Metformin Reduces Systemic Methylglyoxal Levels in Type 2 Diabetes

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Methylglyoxal (MG) is a reactive α-dicarbonyl that is thought to contribute to diabetic complications either as a direct toxin or as a precursor for advanced glycation end products. It is produced primarily from triose phosphates and is detoxified to D-lactate (DL) by the glyoxalase pathway. Because guanidino compounds can block dicarbonyl groups, we have investigated the effects of the diamino biguanide compound metformin and of hyperglycemia on MG and its detoxification products in type 2 diabetes. MG and DL were measured by high-performance liquid chromatography in plasma from 57 subjects with type 2 diabetes. Of these subjects, 27 were treated with diet, sulfonylureas, or insulin (nonmetformin), and 30 were treated with metformin; 28 normal control subjects were also studied. Glycemic control was determined by HbA_{1c}. MG was significantly elevated in diabetic subjects versus the normal control subjects (189.3 ± 38.7 vs. 123.0 ± 37 nmol/l, P = 0.0001). MG levels were significantly reduced by high-dosage (1,500–2,500 mg/day) metformin (158.4 ± 44.2 nmol/l) compared with nonmetformin (189.3 ± 38.7 nmol/l, P = 0.03) or low-dosage (≤1,000 mg/day) metformin (210.98 ± 51.0 nmol/l, P = 0.001), even though the groups had similar glycemic control. Conversely, DL levels were significantly elevated in both the low- and high-dosage metformin groups relative to the nonmetformin group (13.8 ± 7.7 and 13.4 ± 4.6 vs. 10.4 ± 3.9 μmol/l, P = 0.03 and 0.06, respectively). MG correlated with rising HbA_{1c} levels (R = 0.4, P = 0.03, slope = 13.2) in the nonmetformin subjects but showed no increase with worsening glycemic control in the high-dosage metformin group (R = 0.0004, P = 0.99, slope = 0.02). In conclusion, MG is elevated in diabetes and relates to glycemic control. Metformin reduces MG in a dose-dependent fashion and minimizes the effect of worsening glycemic control on MG levels. To the extent that elevated MG levels lead to their development, metformin treatment may protect against diabetic complications by mechanisms independent of its antihyperglycemic effect.
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Dramatically accelerated retinal and renal disease are common complications of long-term diabetes, and as documented by the results of the Diabetes Control and Complications Trial (1), chronic hyperglycemia and its resulting glucose toxicity play an important role in their development. Substantial recent data indicate that glucose toxicity is mediated through increased production of highly chemically reactive α-dicarbonyl precursors of advanced glycation end products (AGEs) (2,3). These toxic α-dicarbonyls, such as methylglyoxal (MG) and 3-deoxyglucosone (3-DG), are extremely reactive as glycating agents for collagen, enzymes, and other important cellular components (4–7) and have been shown to be toxic to cultured cells (7–9). They are elevated in plasma of diabetic individuals (10–14) and are found in increased levels in patients showing evidence of early diabetic nephropathy and retinopathy (13). Both MG and 3-DG can lead to increased AGE formation, which in turn is associated with diabetic vascular complications (15–17). Because of their toxic potential, 3-DG and MG are converted to innocuous products by ubiquitous mechanisms, and both oxidative and reductive pathways exist for their breakdown. MG is oxidized by the glyoxalase pathway to D-lactate and is metabolized reductively to acetol (3,18), whereas the reductive pathway for 3-DG results in the production of 3-deoxyfructose (3-DF) and an oxidative pathway that is not as well characterized leads to the production of 3-deoxy-2-ketogluconic acid (3-DGA) (19,20).

Recently, substantial efforts have been undertaken to develop the guanidino compound aminoguanidine (AG) as a drug to delay or prevent the development of diabetic complications. Using in vitro model systems and animals, several groups have shown that this compound is quite effective in inhibiting the formation of AGEs and alleviating diabetic complications (21–23), and clinical trials are currently underway to evaluate the effect of AG therapy on the progression of diabetic renal and atherosclerotic disease. The mechanism of action of AG appears to involve reactions that inactivate α-dicarbonyls such as 3-DG and MG (24,25). The potential sources of MG and its detoxification pathway to D-lactate (DL) are shown in Fig. 1.

Other guanidino compounds have shown similar, potentially beneficial effects on tissue glycation and the formation of AGEs (26–28). In this context, one of the most useful among these compounds is the safe and effective biguanide antihyperglycemic agent metformin. The similarities of the

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AG, aminoguanidine; AGE, advanced glycation end product; ANOVA, analysis of variance; 3-DF, 3-deoxyfructose; 3-DG, 3-deoxyglucosone; 3-DGA, 3-deoxy-2-ketogluconic acid; DL, D-lactate; D-LDH, D-lactate dehydrogenase; GC-MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; MG, methylglyoxal.

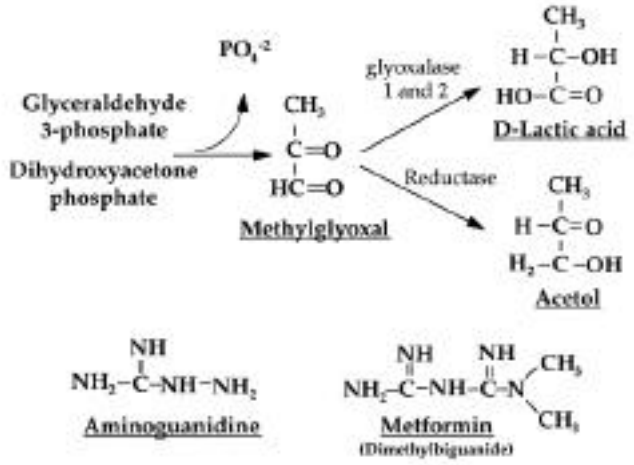

FIG. 1. Schema of the formation and detoxification of MG. The formation of MG occurs by β-elimination of the phosphate group of triose phosphates (glyceraldehyde-3-phosphate [G-3-P] and dihydroxyacetone phosphate [DHAP]), and the major detoxification route is by the glutathione-dependent glyoxalase pathway. The chemical structures of the two guanidino compounds (AG and metformin) are also shown. The condensation products between the amino or guanidino groups on these compounds and the α-dicarbonyl group of MG is proposed to be responsible for the antiglycation effects.

chemical structure of metformin and AG are shown in Fig. 1. Because our preliminary studies have indicated that metformin has an inhibiting effect on in vitro glycation and AGE formation, we have performed studies to determine whether metformin can lower the concentrations of α-dicarbonyls and their metabolites in type 2 diabetes.

RESEARCH DESIGN AND METHODS

We recruited two groups of subjects with type 2 diabetes who were either treated with metformin (500 to 2,500 mg/day for ≥3 months) or not treated with metformin. Subjects were sequentially recruited from the Dartmouth-Hitchcock Medical Center in Lebanon, New Hampshire, using a large population with type 2 diabetes. Inclusion criteria for study subjects were type 2 diabetes, age of 20–70 years, no renal impairment (serum creatinine <140 μmol/l (1.6 mg/dl), no significant liver disease, and stable cardiac status. After the nature of the procedure was explained and informed consent was obtained, each subject underwent a series of clinical and laboratory evaluations on the same day. A complete examination was performed by the same physician (P.J.B.) and included determination of the duration of diabetes, the level of retinopathy as assessed by dilated ophthalmoscopy, and the degree of atherosclerosis and neuropathy, which were graded according to standard clinical criteria. Blood was sampled in the fasting state for determination of glucose, creatinine, and HbA_{1c}. A 24-h urine collection was performed on the preceding day for determination of urinary albumin excretion and creatinine clearance.

To avoid the possible effect of greater or lesser degrees of glycemic control on the levels of α-dicarbonyls, we selected subjects taking or not taking metformin who were paired for similar HbA_{1c} and fasting plasma glucose values. Based on our in vitro quantitative data on the inhibitory effect of metformin on nonenzymatic glycation and AGE formation, we calculated that 60 subjects (30 in each group) would be required to produce sufficient power to demonstrate significant differences between groups if such an effect existed.

Plasma levels of MG and 3-DG, and of their major metabolites (DL, acetol, 3-DF, and 3-DGA), were measured in each subject in the fasting state. Plasma MG and DL levels were also measured in 28 nondiabetic control subjects.

Assay systems. As part of ongoing studies of the role played by glycation in the development of diabetic complications, we have developed assay systems for 3-DG, MG, and their major precursors and metabolites, using high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC-MS), and nuclear magnetic resonance techniques (4,10,29,30). To assess the levels of MG and DL, we used assays developed in our laboratories as detailed below. Their reproducibility, accuracy, and specificity have been rigorously validated.

Determination of MG in plasma. Quantitation of MG is based on a scheme previously described by Ohmori et al. (31) with the following modifications. 2-oxopentanoic acid is added to samples as an internal standard, and extraction of the derivatives is performed with ethyl ether. HPLC determinations are performed on a C-18 Prodigy 5-μm octadecyl silane 250 × 4.6 mm column (Phenomenex, Torrance, CA) with gradient elution from 22 to 45% acetonitrile in 10 mmol/l of potassium phosphate buffer (pH 3.2).

Determination of DL in plasma. The DL HPLC assay was performed on perchloric acid–extracted plasma samples using a modification of a technique described previously (32). 2-Ketobutyric acid was used as an internal standard, and quantitation of the *o*-phenylenediamine derivatized product was performed by HPLC. A Prodigy C-18 250 × 4.6 mm column was used with a mobile phase consisting of 76% 10 mmol/l potassium phosphate and 24% acetonitrile at pH 2.9. DL concentrations are determined by measuring pyruvate concentrations in samples treated with D-lactate dehydrogenase (D-LDH) and in untreated samples. The pyruvate concentrations in treated samples represent the total of DL and pyruvate, whereas untreated samples represent the amount of pyruvate already present in plasma before the D-LDH reaction.

Determination of acetol in plasma. Acetol was determined by the method of Casazza and Fu (33), using 3-hydroxy-2-butanone as an internal standard.

Determination of 3-DG in plasma. 3-DG was assayed by selected ion monitoring by GC-MS as previously described, using U-^{13}C–labeled 3-DG as an internal standard (34).

Determination of 3-DF in plasma. Concentrations of 3-DF were determined by HPLC and pulsed amperometric detection (PAD) of ultrafiltered, deionized samples. A strong anionic column was used with 32 mmol/l sodium hydroxide as eluent. 3-DF was detected electrochemically and quantitated by comparison with an internal standard of 3-*O*-methyl glucose.

Data analysis. We examined the relationship of the α-dicarbonyls, their detoxification products, fasting plasma glucose, and HbA_{1c} to treatment group (metformin or nonmetformin) and to metformin dosage (≤1,000 or >1,000 mg/day) using analysis of variance (ANOVA). The relationship between the plasma levels of the measured products and other possible confounders such as age, duration of diabetes, sex, smoking, and diabetic complications was also analyzed using ANOVA. The relationship between glycemic control (HbA_{1c} and fasting plasma glucose) and levels of α-dicarbonyls and of their detoxification products was determined for subgroups taking >1,000 mg of metformin daily or not taking metformin by linear regression analysis, and the data were adjusted for possible effects of other covariates (including age, duration of diabetes, and weight).

RESULTS

Study population characteristics. We studied 57 subjects with type 2 diabetes, of whom 30 were taking metformin and 27 were given other treatment. The study population consisted of 32 men and 25 women, and the mean age and known duration of diabetes were 62.5 ± 7.5 and 13.6 ± 8.3 years, respectively. The mean age of the control group was 41 ± 14 years, and the group consisted of 13 men and 15 women.

The metformin and nonmetformin subjects were similar in age (61.5 ± 8.6 vs. 63.8 ± 6.1 years, $P = 0.26$). Conversely, the metformin subjects had a somewhat shorter duration of diabetes (11.8 ± 6.9 vs. 15.6 ± 9.3 years, $P = 0.09$) and were somewhat heavier (201.9 ± 38.8 vs. 184.6 ± 34.4 lb, $P = 0.08$), although neither of these differences achieved statistical significance.

Of the 30 subjects in the metformin treatment group, 5 received metformin monotherapy, 16 received metformin plus sulfonylureas, 3 received metformin plus insulin, and 6 received metformin plus insulin and sulfonylurea therapy. Of the 27 subjects in the nonmetformin group, 8 received sulfonylureas alone, 8 received insulin alone, 8 received insulin plus sulfonylureas, and 3 received diet therapy alone.

Effects of other patient characteristics on MG levels. Linear regression and ANOVA analyses showed no significant impact of duration of diabetes ($R = 0.04$, $P = 0.75$), age ($R = 0.186$, $P = 0.16$), and weight ($R = 0.005$, $P = 0.97$) on plasma MG levels. We also found no differences in MG levels between men and women (198.1 ± 54.5 vs. 180.1 ± 37.6 nmol/l, respectively; $P = 0.16$), or between smokers (205.3 ±

TABLE 1
MG and DL plasma levels and HbA_{1c} in diabetes: the effects of metformin

Treatment group	*n*	MG (nmol/l)	DL (μmol/l)	HbA_{1c} (%)
Control subjects	28	123.0 ± 37.0	8.3 ± 3.1	5.2 ± 0.5
Diabetic subjects				
No metformin	27	189.3 ± 38.7*	10.4 ± 3.9†	8.2 ± 1.2*
Metformin				
≤1 g/day	17	210.9 ± 51.0*‡	13.8 ± 7.7§‖	8.0 ± 1.1*‡
>1 g/day	13	158.4 ± 44.2¶‖#	13.4 ± 4.6 §**††	8.3 ± 1.0*‡††

Data are means ± SD. *P < 0.0001, †NS, §P = 0.001–0.003, ¶P = 0.01 vs. control subjects; ‡NS, ‖P = 0.02, **P = 0.06 vs. nonmetformin diabetic subjects; #P = 0.001, ††NS vs. diabetic subjects treated with ≤1 g/day metformin.

57.4 nmol/l) and nonsmokers (180.1 ± 8.9 nmol/l) or former smokers (190.8 ± 51.6 nmol/l) (P = 0.40 across groups).

MG and DL levels in diabetic and control subjects. Plasma levels of MG were significantly elevated (P = 0.0001) in type 2 diabetic subjects who were not taking metformin (n = 28) (189.3 ± 38.7 nmol/l) compared with nondiabetic control subjects (n = 28) (123.0 ± 37.0 nmol/l), although elevation in DL levels in type 2 diabetic subjects (10.4 ± 3.9 μmol/l) versus control subjects (8.3 ± 3.1 μmol/l, P = 0.03) did not achieve statistical significance (P = 0.13) (Table 1).

Relationship between metformin dosage and MG levels. To investigate the relationship between metformin dosage and plasma MG levels, we analyzed differences in mean MG levels between groups receiving no metformin (n = 27), low-dosage metformin (≤1,000 mg/day, n = 17) or higher-dosage metformin (>1,000 mg/day, n = 13). As shown in Table 1, subjects whose metformin dosage was >1,000 mg/day had significantly lower plasma levels of MG (158.4 ± 44.2 nmol/l) than those whose metformin dosage was ≤1,000 mg/day (210.9 ± 51 nmol/l, P = 0.001) or than those not taking metformin (189.3 ± 38.7 nmol/l, P = 0.03). Conversely, as shown in Table 1, the higher dosage of metformin (>1,000 mg/day) was not associated with any difference in HbA_{1c} (8.3 ± 1%) compared with the lower dosage of metformin (8.0 ± 1.1%) or with no metformin treatment (8.2 ± 1.2%).

Metformin therapy and DL levels. Plasma levels of DL, the major detoxification product of MG, were investigated in subjects with type 2 diabetes who were receiving low- or high-dosage metformin and were compared with DL levels of subjects not receiving metformin. As shown in Table 1, the levels of DL were significantly higher in subjects receiving either low-dosage (13.8 ± 7.7 μmol/l) or high-dosage (13.4 ± 4.6 μmol/l) metformin compared with those not receiving metformin (10.4 ± 3.9 μmol/l). We initially screened plasma acetol levels in subjects taking or not taking metformin (n = 10) and found that the levels were low and did not differ between these two groups (0.82 ± 0.32 and 0.82 ± 0.43 μmol/l in the metformin and nonmetformin groups, respectively; P = 0.96) and the control group (0.88 ± 0.25 μmol/l).

Relationship between glycemic control and MG levels according to metformin category. When we examined the relationship between glycemic control and plasma MG levels in the diabetic subjects who were not receiving metformin therapy (Fig. 2*A*), we found significant relationships between HbA_{1c} or fasting plasma glucose and MG levels (R = 0.4, P < 0.03, and R = 0.48, P < 0.01, respectively).Conversely, when we examined these same relationships between HbA_{1c} or fasting glucose levels and MG levels in subjects taking metformin (Fig. 2*B*), we found no relationship between glycemic control and plasma MG (R = 0.0004, P = 0.99, and R = 0.32, P = 0.2, respectively). The slope of the relationship between MG and HbA_{1c} was also essentially flat in subjects taking metformin (slope = 0.02) compared with those not taking metformin (slope = 13.2).

Metformin therapy and 3-DG levels. We did not observe a significant difference in 3-DG levels, or in the levels of its major detoxification product, 3-DF, for subjects taking metformin in any dosage range. Metformin therapy also had no effect on the positive relationship between HbA_{1c} and 3-DG levels. For the relationship between HbA_{1c} and 3-DG in subjects taking metformin, we found R = 0.43 and P = 0.009, and in subjects receiving nonmetformin therapy, we found R = 0.52 and P = 0.004.

DISCUSSION

Metformin is an oral antihyperglycemic agent that is approved by the Food and Drug Administration and has an excellent safety record, and its guanidino structure suggests that it can react with α-dicarbonyls and prevent their toxicity and AGE production. Because of these considerations, we have carried out the current studies to investigate the possible role of metformin in prevention of complications related to diabetes.

Our studies show that in the absence of metformin, plasma levels of MG, one of the major α-dicarbonyls, are significantly elevated in type 2 diabetic subjects relative to nondiabetic control subjects. We also find a strong association between glycemic control, based on HbA_{1c} (Fig. 2*A*) or fasting plasma glucose levels, and MG levels in a population composed of type 2 diabetic subjects who are not taking metformin. This increase in MG levels with rising HbA_{1c} was not seen in subjects taking high dosages of metformin (Fig. 2*B*), suggesting that metformin protects against increased MG production or decreased detoxification associated with increasing degrees of hyperglycemia. Further support for a role of metformin in reducing MG levels comes from our observation that plasma levels are also significantly reduced in subjects treated with high doses (>1,000 mg) of metformin daily (Table 1). The difference in MG levels between subjects treated with >1,000 mg of metformin daily and those treated with a lower metformin dosage or no metformin was not related to differences in glycemic control between the two groups, because they had similar levels of HbA_{1c}. We did not observe significant effects of metformin on plasma levels of

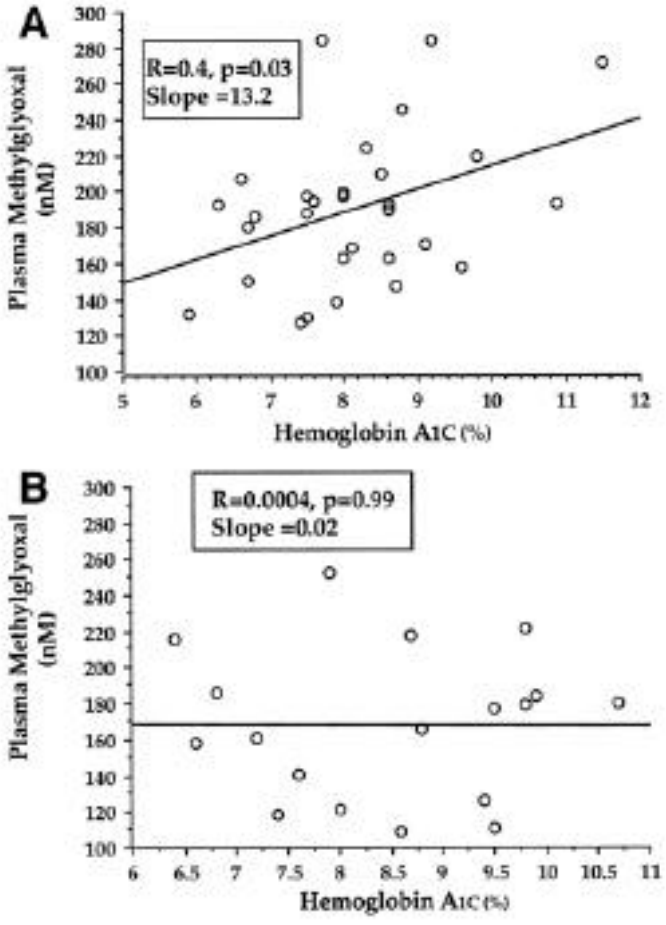

FIG. 2. *A*: Regression analysis of the relationship between plasma MG and glycemic control (HbA_{1c}) in a population consisting of subjects with type 2 diabetes who are not receiving metformin therapy (n = 28). A significant relationship is found, with R = 0.4 and P = 0.03. *B*: Regression analysis of the relationship between plasma MG and HbA_{1c} in subjects with type 2 diabetes receiving high-dosage (>1,000 mg/day) metformin therapy (n = 18). The relationship seen in *A* was not observed with high-dosage metformin (R = 0.0004 and P = 0.99).

the other major α-dicarbonyl, 3-DG, when this compound was measured in our study groups.

The mechanism by which metformin lowers plasma levels of MG most likely relates to a binding effect of the metformin guanidino group with the α-dicarbonyl group of MG. Such binding has been demonstrated in preliminary studies of Ruggiero-Lopez et al. (35) and may be similar to the reaction between another guanidino compound, AG, and MG (24,25). Because of this reactivity, AG has been shown in vitro to inhibit cross-linking of proteins mediated by MG and 3-DG (2,24). We therefore postulate that a reaction between MG and metformin in vivo results in a reduction in MG levels and thereby potentially inhibits tissue glycation.

An alternative explanation for the ability of metformin to reduce MG levels is through an enhancement of MG detoxification by the ubiquitous, high-capacity glyoxalase pathway (18). Support for this hypothesis is provided by our observation that levels of DL, the major end product of this pathway, are significantly increased in the subjects taking metformin. This increase was seen with both low and high dosages of metformin, unlike the effect on plasma MG levels, which were reduced only by higher dosages of metformin (>1,000 mg/day). In contrast, acetol, the major reductive product of the MG detoxification pathway (3), was found in insignificant quantities, and we observed no difference in this metabolite between metformin and nonmetformin groups.

The mechanism by which metformin might increase the activity of the glyoxalase pathway is not known. Because reduced glutathione is required for optimal glyoxalase activity (36), and the oxidative stress associated with the diabetic state may lead to decreased levels of reduced glutathione (37), one possibility is that metformin therapy may lead to increased production of reduced glutathione and enhance MG detoxification.

In contrast to MG, plasma levels of 3-DG, the other α-dicarbonyl compound investigated in our studies, are not significantly affected by metformin treatment. The failure of metformin to decrease the serum concentrations of 3-DG may be due to lower reactivity of 3-DG with metformin, resulting in decreased scavenging activity.

In conclusion, this study suggests that metformin may have the beneficial effect of reducing nonenzymatic glycation by mechanisms that appear to be independent of its antihyperglycemic effect. Considering the proven safety of metformin, confirmation of these results in future studies would support the earlier use of this agent in type 2 diabetes, when prevention of long-term complications may be feasible.

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REFERENCES

1. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
2. Brownlee M: Glycation and diabetic complications (Lilly Lecture 1993). *Diabetes* 43:836–841, 1994
3. Vander Jagt DL, Robinson B, Taylor KK, Hunsaker LA: Reduction of trioses by NADPH-dependent aldo-keto reductases: aldose reductase, methylglyoxal, and diabetic complications. *J Biol Chem* 267:4364–4369, 1992
4. Szwergold BS, Kappler F, Brown TR: Identification of fructose 3-phosphate in the lens of diabetic rats. *Science* 247:451–454, 1990
5. Lal S, Szwergold B, Taylor A, Randall W, Kappler F, Wells-Knecht K, Baynes J, Brown T: Metabolism of fructose-3-phosphate in the diabetic rat lens. *Arch Biochem Biophys* 318:191–199, 1995
6. Kato H, Shin D, Hayase F: 3-Deoxyglucosone crosslinks proteins under physiologic conditions. *Agric Biol Chem* 51:2009–2011, 1987
7. Baskaran S, Balasubramanian KA: Effect of methylglyoxal on protein thiol and amino groups in isolated rat enterocytes and colonocytes and activity of various brush border enzymes. *Indian J Biochem Biophys* 27:13–17, 1990
8. Reiffen K, Schneider F: A comparative study on proliferation, macromolecular synthesis, and energy metabolism of in vitro–grown Ehrlich ascites tumor cells in the presence of glucosone, galactosone and methylglyoxal. *J Cancer Res Clin Oncol* 107:206–210, 1984
9. Shinoda T, Hayase F, Kato H: Suppression of cell-cycle progression during the S-phase of rat fibroblasts by 3-deoxyglucosone: a Maillard reaction intermediate. *Biosci Biotechnol Biochem* 58:2215–2219, 1994
10. Knecht K, Feather M, Baynes J: Detection of 3-deoxyfructose and 3-deoxyglucosone in human urine and plasma: evidence for intermediate stages of the Maillard reaction in vivo. *Arch Biochem Biophys* 294:130–137, 1992
11. McLellan AC, Thornalley PJ, Benn J, Sonksen PH: Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications. *Clin Sci* 87:21–29, 1994
12. Niwa T, Takeda N, Miyazaki T, Yoshizumi H, Tatematsu A, Maeda K, Ohara M,

Tomiyama S, Niimura K: Elevated serum levels of 3-deoxyglucosone, a potent protein-cross-linking intermediate of the Maillard reaction, in uremic patients. *Nephron* 69:438–443, 1995
13. Beisswenger P, Lal S, Howell S, Stevens R, Siegel A, Yeo K, Randall W, Brown T, Szwergold B: The role of 3-deoxyglucosone and the activity of its degradative pathways in the etiology of diabetic microvascular disease. In *The Maillard Reaction in Foods and Medicine*. O'Brien J, Nursten HE, Crabbe MJC, Ames JM, Eds. Cambridge, U.K., Royal Society of Chemistry, 1998, p. 298–303
14. Beisswenger P, Touchette A, Howell S: The effect of hyperglycemia and diabetes on methylglyoxal and its detoxification pathways (Abstract). *Diabetologia* 40 (Suppl. 1):A588, 1997
15. Beisswenger PJ, Moore LL, Truls B-J, Curphey TJ: Increased collagen-linked pentosidine and advanced glycosylation end products in early diabetic nephropathy. *J Clin Invest* 92:212–217, 1993
16. Beisswenger PJ, Makita Z, Curphey TJ, Moore LL, Jean S, Brinck-Johnsen T, Bucala R, Vlassara H: Formation of immunochemical advanced glycosylation end products precedes and correlates with early manifestations of renal and retinal disease in diabetes. *Diabetes* 44:824–829, 1995
17. Monnier V, Vishwanath V, Frank KF, Elmets CAK, Sauthot P, Kohn RR: Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. *N Engl J Med* 314:403–408, 1986
18. Thornalley P: The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. *Biochem J* 269:1–11, 1990
19. Wells-Knecht K, Lyons T, McCance D, Thorpe S, Feather M, Baynes J: 3-Deoxyfructose concentrations are increased in human plasma and urine in diabetes. *Diabetes* 43:1152–1156, 1994
20. Fujii E, Iwase H, Ishii-Karakasa I, Yajima Y, Hotta K: The presence of 2-keto-3-deoxygluconic acid and oxoaldehyde dehydrogenase activity in human erythrocytes. *Biochem Biophys Res Commun* 210:852–857, 1995
21. Brownlee M: Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 15:1835–1843, 1992
22. Hammes H, Martin S, Federlin K, Geisen K, Brownlee M: Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc Natl Acad Sci U S A* 88:11555–11558, 1991
23. Zimmerman G, Meistrell M, Bloom O, Cockroft K, Bianchi M, Risucci D, Broome J, Farmer P, Cerami A, Vlassara H: Neurotoxicity of advanced glycosylation end products during focal stroke and neuroprotective effects of aminoguanidine. *Proc Natl Acad Sci U S A* 92:3744–3748, 1995
24. Lo TWC, Selwood T, Thornalley PJ: The reaction of methylglyoxal with aminoguanidine under physiological conditions and prevention of methylglyoxal binding to plasma proteins. *Biochem Pharmacol* 48:1865–1870, 1994
25. Hirsh J, Petrakova E, Feather M: The reaction of some dicarbonyl sugars with aminoguanidine. *Carbohydr Res* 232:125–130, 1992
26. Menzel EJ, Reihsner R: Alterations of biochemical and biomechanical properties of rat tail tendons caused by non-enzymatic glycation and their inhibition by dibasic amino acids arginine and lysine. *Diabetologia* 34:12–16, 1991
27. Menzel EJ, Reihsner R: Comparison of the effect of different inhibitors of the non-enzymatic glycation of rat tail tendons and bovine serum albumin. *Ann Clin Biochem* 33:241–248, 1996
28. Servetnick D, Bryant D, Wells-Knecht K, Wiesenfeld P: L-Arginine inhibits nonenzymatic glycation and advanced glycosylated end product formation of human serum albumin. *Amino Acids* 11:69–81, 1996
29. Casazza JP, Felver ME, Veech RL: The metabolism of acetone in rat. *J Biol Chem* 259:231–236, 1984
30. Beisswenger P, Jean S, Brinck-Johnsen T, Siegel A, Cavender J: Acetol production is increased in early diabetic nephropathy (Abstract). *Diabetes* 44 (Suppl. 1):23A, 1995
31. Ohmori S, Mori M, Kawase M, Tsuboi S: Determination of methylglyoxal as the 2-methylquinoxaline by high-performance liquid chromatography and its application to biological samples. *J Chromatogr* 414:149–155, 1987
32. Ohmori S, Iwamoto T: Sensitive determination of D-lactic acid in biological samples by high-performance liquid chromatography. *J Chromatogr* 431:239–247, 1988
33. Casazza JP, Fu JL: Measurement of acetol in serum. *Anal Biochem* 148:344–348, 1985
34. Lal S, Kappler F, Walker M, Orchard T, Beisswenger P, Szwergold B, Brown T: Quantitation of 3-deoxyglucosone levels in human plasma. *Arch Biochem Biophys* 342:254–260, 1997
35. Ruggiero D, Lecompte M, Rellier N, Legarde M, Weirnsperger N: Reaction of metformin with reducing sugars and dicarbonyl compounds. *Diabetologia* 40 (Suppl. 1):310, 1997
36. Thornalley P: Modification of the glyoxalase system in disease processes and prospects for therapeutic strategies. *Biochem Soc Trans* 21:531–534,1993
37. Baynes J: The role of oxidative stress in development of diabetic complications. *Diabetes* 40:105–112, 1991

Author Queries (please see Q in margin and underlined text)

Q1: Are affiliations correct as given, and do they correspond correctly with coauthors?
Q2: Should “3-deoxy 2-ketogluconic acid” be changed to “2-keto-3-deoxygluconic acid,” as in reference 20?
Q3: Figure 1 legend—Please spell out DHAP and confirm that G-3-P is spelled out correctly.
Q4: OK to change “D-lactic acid” to “DL” in the sentence beginning “Plasma levels of MG and 3-DG…”?
Q5: OK to change “DGA” to “3-DGA” in sentence beginning “Plasma levels of MG, 3-DG, and their major…”?
Q6: OK to replace “Methods” with “Assay systems,” since the former is included in the primary heading and the discussion of assay systems begins here?
Q7: Is 2-oxopentenoic acid spelled correctly? (Term could not be located for verification.)
Q8: Is PAD correctly spelled out as “pulsed amperometric detection”?
Q9: Is “The relationship between glycemic control…of their detoxification products” OK as edited (as meant?)?
Q10: OK to change “+” to “±” in “vs. 15.6 ± 9.3 years”?
Q11: Is “less than” sign correct in parentheses at end of sentence beginning “When we examined the relationship…”? Original version had “=<” (“equals” sign followed by “less than” sign).
Q12: Because references 24 and 36 were identical, I deleted 36 and changed 37 and 38 to 36 and 37, respectively. Please check text against references to verify that correct sources are cited.
Q13: Acknowledgments—Is NIH grant no. correct as given? (Capital “O” changed to zero in “R01-DK-50364.”)

Reference 6—Please provide all authors’ names. Could not locate reference via MEDLINE.
Reference 9—Please confirm accuracy of reference (not found through MEDLINE).
Reference 13—Have proceedings been published? If so, please provide publisher, city and year of publication, and page range.
Reference 14—Please provide volume no., if possible, and confirm accuracy of journal title. See also query for reference 35; is this abstract from the same source as ref. 35? If so, please supply appropriate information.
Reference 35: Please supply publisher, city and year of publication, and page range. See also reference 14.