months. One found an 18% reduction in GHb [19], whereas the other reported no effect [25]. In two intervention trials with diabetics, one showed a lowering of GHb with 1 g AA/day for 4 months [26], whereas 0.5 g AA/day for 2 months showed no difference in mean GHb compared to controls [27]. Additionally, Shoff et al. [20] reported no difference in mean GHb among the highest vs. lowest quintiles of AA intake in a study of over 2000 subjects. However, a linkage between AA intake and GHb has been observed in several large population studies. In non-diabetics consuming total AA (dietary plus supplement) $\leqslant$0.20 g AA/day, GHb was negatively associated with AA intake [20,21]. Moreover, Sargeant et al. [28] reported an inverse relationship between GHb and plasma AA in a study of over 6000 non-diabetics (GHb $\leqslant$7.0). Thus, the inhibitory effect of AA on hemoglobin glycation, especially in cases of multigram AA supplementation, has not been fully clarified.

Ascorbic acid supplementation and decreased glycation

More than 11% of adults in the western United States take an individual AA supplement daily and it is the most commonly consumed nutritional supplement after multivitamins [29]. In light of this and the importance of adequate AA intake and blood glucose (BG) control in many aspects of human health including immunity, aging, birth defects, diabetes, etc., the uncertainty regarding the influence of AA on GHb mandated investigation.

Our results from a pilot study of 138 subjects who were supplementing up to 20 g of AA/day provided insight into this question. The subjects were outpatients at a private oncology clinic where over 1000 patients who had achieved sustained remission were seen periodically in routine follow-up. All patients arriving on the days of research specimen collection were invited to participate and written informed consent was obtained from all consenting subjects. Each provided blood samples and information on diet and vitamin supplement regimens.

The mean, median and range of AA intakes, plasma AA and GHb are shown in Table 1. A significant linear relationship was found between plasma AA concentrations and self-reported AA

Table 1 Ascorbic acid (AA) intake and plasma concentrations, and glycohemoglobin (GHb) in 138 subjects.

	AA intake (g/day)	Plasma AA (μmol/L)	GHb (%)
Mean ± SD	5.6 ± 4.5	159 ± 95.7	6.7 ± 1.4
Range	0—20	11.4—517	3.8—13
Median	4	131	6.5

intake ($p = .0001$). This was consistent with other studies [30] which have shown that plasma AA concentration is correlated with AA intake. Linear regression analysis also showed that there was a statistically significant inverse association between plasma AA and GHb (slope = −0.003; $p = 0.0061$). The mean plasma AA in our subjects was more than 2-fold greater than values found in a large population study (e.g., 45—61 μmol/L [28]). Individuals in that study were consuming the average diet (∼65 mg AA/day) and not supplementing AA. Subjects in other studies [19,25] who supplemented 1—1.5 g AA/day exhibited plasma concentrations (i.e., ∼120 μmol/L) nearer to our subjects consuming the median 4 g AA/day.

Two large population studies [20,21] cited above suggest that AA may play a role in decreasing the formation of glycation products (i.e., GHb). Moreover, there is much additional evidence that supplemental AA influences in vivo glycation of proteins, including hemoglobin, and thus, can produce a "false" GHb. In humans [19,25,26,31] and animals [22,23], supplementation of AA for several months did not lower fasting blood glucose concentrations. This suggests that glycemia is not being influenced by AA. Nevertheless, all but one of these studies [25] reported a significantly diminished GHb. If AA is distorting GHb measurements by interfering with glycation, this could have important ramifications for clinical medicine and public health.

Consequences of underestimation of glycohemoglobin

Sargent et al. [28] found a decrease in GHb of about 0.1 for every 20 μmol/L rise in plasma AA. Our results indicated a decrease of 0.07 for a 20 μmol/L increase in plasma AA. The subjects in our study encompassed a much wider range of AA intakes and plasma AA than the population studies. In supplementing humans, a 1 g oral dose of AA can raise plasma AA to 130 μmol/L within an hour and such doses at intervals of about 2 h throughout the day can maintain ∼230 μmol AA/L [32]. A higher and more constant plasma AA level can be attained using larger doses of sustained-release AA tablets, especially those with 6—8 h release times. Even with the dosage scheme that produces ∼230 μmol AA/L, an approximate 0.7 depression in GHb would occur.

One could speculate that an AA-related decrease in GHb of this magnitude could mask a higher average blood sugar and would have significance in assessing disease risk. For example, when GHb was examined in >4600 males, it was found to be a continuous risk factor and predictor of mortality throughout the full range of GHb concentrations (i.e., there was no threshold value for GHb) [33]. The results suggested that small changes in population GHb could shift many people to lower risk categories and considerably decrease total mortality. A lowering of 0.2 in the population GHb would reduce total mortality by 10% [33].

Similarly, relatively small increases in average blood sugar can accompany adverse reproductive effects [13,34]. A difference in mean maternal GHb of 0.8 was found for women giving birth to infants without or with congenital malformations (i.e., 8.3 vs. 9.1, respectively) [34]. The prognostic value of GHb in predicting congenital fetal heart defects has also been evaluated [35]. An increased risk of both cardiac and non-cardiac anomalies was found at a GHb of 6.3%, only slightly above the normal upper limit (6.1%). That study concluded that there is no critical lower cutoff value in GHb for detecting congenital anomalies [35]. Our data suggested that supplementing the median amount of AA (4 g/d) would increase plasma AA by ∼100 μmol/L and, thus, on average, depress the measured GHb value by 0.35. A bias and error of this size could be clinically important. Women at risk for producing offspring with birth defects might not be recognized and not receive the more aggressive intervention needed for prevention.

Impaired glucose tolerance has long been observed in cancer patients [17,36,37] and only patients who improve glucose tolerance achieve sustained remission [18]. Hyperglycemia (i.e., elevated GHb) in children with acute lymphoblastic leukemia (ALL) is well documented [38]. Following treatment for ALL, GHb values returned to normal after achievement of complete remission [38]. In

murine models, susceptibility to an aggressive mammary tumor is significantly influenced by average blood sugar [16]. With GHb elevated only 15% (5.36 vs. 4.67), the 10-week mortality due to the tumor was 200% greater than mortality in normoglycemic animals. Moreover, in hypoglycemic animals with GHb 21% below controls (3.69 vs. 4.67), mortality was only 15% of controls. Thus, elevated GHb appears to be associated with susceptibility to, and presence of, active neoplastic disease. Whereas, caloric restriction (i.e., lower BG) is inversely associated with susceptibility to neoplasms [2,39]. Thus, if AA supplementation were obscuring the true average BG and leading to an underestimation of GHb, treatment of cancer and its outcome in patients could be impacted.

Conclusions

Our data suggest that the inverse relationship of GHb and plasma AA extends over a wider range of GHb, plasma AA and AA intake than reported previously. It also is consistent with the concept that AA is decreasing glycation and genuinely lowering GHb. This would mean that average blood sugar, as measured by GHb, may be underestimated in subjects consuming multi-gram doses of AA. Accurate knowledge of average blood sugar (i.e., a "true" unbiased GHb) is important in cancer, diabetes, prevention of birth defects, etc. Because AA supplementation is common, it seems prudent for primary care health providers to inquire regarding the AA intake of patients, especially diabetics, when using GHb for diagnosis or treatment monitoring.

Furthermore, a decrease in glycation has been suggested to slow aging. The mechanism by which caloric restriction may retard aging, in part, could be related to the diminished glycation of proteins that accompanies the lower blood sugar in calorie restricted animals and humans. Similarly, if AA can interfere with glycation, one might speculate that AA would have a delaying effect on aging. Recently an enzyme activator [40] that accelerates the pentose phosphate cycle was found to inhibit production of advanced glycation endproducts. Activation of the enzyme transketolase, enhances conversion of glyceraldehyde-3-phosphate and fructose-6-phosphate to pentose-5-phosphates and other sugars [40]. Glyceraldehyde-3-phosphate is reported to be a more potent glycation agent than glucose. For over 30 years it has been known that AA stimulates the pentose phosphate cycle, its rate being proportional to intracellular AA concentration [41]. Thus, elevated levels of AA could be functioning to reduce amounts of highly reactive glucose metabolites, resulting in less glycation and aging.

Several other mechanisms can be hypothesized for AA in countering the changes of aging. Through its antioxidant properties, AA could oppose the processes involved in Denham Harman's free radical theory of aging [42]. In addition, we have postulated [43] that AA, along with lysine, can enhance the replacement and repair of damaged structural proteins, and further oppose age-related dysfunction.

Acknowledgements

This study was supported in part by Northwest Oncology Foundation and Applied Research Institute. We thank Pierce Chemical Company for the Glycotest Kits; Professor D.H. Read and S. Belcher at Seattle University for blood assays; and H. F. Krone for manuscript preparation. Glenn A. Warner, MD, of NW Oncology Clinic is gratefully acknowledged for overseeing the study subjects.

References

[1] Cerami A, Vlassare H, Brownlee M. Glucose and aging. Sci Am 1987;256(5):90—6.
[2] Ramamurthy B, Höök P, Jones AD, Larsson L. Changes in myosin structure and function in response to glycation. FASEB J 2001;15:2415—22.
[3] Cheng R, Lin B, Ortwerth BJ. Rate of formation of AGEs during ascorbate glycation and during aging in human lens tissue. Biochim Biophys Acta 2002;1587(1):65—74.
[4] Aronson D. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J Hypertens 2003;21(1):3—12.
[5] Kikuchi S, Shinpo K, et al. Glycation — a sweet tempter for neuronal death. Brain Res Brain Res Rev 2003;41(2-3):306—23.
[6] Brownlee M. Advanced protein glycosylation in diabetes and aging. Annu Rev Med 1995;46:223—34.
[7] Hodge JE. Chemistry of browning reactions in model systems. J Agr Food Chem 1953;1:928—35.
[8] Kurata T, Fujimaki M, Sakurai T. Red pigment produced by the reaction of dehydro-L-ascorbic acid with ε-amino acid. Agric Biol Chem 1973;37:1471—3.
[9] Carr AC, Frei B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am J Clin Nutr 1999;69:1086—107.
[10] Ely JTA. Unrecognized pandemic subclinical diabetes of the affluent nations: causes, cost and prevention. J Orthomol Med 1996;11:95—9.
[11] Kritchevsky D. Diet in heart disease and cancer. Adv Exp Med Biol 1995;369:201—9.
[12] Ely JTA. Hyperglycemia and major congenital anomalies. New Engl J Med 1981;305:833.
[13] Schaefer-Graf UM, Buchanan TA, Xiang A, Songster G, Montoro M, Kjos SL. Patterns of congenital anomalies and relationship to initial maternal fasting glucose in pregnan-

cies complicated by type 2 and gestational diabetes. Am J Obstet Gynecol 2000;182:313—20.
[14] Diabetes and Nutrition Study Group of the European Association for the Study of Diabetes. Recommendations for the nutritional management of patients with diabetes mellitus. Eur J Clin Nutr 2000;54:353—5.
[15] Ely JTA. Glycemic modulation of tumor tolerance. J Orthomol Med 1996;11(1):23—34.
[16] Santisteban GA, Ely JTA, Hamel EE, Read DH, Kozawa SM. Glycemic modulation of tumor tolerance in a mouse model of breast cancer. Biochem Biophys Res Commun 1985;132(3):1174—9.
[17] Cheraskin E, Ringsdorf WM, Hutchins K, Setyaamadja ATSH, Wideman GL. Carbohydrate metabolism and cervical (uterine) carcinoma. Am J Obst Gyn 1967;97:817—20.
[18] Marks PA, Bishop JS. Glucose metabolism in subjects with neoplastic disease: response to insulin and glucose tolerance followup studies. Proc Am Assoc Cancer Res 1957;2:228—9.
[19] Davie SJ, Gould BJ, Yudkin JS. Effect of vitamin C on glycosylation of proteins. Diabetes 1992;41:167—73.
[20] Shoff SM, Mares-Perlman JA, Cruickshanks KJ, Klein R, Klein BEK, Ritter LL. Glycated hemoglobin concentrations and vitamin E, vitamin C, and beta-carotene intake in diabetic and nondiabetic older adults. Am J Clin Nutr 1993;58:412—6.
[21] Boeing H, Weisgerber UM, Jeckel A, Rose H-J, Kroke A. Association between glycated hemoglobin and diet and other lifestyle factors in a nondiabetic population: cross-sectional evaluation of data from the Potsdam cohort of the European Prospective Investigation into Cancer and Nutrition Study. Am J Clin Nutr 2000;71:1115—22.
[22] Gembal M, Druzynska J, et al. The effect of ascorbic acid on protein glycation in streptozotocin-diabetic rats. Diabetologia 1994;37:731.
[23] Krone CA, Ely JTA. Vitamin C and glycohemoglobin revisited. Clin Chem 2001;47(1):148.
[24] Smith D, Shang F, et al. Decreasing ascorbate intake does not affect the levels of glutathione, tocopherol or retinol in the ascorbate-requiring osteogenic disorder Shionogi rats. J Nutr 1999;129:1229—32.
[25] Weykamp C, Penders TJ, Baadenhuijsen H, Muskiet AJ, Martina W, van der Slik W. Vitamin C and glycohemoglobin. Clin Chem 1995;41:713—6.
[26] Paolisso G, Balbi V, et al. Metabolic benefits deriving from chronic vitamin C supplementation in aged non-insulin dependent diabetics. J Am Coll Nutr 1995;14(2):387—92.
[27] Bishop N, Schorah CJ, Wales JK. The effect of vitamin C supplementation on diabetic hyperlipidemia: a double-blind, crossover study. Diabetic Med 1985;2:121—4.
[28] Sargeant LA, Warham NJ, et al. Vitamin C and hyperglycemia in the European prospective investigation in cancer — Norfolk (EPIC-Norfolk) study. Diabetes Care 2000; 23(6):726—32.
[29] Selsinski MJ, Subar AF, Kahle LL. Dietary intake of fat, fiber and other nutrients is related to the use of vitamin and mineral supplements in the United States: the 1992 National Health Interview Study. J Nutr 1996;126: 3001—8.
[30] Jacob RA. Assessment of human vitamin C status. J Nutr 1990;120:1480—5.
[31] Ely JTA, Warner GA, Read DH, Santisteban GA. Protein glycation: ascorbate antagonism. Bull Am Phys Soc 1988; 33(abstr):296.
[32] Lewin S. Vitamin C: its molecular biology and medical potential. New York: Academic Press; 1976. p. 231.
[33] Khaw KT, Wareham N, et al. Glycated haemoglobin, diabetes, and mortality in men in Norfolk cohort of European Prospective Investigation of Cancer and Nutrition (EPIC-Norfolk). BMJ 2001;322:1—6.
[34] Rosenn B, Miodovnik M, Dignan PSJ, Siddiqi TA, Khoury J, Mimouni F. Minor congenital malformation in infants of insulin-dependent diabetic women: association with poor glycemic control. Obstet Gynecol 1990;76:745—9.
[35] Shields LE, Gan EA, Murphy HF, Sahn DJ, Moore TR. The prognostic value of hemoglobin A1c in predicting fetal heart disease in diabetic pregnancies. Obstet Gynecol 1993;81(6):954—7.
[36] Glicksman AS, Myers WPL, Rawson RW. Diabetes mellitus and carbohydrate metabolism in patients with cancer. Med Clin North Am 1956;40:887—900.
[37] Rofe AM, Bourgeois CS, Coyle P, Taylor A, Abdi EA. Altered insulin response to glucose in weight-losing cancer patients. Anticancer Res 1994;14:647—50.
[38] Yetgin S, Yalcin SS, Ozbek N. Clinical value of glycated hemoglobin and fructosamine in the long-term glycemic control of children with acute lymphoblastic leukemia. Acta Paediatr Jpn 1998;40(1):52—6.
[39] Zhu Z, Haegele A, Thompson H. Effect of caloric restriction on pre-malignant and malignant stages of mammary carcinogenesis. Carcinogenesis 1997;18(5): 1007—12.
[40] Hammes HP, Du X, et al. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat Med 2003;9(3): 294—9.
[41] Cooper MR, McCall CE, DeChatelet LR. Stimulation of leukocyte hexose monophosphate shunt activity by ascorbic acid. Infect Immun 1971;3:851—3.
[42] Harman D. Aging: overview. Ann NY Acad Sci 2001;928: 1—21.
[43] Ely JTA, Krone CA. Aging: predictions of a new perspective on old data. Exp Biol Med 2002;227(12):939—42.