3-Deoxyfructose Concentrations Are Increased in Human Plasma and Urine in Diabetes

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3-Deoxyglucosone (3-DG) is a reactive dicarbonyl sugar thought to be a key intermediate in the nonenzymatic polymerization and browning of proteins by glucose. 3-DG may be formed in vivo from fructose, fructose 3-phosphate, or Amadori adducts to protein, such as N^{ϵ}-fructoselysine (FL), all of which are known to be elevated in body fluids or tissues in diabetes. Modification of proteins by 3-DG formed in vivo is thought to be limited by enzymatic reduction of 3-DG to less reactive species, such as 3-deoxyfructose (3-DF). In this study, we have measured 3-DF, as a metabolic fingerprint of 3-DG, in plasma and urine from a group of diabetic patients and control subjects. Plasma and urinary 3-DF concentrations were significantly increased in the diabetic compared with the control population (0.853 ± 0.189 vs. 0.494 ± 0.072 μM, $P < 0.001$, and 69.9 ± 44.2 vs. 38.7 ± 16.1 nmol/mg creatinine, $P < 0.001$, respectively). Plasma and urinary 3-DF concentrations correlated strongly with one another, with HbA_{1c} ($P < 0.005$ in all cases), and with urinary FL ($P < 0.02$ and $P = 0.005$, respectively). The overall increase in 3-DF concentrations in plasma and urine in diabetes and their correlation with other indexes of glycemic control suggest that increased amounts of 3-DG are formed in the body during hyperglycemia in diabetes and then metabolized to 3-DF. These observations are consistent with a role for increased formation of the dicarbonyl sugar 3-DG in the accelerated browning of tissue proteins in diabetes. *Diabetes* 43:1152–1156, 1994

Increased protein glycation and accumulation of advanced glycation end products (AGEs) in tissue proteins are chemical consequences of chronic hyperglycemia in diabetes. These modifications of protein affect protein structure and function and are thought to contribute to the gradual development of pathophysiology and the long-term complications of diabetes (1,2). The oxidation of glucose, leading to formation of reactive ketoaldehyde or dicarbonyl intermediates, is proposed to have a major role in the browning and cross-linking of proteins by glucose (3,4) and in the formation of AGEs in protein (5). However, other reactive dicarbonyl sugars, such as 1-deoxyglucosone (6,7) and 3-deoxyglucosone (3-DG) (6,8–10), may also be formed nonoxidatively by rearrangement and hydrolysis of Amadori compounds. 1-Deoxyglucosone has never been isolated from Maillard reaction mixtures; evidence for its formation is based on the analysis of degradation products (6) and detection of the quinoxaline derivative by trapping with *o*-phenylenediamine (7). However, Kato and colleagues (11,12) have identified 3-DG in reactions of glucose with proteins under physiological conditions (pH 7.4, 37°C) and have shown that 3-DG is a potent chemical modifier of lysine, arginine, and tryptophan residues in protein and causes rapid cross-linking and formation of fluorescent products in proteins (13–15).

We have recently detected 3-DG as a product formed on decomposition of the Amadori compound, N^{α}-formyl-N^{ϵ}-fructoselysine, under physiological conditions (unpublished observations), providing evidence that Amadori adducts to protein may also be precursors of 3-DG. However, sugars other than glucose may also be important in the browning of proteins in vivo and may also be sources of 3-DG. Suarez et al. (16) and McPherson et al. (17) observed that fructose, which is increased in many tissues in diabetes as a result of increased sorbitol pathway activity, was as reactive as glucose in the glycation of proteins in vitro, but about 10 times as reactive as glucose in the formation of fluorescent products. Shin et al. (11) presented evidence that the rapid cross-linking of proteins by fructose under physiological conditions was not dependent on the initial formation of an Amadori adduct but rather originated from the direct formation of 3-DG from fructose. In other work, Szwergold et al. (18) and Petersen et al. (19) detected increased levels of fructose 3-phosphate in diabetic compared with normal rat lenses and erythrocytes. These investigators hypothesized that the increase in fructose 3-phosphate in the diabetic lens, and its subsequent conversion to 3-DG, contributed to the accelerated browning of lens proteins and development of cataracts in diabetes. Thus, three possible sources of 3-DG in vivo are Amadori adducts to protein, fructose, and fructose 3-phosphate, each of which is elevated in diabetes and may produce 3-DG for accelerating the modification of proteins via the Maillard reaction.

Liang et al. (20) and Kato et al. (21) have characterized a hepatic NADPH-dependent 2-oxoaldehyde reductase that converts 3-DG to the less reactive 3-deoxyfructose (3-DF) and have suggested that this enzyme has a metabolic role in protecting against protein modification and formation of AGEs during the advanced stages of the Maillard reaction.

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AGE, advanced glycosylation end product; 3-DG, 3-deoxyglucosone; 3-DF, 3-deoxyfructose; SIM-GC/MS, selected ion monitoring gas chromatography-mass spectrometry; $NaBD_4$, sodium borodeuteride; 3-DH, 3-deoxyhexitol; FL, N^{ϵ}-fructoselysine; ICA, islet cell autoantibody.

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3-DF was also identified as the major metabolite or detoxification product of 3-DG in the urine of rats administered 3-DG (22). Kanazu et al. (23) have shown that the enzyme with 3-DG reductase activity is identical to an aldehyde reductase widely distributed among animal tissues, and Igaki et al. (24) have reported that aldose reductase can also reduce 3-DG to 3-DF, providing an additional level of protection against 3-DG–mediated damage to protein during hyperglycemia.

We recently reported a selected ion monitoring gas chromatography–mass spectrometry (SIM-GC/MS) (25) assay for measuring 3-DG and 3-DF in urine and plasma based on comparison of the mass spectra of $NaBH_4$ and sodium borodeuteride ($NaBD_4$)-reduced samples. This study showed that the 3-deoxyhexitol (3-DH) obtained on reduction of 3-DG and 3-DF was derived exclusively from 3-DF in urine and >85% from 3-DF in plasma, consistent with the observation of Kato et al. (22) that 3-DG was metabolized to 3-DF in vivo. In this study, we have measured 3-DF in human urine and plasma as a metabolic fingerprint of 3-DG to determine the effect of diabetes and glycemic control on the formation of 3-DG.

RESEARCH DESIGN AND METHODS

A total of 64 patients were enrolled in the study, 35 with diabetes (25 type I; 10 type II) 13–73 years of age (mean ± SD, 43.4 ± 17.2 years) and 29 control subjects 22–60 years of age (mean ± SD, 35.7 ± 10.6 years). Samples were collected from two sources: the Royal Victoria Hospital, Belfast, Northern Ireland, and the Medical University of South Carolina, Charleston, South Carolina. Informed consent of volunteers was obtained, and the study was approved by the respective institutional human subjects review boards. For a limited number of patients, both plasma and urine samples were available for all analyses; the actual number of samples analyzed is indicated in the figure legends. One individual recruited as a nondiabetic subject was found to have fasting blood glucose and HbA_{1c} values well within the normal range, but urinary N^{ϵ}-fructoselysine (FL) and 3-DF and plasma 3-DF were in the diabetic range. At a time when our codes were sealed, an unrelated study found this subject to be positive for islet cell autoantibodies (ICAs); this subject is also the first-degree relative of a type I diabetic patient. Assay results for this individual are classified in all figures as nondiabetic, but with a unique symbol (⊙), and statistical analyses do not include data from this individual.

Materials. Reagents were obtained from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI). [U-^{13}C]3-DG was synthesized from [U-^{13}C]glucose as described by Madson and Feather (26). The internal standard used for SIM-GC/MS analyses was [U-^{13}C]3-DH prepared by $NaBH_4$ reduction of [U-^{13}C]3-DG. The concentration of [U-^{13}C]3-DH in the standard solutions was determined by measurement of malondialdehyde formed upon periodate oxidation (27), using the fluorescent thiobarbituric acid assay described by Steinbrecher et al. (28). Urinary creatinine and plasma glucose were determined with diagnostic kits from Sigma and American Research Products (Solon, OH), respectively. HbA_{1c} was measured using cation-exchange micro columns (Bio-Rad, Hercules, CA).

Sample preparation and analysis. Procedures for sample collection and analysis of 3-DF were as described in detail previously (25), except for the use of [U-^{13}C]3-DH as an internal standard. Briefly, second voided, fasting urine and plasma samples were collected, reduced with $NaBD_4$, desalted by ion-exchange chromatography, then converted to per-*O*-acetylated deoxyalditol derivatives for analysis by SIM-GC/MS. The *m/z* 232 and 235 ions were monitored for the sample and internal standard, respectively. Authentic 3-DF is not available for recovery experiments; however, the amount of 3-DF measured in plasma samples was unchanged after 6 h of incubation at 37°C and in urine samples was unchanged during storage for 6 weeks at 4°C. Intra- and interassay coefficients of variation for the assay ranged from 3 to 6% (25). Urinary FL was measured in a separate aliquot of urine as described previously (29). Data are expressed as means ± SD. Correlations and significance were evaluated with nonparametric statistics by the Spearman rank correlation method and Mann-Whitney *U* test, respectively, using SigmaStat (Jandel Scientific, San Rafael, CA).

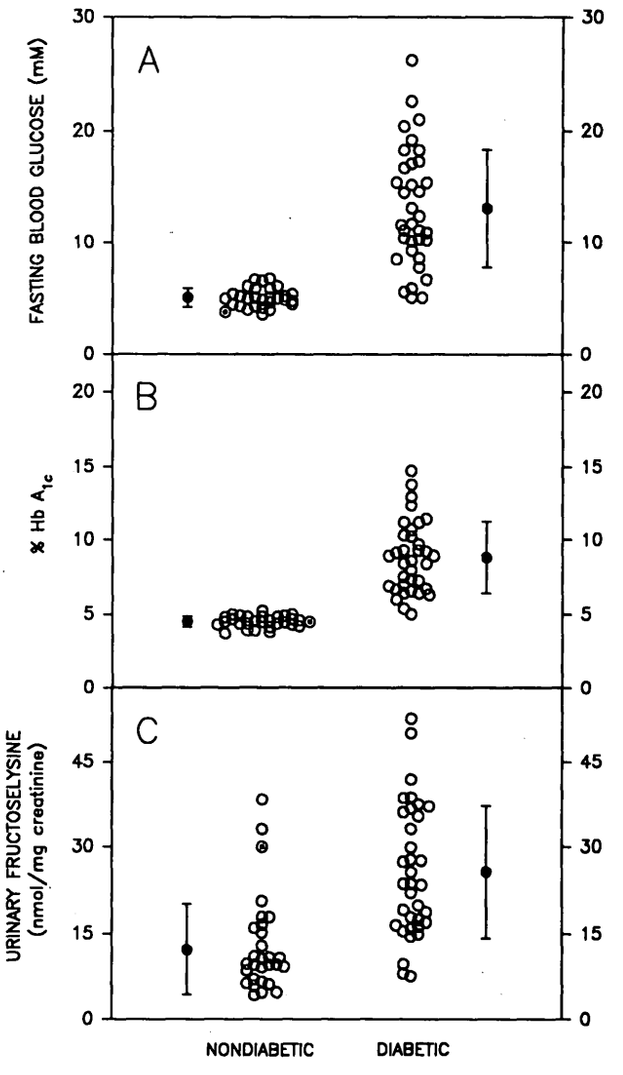

FIG. 1. Fasting blood glucose, HbA_{1c}, and urinary FL concentrations in diabetic and nondiabetic subjects. Error bars and ● represent the mean ± 1 SD. An ICA-positive individual is classified on all graphs as nondiabetic, but with a unique symbol (⊙), and is not included in the statistical analyses. Results for diabetic patients (n = 35) compared with nondiabetic subjects (n = 29) were *A*: fasting blood glucose: 13.1 ± 5.3 vs. 5.1 ± 0.8 mM, $P < 0.001$; *B*: HbA_{1c}: 8.8 ± 2.4 vs. 4.5 ± 0.4%, $P < 0.001$; and *C*: urinary FL: 25.7 ± 11.4 vs. 12.2 ± 8.0 nmol/mg creatinine, $P < 0.001$.

RESULTS

Analyses of fasting blood glucose, HbA_{1c}, and urinary FL concentrations in the diabetic and control groups are summarized in Fig. 1. The differences between the means of the two groups were highly significant: fasting blood glucose was 13.1 vs. 5.1 mM ($P < 0.001$), HbA_{1c} was 8.8 vs. 4.5% ($P < 0.001$), and urinary FL was 25.7 vs. 12.2 nmol/mg creatinine ($P < 0.001$). Two nondiabetic individuals, in addition to the ICA-positive patient, exhibited increased urinary FL, possibly from an inadequate dietary fast. Data in Fig. 2 show a similar increase in the mean concentration of 3-DF in diabetic urine compared with control urine (69.9 vs. 38.7 nmol/mg creatinine, $P < 0.001$) and plasma (0.853 vs. 0.494 μM, $P < 0.001$). The range of 3-DF values for the diabetic group was greater than that for the control group and consistent with the range of glycemic control and protein glycation in the diabetic patients (Fig. 1). Figure 3 also shows that a good correlation ($P < 0.005$) was found between 3-DF concentrations in urine and plasma.

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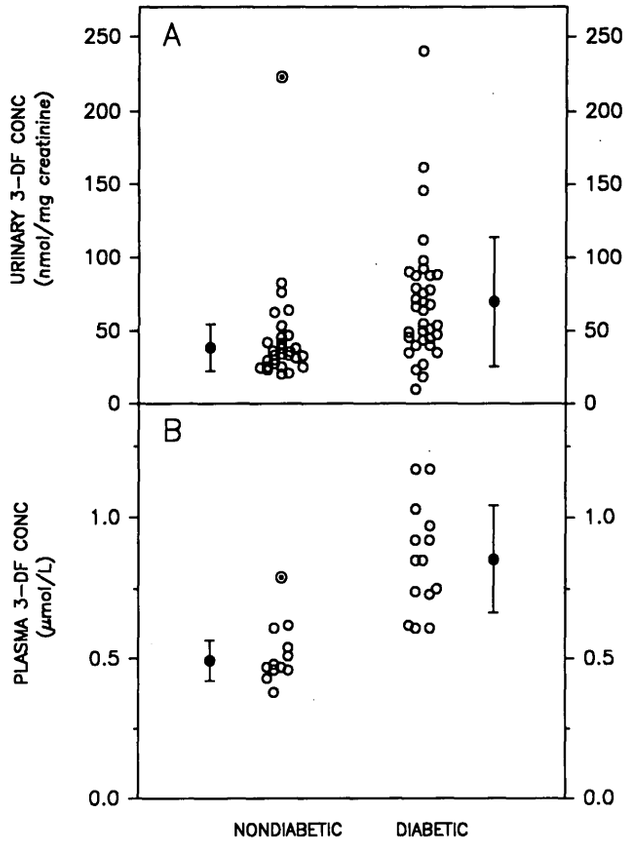

FIG. 2. Urinary and plasma 3-DF concentrations in diabetic and nondiabetic subjects. *A*: urinary 3-DF was elevated in diabetic patients (n = 35) compared with nondiabetic subjects (n = 29) (69.9 ± 44.2 vs. 38.7 ± 16.1 nmol/mg creatinine, $P < 0.001$). *B*: plasma levels of 3-DF were also elevated in diabetic subjects (n = 14) compared with nondiabetic subjects (n = 12) (0.853 ± 0.189 vs. 0.494 ± 0.072 μM, $P < 0.001$).

If 3-DF concentrations were dependent on glycemic control, then levels of 3-DF in urine and plasma should increase in concert with increases in protein glycation. In fact, a good correlation was found between urinary 3-DF and two indexes of protein glycation, HbA_{1c} (Fig. 4*A*: $r = 0.50$; $P < 0.005$) and urinary FL (Fig. 4*B*: $r = 0.55$; $P < 0.005$). Plasma 3-DF also correlated well with HbA_{1c} (Fig. 5*A*: $r = 0.64$; $P < 0.005$) and urinary FL (Fig. 5*B*: $r = 0.47$; $P = 0.02$).

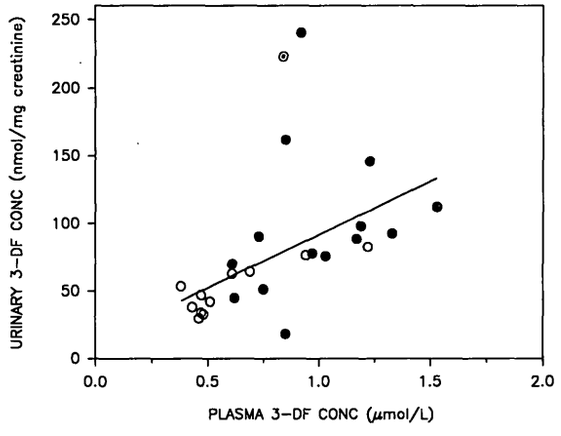

FIG. 3. Relationship between urinary and plasma 3-DF in diabetic patients (●, n = 14) and nondiabetic subjects (○, n = 12). The line is the least-squares fit to the data and has the equation: urinary 3-DH = 78.4 × plasma 3-DH + 13.1 ($r = 0.75$, $P < 0.005$).

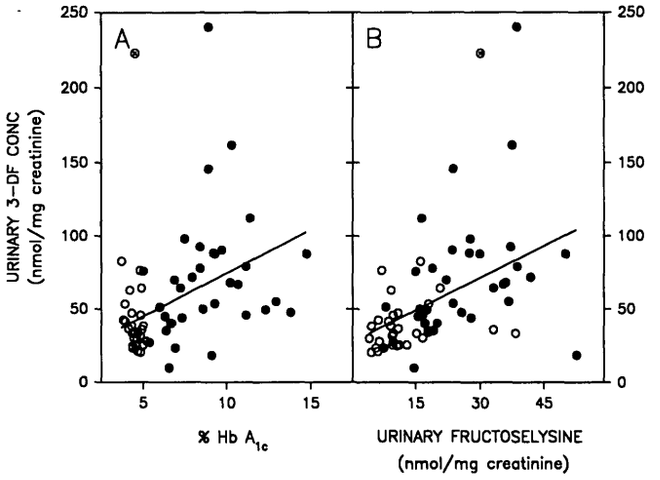

FIG. 4. Relationships between urinary 3-DF and HbA_{1c} (*A*) and urinary FL (*B*) in diabetic (●, n = 35) and nondiabetic subjects (○, n = 29). *A*: the equation of the line is urinary 3-DF = 5.91 × HbA_{1c} + 15.3 ($r = 0.50$, $P < 0.005$). *B*: the equation of the line is urinary 3-DF = 1.47 × FL + 27.1 ($r = 0.55$, $P < 0.005$).

DISCUSSION

In this study, we show that levels of 3-DF in plasma and urine are increased in diabetes and correlate with one another and with indexes of glycemic control, such as HbA_{1c} and urinary FL concentration. These relationships indicate that the increases in 3-DF are dependent, at least in part, on the increase in glycemia in diabetes. Our experiments do not permit us to determine whether 3-DG, the presumed source of 3-DF, is derived from Amadori compounds, fructose, fructose 3-phosphate, or all of these sources. Concentrations of each of these products change in response to glycemic control, and all may contribute to the formation of 3-DG and 3-DF. Our observation that the amounts of plasma and urinary 3-DF are 1.5–2 times higher in the diabetic group compared with the control group (Figs. 2 and 5*B*) agrees with work by Hayase et al. (30), who reported a 1.6-fold increase in pyrraline in plasma albumin from diabetic patients compared with control subjects, and by Odetti et al. (31), who reported a 2.5-fold increase in pentosidine in plasma proteins from diabetic patients compared with con-

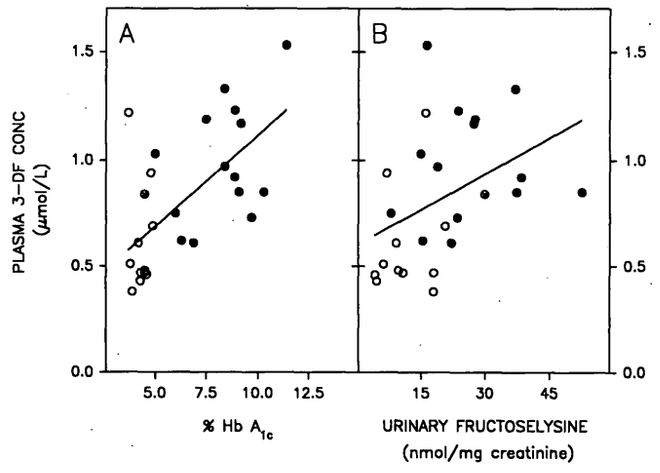

FIG. 5. Relationships between plasma 3-DF and HbA_{1c} (*A*) and urinary FL (*B*) in diabetic patients (●, n = 14) and nondiabetic subjects (○, n = 12). *A*: the equation of the line is plasma 3-DF = 0.086 × HbA_{1c} + 0.258 ($r = 0.67$, $P < 0.005$). *B*: the equation of the line is plasma 3-DF = 0.011 × FL + 0.60 ($r = 0.47$, $P = 0.02$).

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trol subjects. Pyrraline (30) and pentosidine (31–33) are the only structurally characterized AGEs that are known to increase in plasma proteins in diabetes and that can be formed by the reaction of 3-DG with protein. If these and other AGEs formed from dicarbonyl sugars are involved in the pathogenesis of diabetic complications, then individual differences in ability to detoxify α-dicarbonyl sugars may provide a genetic basis for differences in the severity of complications among diabetic patients with comparable glycemic control.

Efforts to measure 3-DG itself in plasma samples were compromised by two problems that we have encountered with our original assay procedure (25). First, we observed that the level of 3-DG increased gradually in hyperglycemic samples during storage at room temperature and during the 2-h treatment at 37°C with glucose oxidase/peroxidase reagent, used to convert glucose to gluconic acid (25). The formation of 3-DG during storage and workup may have resulted from formation of Amadori adducts in situ and their rearrangement to release 3-DG (6). A second problem we encountered was that recovery of added 3-DG also decreased during sample workup, perhaps because of its instability or reaction with serum protein. To some extent, these errors canceled one another, although their net effect on the accuracy of measurements of 3-DG in plasma is uncertain and probably varies with the glucose concentration in the sample. Before we were aware of these problems, Niwa et al. (34) used our original assay procedure to measure 3-DG in human serum and reported increased levels of 3-DG in diabetic serum compared with nondiabetic serum. Recent work in our laboratory, using a revised assay procedure, supports the general conclusion of Niwa et al. (34) that blood levels of 3-DG are increased in diabetes, although our preliminary data suggest that levels of 3-DG are significantly lower than reported by these authors, i.e., closer to the values we originally reported (1 ± 0.2 μg 3-DG/dl plasma) rather than the 30 μg 3-DG/dl serum reported by Niwa et al. (34).

3-DG is a potent protein cross-linking agent (13,14), and increased levels of 3-DG in the blood of diabetic patients (34) may accelerate the modification of proteins, formation of AGE products, and development of pathological conditions in diabetes. Note that all of the AGE products that have been characterized thus far and that are known to accumulate in tissue proteins with age and at an accelerated rate in diabetes are also oxidation products, e.g., N^{ϵ}-(carboxymethyl)lysine, N^{ϵ}-(carboxymethyl)hydroxylysine, and pentosidine (4). These AGEs have been termed glycoxidation products because of the involvement of both glycation and oxidation reactions in their formation (4). They are thought to be formed by oxidation of either glucose or Amadori adducts to protein, yielding reactive dicarbonyl sugars either free in solution or bound to protein. In contrast, 3-DG and other deoxyglucosones represent reactive dicarbonyl sugars formed from Amadori compounds, fructose, or fructose 3-phosphate by nonoxidative rearrangement and hydrolysis reactions. To assess the relative importance of oxidative and nonoxidative sources of dicarbonyl sugar intermediates in the Maillard reaction in vivo, it will be necessary to distinguish between products formed by reaction of protein with glucose autoxidation products and those formed by reaction of protein with deoxyglucosones and to determine the extent to which oxidation reactions are involved in the cross-linking of proteins by deoxyglucosones.

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