α-Dicarbonyls Increase in the Postprandial Period and Reflect the Degree of Hyperglycemia

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OBJECTIVE — Chronic hyperglycemia is known to increase tissue glycation and diabetic complications, but controversy exists regarding the independent role of increased postprandial glucose excursions. To address this question, we have studied the effect of postprandial glycemic excursions (PPGEs) on levels of methylglyoxal (MG) and 3-deoxyglucosone (3-DG), two highly reactive precursors of advanced glycation end products (AGEs).

RESEARCH DESIGN AND METHODS — We performed 4-month crossover studies on 21 subjects with type 1 diabetes and compared the effect of premeal insulin lispro or regular insulin on PPGEs and MG/3-DG excursions. PPGE was determined after standard test meal (STMs) and by frequent postprandial glucose monitoring. HbA_{1c} and postprandial MG and D-lactate were measured by HPLC, whereas 3-DG was determined by gas chromatography/mass spectroscopy.

RESULTS — Treatment with insulin lispro resulted in a highly significant reduction in PPGEs relative to the regular insulin–treated group ($P = 0.0005$). However, HbA_{1c} levels were similar in the two groups, and no relationship was observed between HbA_{1c} and PPGE ($P = 0.93$). Significant postprandial increases in MG, 3-DG, and D-lactate occurred after the STM. Excursions of MG and 3-DG were highly correlated with levels of PPGE ($R = 0.55$, $P = 0.0002$ and $R = 0.61$, $P = 0.0004$; respectively), whereas a significant inverse relationship was seen between PPGE and D-lactate excursions ($R = 0.40$, $P = 0.01$). Conversely, no correlation was observed between HbA1c and postprandial MG, 3-DG, or D-lactate levels.

CONCLUSIONS — Increased production of MG and 3-DG occur with greater PPGE, whereas HbA1c does not reflect these differences. Reduced PPGE also leads to increased production of D-lactate, indicating a role for increased detoxification in reducing MG levels. The higher postprandial levels of MG and 3-DG observed with greater PPGE may provide a partial explanation for the adverse effects of glycemic lability and support the value of agents that reduce glucose excursions.

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Several major studies have shown a strong correlation between long-term average glycemia and the development of diabetic sequelae, such as retinopathy, nephropathy, neuropathy, and cardiovascular disease, in both type 1 and type 2 diabetes (1,2). The primary method used to assess long-term glycemic control in these studies has been the measurement of HbA_{1c} (3). Whereas this assay is useful in monitoring mean glycemia, a number of published studies, as well as data from our laboratory, suggest that HbA_{1c} is relatively insensitive to glycemic fluctuations, because diabetic populations with high or low glycemic excursions but with equal mean glucose levels have similar levels of HbA_{1c} (4,5). In particular, recent studies have demonstrated that HbA_{1c} levels may remain within the normal or minimally elevated range even when postprandial glucose values exceed 200 mg/dl (6). In agreement with this data, controlled studies comparing the efficacy of insulin lispro with biosynthetic human regular insulin have demonstrated a significant reduction in postprandial glucose concentrations 1 and 2 h after a meal in the insulin lispro group without any significant changes in HbA_{1c} levels (7,8). Overall, these observations and the frequent dissociation between glucose values observed by patient self-monitoring of glucose and HbA_{1c} values in the clinical setting (9) suggest that hemoglobin glycation, as currently measured, may not provide a fully accurate picture of temporal glycemic patterns.

Partly because of these methodological problems, the possible independent role of fluctuations in blood glucose (particularly due to postprandial hyperglycemia) in the development of diabetic vascular damage has not been as closely examined as that of mean hyperglycemia. Nevertheless, there are data to suggest that glycemic fluctuations may be important. In particular, it is well known that in type 1 diabetes, in which glycemic fluctuations are greater than those in type 2 diabetes, there is a significantly higher prevalence of diabetic complications, suggesting that transient glucose excursions may be an important independent variable (10–12). The mechanisms whereby repeated episodes of transient hyperglycemia may promote tissue damage is not clear. However, there are substantial data indicating that glucose toxicity in diabetes may be mediated through an increased production of α-ketoaldehydes such as 3-deoxy-

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Abbreviations: 3-DG, 3-deoxyglucosone; AGE, advanced glycosylation end product; DHAP, dihydroxyacetone phosphate; DHMC, Dartmouth-Hitchcock Medical Center; FL, fructoselysine; GC/MS, gas chromatography/mass spectroscopy; GSH, glutathione; HPLC, high-performance liquid chromatography; MG, methylglyoxal; PPGE, postprandial glycemic excursion; STM, standard test meal.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Goals for glycemic control*

	Regular insulin (mg/dl)	Insulin lispro (mg/dl)
Before meals	80–140	80–140
1 h after meals	120–180	100–160
2 h after meals	100–160	80–140
Before bedtime snack	100–160	100–160

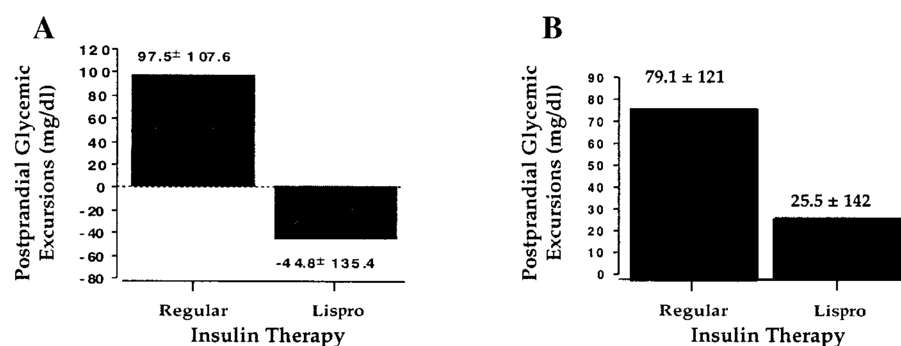

Figure 1—A: *Mean long-term postprandial glycemic excursions (over the second month of each study period) in subjects on insulin lispro and biosynthetic human regular insulin. The excursions in the insulin lispro group were expressed as means* ± *SD and were significantly lower than those in the regular insulin group* (P = *0.0005*). B: *Mean* ± *SD post-STM glycemic excursions at the end of each study period in the insulin lispro and biosynthetic human regular insulin groups* (P = *0.20*).

glucosone (3-DG) and methylglyoxal (MG), which are extremely reactive in the glycation of proteins (13–16) and are consequently toxic to cultured cells (16–18). Both of these sugars are elevated in diabetic plasma (19–22) in proportion to the degree of hyperglycemia and are involved in increased production of advanced glycosylation end products (AGEs), which are in turn associated with diabetic vascular and neuropathic complications (23–27).

Our recent data on the postprandial levels of 3-DG and MG in diabetic populations indicate that the concentrations of these compounds correlate well with postprandial glucose but show no correlation with HbA_{1c}. Considering the potential toxicity of α-ketoaldehydes, reducing postprandial elevations of these compounds may be beneficial in decreasing the incidence and rate of progression of retinopathy and nephropathy. Insulin lispro provides a more effective method to reduce postprandial glycemia than traditional crystalline zinc (regular) insulin and therefore provides a tool to perform clinical studies to test the hypothesis that reducing postprandial glycemia can also reduce dicarbonyl levels during this time period (8,28). The current study is designed to determine whether reducing postprandial glycemia and stabilizing glycemic fluctuations with insulin lispro would reduce the formation of reactive α-dicarbonyls.

RESEARCH DESIGN AND METHODS

Subjects

We studied subjects with type 1 diabetes who were currently on multiple insulin injections or who were willing to initiate such a regimen. To be eligible, subjects had to be between 15 and 65 years of age, be free of advanced diabetic complications, and have normal renal function (serum creatinine <1.5 mg/dl.) They were recruited from the Dartmouth-Hitchcock Medical Center (DHMC), which is the regional center for intensive diabetes care for a large geographic area in northern New England.

Study protocol. We studied 21 subjects by using a double-blind crossover design. Insulin lispro or humulin regular, combined with intermediate (NPH or lente) or long-acting (ultralente) insulins, was given over an initial 2-month period before switching to the alternate insulin. Subjects performed self-monitoring of blood glucose with an Accucheck Advantage Meter and recorded their results on a daily basis. Glucose values were determined five times daily to facilitate adjustments of insulin dosage, and seven blood glucose readings per day were measured once weekly to assess long-term postprandial glucose excursions. The former (five) were determined before breakfast, lunch, and dinner and 2 h after breakfast and dinner, and the latter (seven) were performed before each meal, 1 and 2 h after breakfast, and 1 and 2 h after dinner. Glucose values were recorded on study flow sheets and were faxed weekly to the study coordinator. Based on these records, glycemic control was managed by one of two certified diabetes educators who adjusted regular insulin, insulin lispro, and intermediate- and long-acting insulins to maintain optimal values with minimal episodes of hypoglycemia (see guidelines in Table 1).

At the end of each 2-month study period, a standard test meal (STM) was ingested, and fasting, 1-h, and 2-h blood glucose values were determined. The composition of the test meal was as follows: women, 369 calories (50 g carbohydrate [54%], 13 g protein [14%], and 13 g fat [32%]); and men, 529 calories (68 g carbohydrate [52%], 17 g protein [13%], and 21 g fat [35%]).

Glycemic profiles. Long-term postprandial glycemic excursions (PPGEs) were expressed as the mean of the sum of the difference between preprandial glucose values and the postbreakfast and postsupper plasma glucose values during the second month of each study period. The PPGEs after a standardized test meal performed at the end of each 2-month study period were calculated in a similar fashion (post-STM).

Protocol for assessment of 3-DG, MG, and D-lactate. Plasma levels of 3-DG, MG, and the MG metabolite D-lactic acid were measured initially and at the end of each 2-month study period in the fasting state and 1 and 2 h after the standard test meal (post-STM). Their postprandial excursions were calculated in a similar fashion to the glucose values.

Assessment of HbA_{1c}. At baseline and at the end of each 2-month study period, HbA_{1c} was measured by high-performance liquid chromatography (HPLC) (Diamat method; BioRad Irvine, CA) in the clinical laboratory at the DHMC (29).

Laboratory methods

Determination of 3-DG in plasma. 3-DG was assayed by a modification of a method described previously (30). 3-DG is reacted with 2,3-diamino naphthalene, extracted by ethyl acetate, and derivatized to its trimethylsilyl derivative for analysis by gas chromatography/mass spectroscopy (GC/MS). The major fragments generated by this adduct are at m/z of 295 and

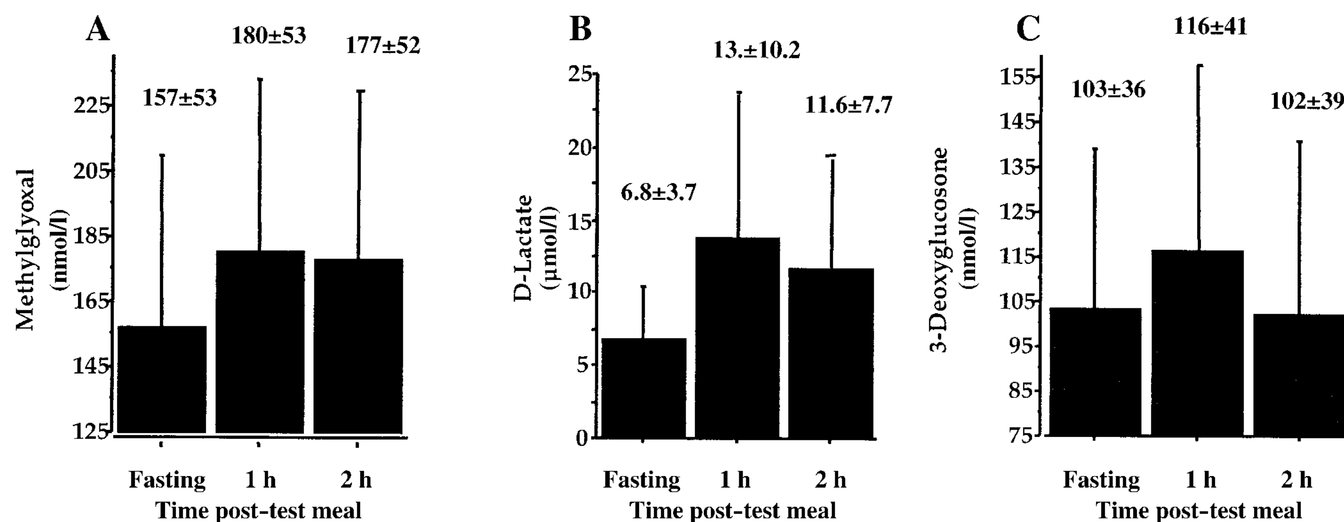

Figure 2—*Mean ± SD integrated postprandial excursions of methylglyoxal (A), D-lactate (B), and 3-deoxyglucosone (C) after an STM. The overall excursions (sum of 1- and 2-h values minus the baseline value) were 43.3 ± 75.3 nmol/l for MG, 11.8 ± 14.9 μmol/l for D-lactate, and 12.1 ± 46.9 nmol/l for 3-DG. As noted in RESULTS, these excursions were highly statistically significant.*

306. Using [U-^{13}C]-labeled 3-DG as an internal standard, 3-DG was quantitated by selected ion monitoring by the GC/MS method.

Determination of MG in plasma. Quantitation of MG was based on a method described by Ohmori et al. (31) using HPLC of the *O*-phenylenediamine derivative.

Determination of D-lactate in plasma. The D-lactate assay was performed on plasma using a modification of a technique described previously (32). D-Lactate concentrations were determined by measuring pyruvate on samples treated with D-lactate dehydrogenase and untreated samples. 2-Ketobutyric acid is used as an internal standard, and the *O*-phenylenediamine derivatized product was quantified by HPLC.

Statistical analysis

Analysis of differences in long-term PPGEs during insulin lispro or human regular insulin therapy were performed by the unpaired *t* test, which compared the means of the postprandial glucose values determined for the second month of each study period. Similar comparisons were performed for post-STM PPGEs at the end of each 2-month period. We also evaluated differences in the post-STM parameters by subtracting values obtained during insulin lispro from those obtained during regular insulin therapy and performing single-number T testing on the difference. The null hypothesis for these analyses was that no differences would exist between the groups. Using linear regression analysis, we also examined the relationship between post-STM glycemic excursions and post-STM levels of plasma MG, 3-DG, and D-lactate for the two major treatment periods. These data were corrected for possible confounding by other variables, such as age and duration of diabetes, by multivariate regression analysis.

RESULTS

Long-term postprandial glucose excursions, post-STM glucose excursions, and HbA_{1c}

We observed significantly lower mean postprandial glucose excursions during the second month of insulin lispro therapy relative to the values observed during regular insulin therapy. The mean long-term postprandial glucose excursions for the insulin lispro group during that time period was −44.8 ± 135.4 mg/dl, whereas PPGE in the regular insulin group was 97.5 ± 107.6 mg/dl (Fig. 1*A*). The mean difference between groups was 142.3 mg/dl, with a *P* value of 0.0005. The mean PPGE after the STM performed at the end of the insulin lispro treatment period (Fig. 1*B*) was also lower but not statistically different from that observed for the regular insulin group (25.5 ± 142.6 vs. 79.1 ± 120.9, $P = 0.20$). Similar results were observed for the mean long-term and post-STM postprandial glucose excursions when the difference between the regular and insulin lispro groups was analyzed by one-sample *t* testing ($P = 0.0024$ and 0.18, respectively). In agreement with previous reports, the HbA_{1c} levels determined at the end of the regular and insulin lispro treatment periods showed no difference between the two groups, despite the significant reductions in postprandial glucose levels in the lispro group (mean HbA_{1c} 7.83 ± 0.86% for regular and 7.59 ± 0.90% lispro, $P = 0.39$).

α-Dicarbonyls

The postprandial MG excursion, based on values obtained after the standardized test meal, showed a significant rise, as noted in Fig. 2*A* (excursion of 43.3 ± 75.3 nmol/l). When analyzed by paired *t* testing, the increase in MG levels at 1 and 2 h (22.95 and 20.38 nmol/l) was significant relative to baseline ($P = 0.0004$ and 0.004, respectively). When linear regression analysis was performed to assess the relationship between post–test meal PPGEs and postprandial MG excursions (Fig. 3*A*), a highly significant correlation was observed ($R = 0.55$, $P = 0.0002$). No correlation was found between HbA_{1c} levels measured at the end of each treatment period and postprandial plasma MG excursions ($R = 0.01$, $P = 0.95$; data not shown)

Plasma levels of D-lactate also increased in the post–test meal period (mean integrated postprandial excursion 11.8 ± 14.9 μmol/l), as seen in Fig. 2*B*. These values were significantly higher at 1 and 2 h (excursions of 7.04 and 4.74 μmol/l) relative to baseline ($P < 0.0001$

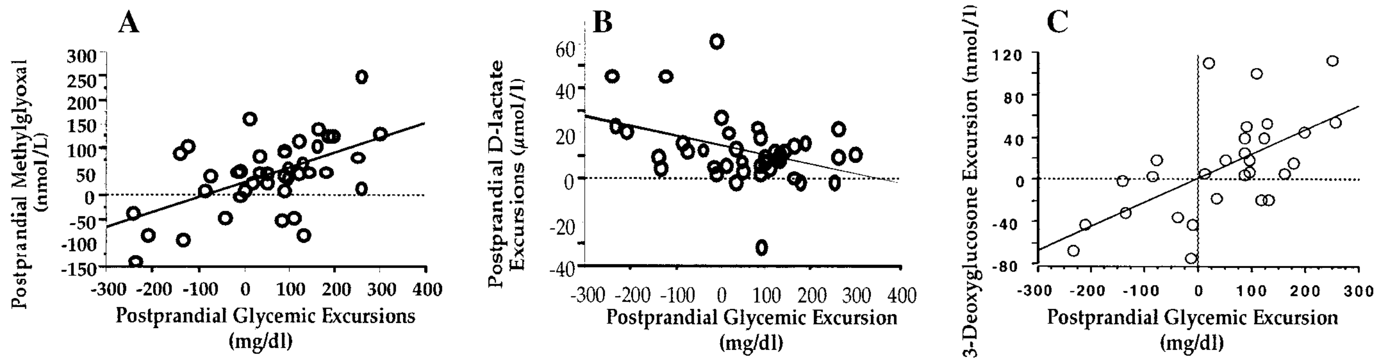

Figure 3—*The relationships between mean ± SD of post-STM glycemic excursions and the post-STM excursions of MG* (A), *D-lactate* (B), *and 3-DG* (C). *Highly significant relationships were observed between PPGEs and MG* (R = *0.55,* P = *0.0002*), *D-lactate* (R = *–0.4,* P = *0.01*), *and 3-DG* (R = *0.61,* P = *0.0004*).

and $P = 0.0001$, respectively). Higher values were observed in the insulin lispro group relative to the regular insulin group (15.7 ± 15.7 vs. 7.7 ± 13 μmol/l), and the difference between the two groups (−8.32 μmol/l) was significant at $P = 0.02$ by one-sample *t* testing. PPGEs and mean postprandial D-lactate excursions showed a significant inverse relationship ($R = 0.4$, $P = 0.01$) when linear regression analysis was performed (Fig. 3*B*).

Plasma levels of 3-DG also increased in the post–test meal period, with a mean integrated excursion of 12.1 ± 46.9 nmol/l (Fig. 2*C*). This increase was significant at 1 h (13.1 nmol/l, $P = 0.007$) relative to baseline but returned to baseline at 2 h (102 ± 39 vs. 103 ± 36 nmol/l). A significant correlation was also observed between PPGEs and postprandial 3-DG excursions ($R = 0.61$, $P = 0.0004$) when linear regression analysis was performed (Fig. 3*C*).

To account for possible confounding variables of age, duration of diabetes, and HbA_{1c}, the data were also analyzed by multiple regression analysis. Taking into consideration these potential confounders, the independent relationships between postprandial MG, 3-DG, and D-lactate excursions and PPGEs remained highly significant, with respective *P* values of 0.0008, 0.024, and 0.02. As expected, no relationship was observed between age, duration of diabetes, or HbA_{1c} levels and PPGEs.

CONCLUSIONS — There is increasing evidence that postprandial glucose may play an important role in the micro- and macrovascular complications of diabetes. This is evident in the Chicago and Honolulu Heart Studies and the Verona Study, all of which show an elevated risk of cardiovascular disease and all-cause mortality with increasing postprandial glucose values (33,34). In the Diabetes Intervention Study (35), increased postprandial glucose was associated with significantly increased 10-year cardiovascular mortality, whereas no such effect was seen with elevation of fasting glucose. The Diabetes Control and Complications Trail reported that a decrease in 2-h postprandial glucose levels, as well as in fasting glucose, led to a reduced risk of retinopathy, nephropathy, and neuropathy (36).

The mechanism by which postprandial hyperglycemia may mediate chronic complications in diabetes is speculative, and the means by which intensive insulin therapy may ameliorate or reduce the risk of complications is not known. Emerging evidence supports an important role for reactive α-dicarbonyls as intermediates in protein glycation and AGE formation, which in turn lead to diabetic complications (37–41). In the current study, we investigated the impact of postprandial glucose reduction with insulin lispro versus regular human insulin on the formation of α-dicarbonyls in patients with type 1 diabetes in which HbA_{1c} is not statistically different.

We demonstrated significant reduction in mean glycemic excursions in the 2-h postprandial interval in the insulin lispro treatment group relative to values obtained during therapy with biosynthetic human insulin. This confirms the findings from previous double-blind studies (7,8), although the differences in glycemic excursions between treatment groups were greater in the current study, possibly because of the more intensive clinical management of this smaller study group. Also, as observed previously, no significant difference was shown in HbA_{1c} when the insulin lispro and human regular insulin treatment periods were compared, despite the significant reduction in postprandial values in the lispro group. Both MG and 3-DG showed significant increases in the postprandial period, consistent with rapid meal-related changes in these dicarbonyls. Both dicarbonyls peaked at 1 h after a standardized test meal and returned toward baseline at 2 h, although the magnitude of the postprandial increase in MG levels was greater that that seen for 3-DG. There was a strong correlation between elevation of MG and 3-DG and glycemic excursion (Fig. 3*A* and *C*), indicating that the mean postprandial increase seen in both dicarbonyls was reduced by therapy that reduces postprandial glycemia. Multiple postprandial glucose measurements performed during the second month of each study period showed that the insulin lispro group demonstrated significant reductions in postprandial glycemia relative to regular insulin therapy. These repeated reductions in glycemia could lead to lower MG and 3-DG levels during the postprandial period in the insulin lispro group over time.

Glucose is an important source of MG, and it is not surprising that postprandial elevation of plasma glucose leads to an increase in plasma MG. Production of MG occurs primarily from the triosephosphate intermediates in the glycolytic pathway (dihydroxyacetone phosphate [DHAP] and glyceraldehyde-3-phosphate) by spontaneous nonenzymatic elimination of the phosphate group of

DHAP. Consequently, an increased flux through the glycolytic pathway associated with the ingestion of carbohydrate could account for the significant increase in postprandial MG levels observed in diabetic subjects (42,43). Because the reduction in PPGEs observed with insulin lispro is at least partially due to greater intracellulose transport, the observed decrease in MG production would have to be explained by improved downstream glycolytic flux resulting in lower levels of MG triosephosphate precursors. Increased flux through other metabolic pathways could also play a role in increasing MG production, including cytochrome P4502E1 catalyzed oxidation of acetone from ketone bodies (40), cleavage of Amadori products (44), or catabolism of threonine via aminoacetone (45). Some of these pathways have been shown to be increased in diabetes and may also be important in diabetic complications. Therefore, reductions in postprandial glucose, as demonstrated in the insulin lispro group, may also reduce MG by these mechanisms.

In addition to increased production, elevated levels of MG may be the consequence of decreased clearance of this compound by detoxification pathways. Most detoxification of MG is catalyzed by the oxidative glyoxalase system, leading to the production of the inert end product D-lactate (46). Previous studies to investigate the effect of diabetes on the activity of glyoxalase have produced mixed results: some showed a modest increase in activity in red blood cells and leukocytes (21,47), whereas others found reduced activity in renal cortex and liver in diabetic animals (48). Optimal activity of the glyoxalase system is dependent on adequate levels of reduced glutathione (GSH) (49), and the known association of hyperglycemia and depletion of GSH (50,51) could lead to impaired MG detoxification. Because oxidative stress is known to deplete GSH (37), its increase in the postprandial period could rapidly effect GSH levels (52).

In the current study, a significant increase in D-lactate was associated with reduced postprandial glycemia (Fig. 3*B*), supporting a role for increased rates of detoxification in decreasing MG levels. We also observed higher mean D-lactate levels in the insulin lispro group relative to the regular insulin group, further supporting this mechanism. Rapid repletion of GSH associated with improved postprandial glycemia during insulin lispro therapy, with an associated increased glyoxalase activity, could provide one possible explanation for the lower levels of MG observed. These observations suggest that enhanced MG detoxification by the glyoxalase system (53) may be at least partially responsible for the reduction in postprandial MG levels seen with treatments that reduce postprandial glycemia (48,51). An important role for overproduction of MG in these studies, however, is confirmed by the finding of significant MG elevations, even in the face of apparent increased glyoxalase activity.

The mechanisms responsible for increased levels of 3-DG observed in the postprandial period are less obvious than those for MG. One possible source is fructose-3-phosphate, which has been shown to derive from a ubiquitous fructose-3-kinase. Recent data from Delpierre et al. (54) and from our laboratory (B.S., S.H., P.B., unpublished data) suggest that fructoselysine (FL) is the preferred substrate for this enzyme and is more likely to be the major 3-DG precursor. Because the time frame of the formation of FL from glucose exceeds that observed in the postprandial period, it is unlikely that increased endogenous production of FL is responsible for the observed increase. The increase in postprandial 3-DG levels may result from conversion of dietary FL in the postprandial period (55,56). Because detoxification of 3-DG proceeds via reductive and oxidative pathways and we have previously shown that reductive conversion of 3-DG to 3-deoxyfructose can be impaired in diabetes (38,57), reduced detoxification of dietary FL associated with increased postprandial glycemia could be responsible for the observed increase. Because we have not measured the major detoxification products (3-deoxyfructose and 2-keto 3-deoxygluconate) in these studies, the role of decreased detoxification in the observed increase in postprandial 3-DG remains uncertain.

In summary, significant elevations of MG and 3-DG levels occur during the postprandial period in patients with type 1 diabetes. These elevations were closely correlated with the degree of postprandial glycemia, suggesting an important role for glycemic control during this period for the formation of α-dicarbonyls. Hyperglycemia, resulting in overproduction of MG and 3-DG, plays an important role in the observed elevations, although indirect evidence suggests that impaired detoxification of MG could partially account for increased MG levels. A significant reduction in postprandial glycemia in the group receiving insulin lispro therapy may selectively reduce α-dicarbonyl formation, resulting in reduced AGE production and the prevention of diabetic complications.

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