LEADER 3—Lipase and Amylase Activity in Subjects With Type 2 Diabetes

Baseline Data From Over 9000 Subjects in the LEADER Trial

William M. Steinberg, MD,* Michael A. Nauck, MD,† Bernard Zinman, MD,‡ Gilbert H. Daniels, MD,§ Richard M. Bergenstal, MD,∥ Johannes F.E. Mann, MD,¶ Lasse Steen Ravn, MD, PhD,† Alan C. Moses, MD,¶ Mette Stockner, MD,¶ Florian M.M. Baeres, MD,¶ Steven P. Marso, MD,** and John B. Buse, MD, PhD†† on behalf of the LEADER Trial investigators

From the *Department of Medicine, George Washington University Medical Center, Rockville, MD; †Department of Internal Medicine/Diabetology, Diabeteszentrum Bad Lauterberg, Bad Lauterberg im Harz, Germany; ‡Department of Medicine, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada; §Thyroid Unit and Department of Medicine, Massachusetts General Hospital Harvard Medical School, Boston, MA; ¶Park Nicollet Institute for Research and Education, International Diabetes Center, Minneapolis, MN; ∥Department of Medicine, Friedrich Alexander University of Erlangen, Erlangen, Germany; #Novo Nordisk A/S, Bagsvaerd, Denmark; **Division of Cardiology, Department of Internal Medicine, University of Texas Southwestern, Dallas, TX; and ††Division of Endocrinology, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC.

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Reprints: William M. Steinberg, MD, Department of Medicine, George Washington University, Rockville Internal Medicine Group, 1201 Seven Locks Rd, Rockville, MD 20854 (e-mail: wstein6905@aol.com).

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Dr Bergenstal has served on a scientific advisory board and consulted or performed 479 clinical researches with Abbott Diabetes Care, Amlyn, Bayer, Becton Dickinson, Boehringer Ingelheim, Bristol-Myers Squibb/AstraZeneca, Intuity, Calibra, DexCom, Eli Lilly and Company, Halozyme, Helmsley Trust, Hygieia, Johnson & Johnson, Medtronic, Merck, NIH, Novo Nordisk, ResMed, Roche, Sanofi, and Takeda. His employer, nonproprietary Park Nicollet Institute, contracts for his services, and no personal income goes to Dr Bergenstal. He has inherited stock in Merck.

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Conclusions: In this large study of type 2 diabetic patients, nearly 25% had elevated lipase or amylase levels without symptoms of acute pancreatitis. The clinician must take these data into account when evaluating abdominal symptoms in type 2 diabetic patients.

Key Words: pancreatitis, lipase, amylase, type 2 diabetes

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Subjects and Study Design

The LEADER trial is an international double-blind placebo-controlled trial currently evaluating the cardiovascular safety of liraglutide (www.clinicaltrials.gov; NCT01179048). There are 9340 subjects with type 2 diabetes who are randomized to receive liraglutide or placebo. The purpose of the current study is to assess baseline amylase and lipase activity in the LEADER population. We believe that this is the largest study of lipase and amylase activity in subjects with type 2 diabetes.

MATERIALS AND METHODS

Subjects and Study Design

The LEADER trial is an international double-blind placebo-controlled trial currently evaluating the cardiovascular safety of liraglutide (www.clinicaltrials.gov; NCT01179048). There are 9340 subjects with type 2 diabetes at high risk for cardiovascular events (with or without existing cardiovascular disease) enrolled 9309, with mean (SD) and median values of 66.2 (36.3) and 47.5 (43.7) U/L and 38.3 (28.2) U/L, respectively, of all subjects (n = 9273). The distribution of lipase values was skewed; therefore a logarithmic transformation was applied before analysis. Explanatory factors were prespecified and included sex, age, body mass index (BMI), smoking status, race, duration of diabetes, glycemic control (HbA1c), diabetes pretreatment, eGFR (modified diet in renal disease formula), autoimmune disease, thyroid disease, lipid levels, and use of certain medications (proton pump inhibitors, β-blockers, H2 receptor antagonists, and glucocorticoids). A multivariable linear normal analysis of covariance model was created, with the logarithmically transformed lipase and amylase values as dependent variables. The effect of each covariate was first derived on the log scale and then exponentially transformed to obtain an interpretation as relative activity of lipase and amylase. Effects of each factor are therefore adjusted to the set of all other factors and expressed in relative terms, with an effect equal to 1.0 representing “neutral” (also expressed as percent change = 0%). All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC).

All authors had access to the study data and reviewed and approved the final article.

RESULTS

Baseline characteristics of the LEADER population stratified by sex are presented in Table 1. No subjects had symptoms of pancreatitis at baseline, and lipase activity was available in 99.3% of all subjects (n = 9273). The distribution of lipase values is depicted in Figure 1A. Mean (SD) and median (interquartile range) values were 47.5 (43.7) U/L and 38.3 (28.2–53.9) U/L, respectively. Baseline amylase was available in 99.7% of all subjects (n = 9309), with mean (SD) and median values of 66.2 (36.3) and 59.0 (44.0–79.0) U/L, respectively (Fig. 1B). There were 9273 subjects with both valid amylase and lipase values.

Among all subjects with available baseline data for both enzymes, 16.6% (n = 1540) and 11.8% (n = 1094), respectively, had lipase and amylase activity above the ULN. Measurements greater
than 3 times the ULN for lipase were seen in 1.2% of subjects and 0.2% for amylase (Figs. 1A, B; Table 2).

Results of the same multivariable regression model performed separately for lipase and amylase are presented in Table 3A and B.

 Severely reduced eGFR (<30 mL/min per 1.73 m², corresponding to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative chronic kidney disease [CKD] stage 4–5) was associated with the largest effect on increasing lipase and amylase.

### TABLE 1. Baseline Demographics

|                          | Total (N = 9340) | Female (n = 3337) | Male (n = 6003) |
|--------------------------|------------------|-------------------|-----------------|
| Age, y                   | 64.3 (7.2); 64.0 | 64.3 (7.2); 64.0  | 64.2 (7.3); 64.0 |
| Body weight, kg          | 91.8 (21.0); 89.9 | 84.7 (19.5); 82.4 | 95.7 (20.8); 93.2 |
| BMI, kg/m²               | 32.5 (6.3); 31.7 | 33.6 (6.8); 32.8  | 31.9 (5.9); 31.2 |
| HbA1c, %                 | 8.7 (1.5); 8.3   | 8.8 (1.6); 8.4    | 8.6 (1.5); 8.2  |
| Diabetes duration, y     | 12.7 (8.0); 11.3 | 13.3 (8.3); 11.8  | 12.4 (7.8); 11.1 |
| Blood pressure, mm Hg    |                  |                   |                 |
| Systolic                 | 137.7 (18.6); 137.0 | 139.1 (19.2); 138.0 | 136.9 (18.2); 136.0 |
| Diastolic                | 77.9 (10.5); 78.5 | 78.1 (10.7); 79.0 | 77.8 (10.3); 78.5 |
| Heart rate, beats per minute | 73.2 (11.4); 72.0 | 74.4 (10.9); 74.0 | 72.5 (11.6); 72.0 |
| eGFR, mL/min per 1.73 m² |                  |                   |                 |
| <30                      | 177 (1.9)        | 76 (2.3)          | 101 (1.7)       |
| 30–59                    | 1854 (19.9)      | 700 (21.0)        | 1154 (19.2)     |
| 60–89                    | 3860 (41.3)      | 1353 (40.5)       | 2507 (41.8)     |
| ≥90                      | 3447 (36.9)      | 1207 (36.2)       | 2240 (37.3)     |
| Glucose lowering treatment |                |                   |                 |
| Diet or no treatment     | 504 (5.4)        | 128 (5.3)         | 376 (5.5)       |
| Insulin alone            | 665 (7.1)        | 279 (8.4)         | 386 (6.4)       |
| Oral glucose lowering*   | 4931 (52.8)      | 1687 (50.6)       | 3244 (54.0)     |
| Oral glucose lowering + insulin | 3240 (34.7)    | 1195 (35.8)       | 2045 (34.1)     |
| Prior cardiovascular disease | 7592 (81.3)    | 2544 (76.2)       | 5048 (84.1)     |
| No. oral antihyperglycemic medications used |                |                   |                 |
| 1                        | 1917 (20.5)      | 679 (20.3)        | 1238 (20.6)     |
| 2                        | 2686 (28.8)      | 914 (27.4)        | 1772 (29.5)     |
| >2                       | 328 (3.5)        | 94 (2.8)          | 234 (3.9)       |
| Smoking                  |                  |                   |                 |
| Current                  | 1130 (12.1)      | 281 (8.4)         | 849 (14.1)      |
| Previous                 | 4337 (46.4)      | 926 (27.7)        | 3411 (56.8)     |
| Never                    | 3873 (41.5)      | 2130 (63.8)       | 1743 (29.0)     |

Data shown are mean (SD); median for continuous variables and n (%) for groupings.

*Not used in combination with insulin.

than 3 times the ULN for lipase were seen in 1.2% of subjects and 0.2% for amylase (Figs. 1A, B, Table 2).

Results of the same multivariable regression model performed separately for lipase and amylase are presented in Table 3A and B.

Severely reduced eGFR (<30 mL/min per 1.73 m², corresponding to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative chronic kidney disease [CKD] stage 4–5) was associated with the largest effect on increasing lipase and amylase.

### FIGURE 1. Distribution of (A) lipase and (B) amylase values in all randomized subjects. Horizontal lines (from lowest to highest value on y-axis) denote 1, 2, 3, and 10 times ULN values.
activity. There were no effects on either lipase or amylase activity from history of CVD, thyroid disease, autoimmune disease, pancreatitis, or gallstones; diabetes duration; or glucocorticoid use. Several characteristics unrelated to eGFR were associated with smaller but statistically significant changes in serum lipase and amylase activity. For lipase, Asian race, higher HbA1c, higher serum triglyceride concentrations, presence of diabetic nephropathy, and treatment with oral antihyperglycemic drugs were associated with increasing activity, whereas female sex, black race, increasing age, higher BMI, use of proton pump inhibitors, higher serum low density lipoprotein (LDL) cholesterol concentrations, and insulin treatment were associated with decreasing activity. For amylase, Asian and black races, higher serum high density lipoprotein (HDL) cholesterol concentrations, and presence of diabetic nephropathy were associated with increasing activity, whereas female sex, black race, increasing age, higher BMI, increasing HbA1c, history of smoking, increasing age, increasing BMI, increasing HbA1c, history of cholecystitis, gallstone disease, and use of β-blockers, H2 receptor antagonists, and proton pump inhibitors were associated with decreasing activity.

The distribution of baseline lipase and amylase values by eGFR in subjects with known values for eGFR, lipase, and amylase (n = 9271) is shown in Table 2. There was an association between progressive eGFR reductions and elevations in both (either >ULN or >3× ULN). However, a majority of the 177 subjects with eGFR less than 30 mL/min per 1.73 m² (corresponding to >ULN) had normal lipase (65%) and normal amylase (73%) activity. Of those subjects with normal eGFR (≥90 mL/min per 1.73 m²), 12.2% had elevated lipase and 7.7% had elevated amylase activity.

Table 4 analyzes subjects with normal or elevated levels of lipase and amylase. Logistic regression indicated that worsening eGFR was associated with larger proportions of subjects with elevations in either enzyme, findings recognized in other studies. However, of those subjects with normal renal function (eGFR ≥90 mL/min per 1.73 m²), 12.2% had elevated levels of lipase and 7.7% had elevated levels of amylase activity. Other factors shown to have a smaller impact on either increasing or decreasing lipase or amylase activity included certain drugs (proton pump inhibitors, insulin, and oral antihyperglycemics), BMI, HbA1c level, age, and race. Risk factors, known to be associated with pancreatitis, such as a history of pancreatitis or gallstones, did not seem to affect baseline activity of either lipase or amylase. Other environmental factors such as alcohol intake and smoking have been described as risk factors for the development of pancreatitis. Although a history of alcohol consumption was not systematically collected for the present population, smoking was analyzed and did not have an independent effect on pancreatic enzyme activity.

It is unclear why lipase and amylase activity is elevated in a significant portion of subjects with type 2 diabetes. To understand these findings, the complex physiologic nature of these biochemical markers requires comment. It is known that there are many sources of amylase and lipase in the human body. Amylase exists in the small intestine, liver, and salivary glands and is released as a result of trauma and disease. Lipase is also released from the pancreas, but its exact source is still under investigation. It is believed that lipase is released in response to the presence of triglycerides in the diet, and that amylase is released in response to the presence of carbohydrates in the diet. It is also possible that there is a genetic component to the elevated levels of lipase and amylase in the population of type 2 diabetes subjects.

### Table 2. Distribution of Baseline Lipase and Amylase Values by eGFR (mL/min per 1.73 m²) in Subjects With Known eGFR, Lipase, and Amylase Measures (n = 9271)

| A. Lipase, U/L | eGFR | >ULN | >3× ULN |
|---------------|------|------|--------|
| <30 (n = 177) | 35.03 | 1.69 |
| 30–59 (n = 1840) | 24.02 | 1.25 |
| 60–89 (n = 3837) | 16.13 | 1.49 |
| ≥90 (n = 3417) | 12.17 | 0.85 |
| Total (n = 9271) | 16.60 | 1.21 |

ULN = 63 U/L

| B. Amylase | eGFR | >ULN | >3× ULN |
|-----------|------|------|--------|
| <30 (n = 177) | 26.55 | 0.57 |
| 30–59 (n = 1840) | 18.70 | 0.16 |
| 60–89 (n = 3837) | 11.31 | 0.26 |
| ≥90 (n = 3417) | 7.72 | 0.06 |
| Total (n = 9271) | 11.75 | 0.17 |

ULN = 100 U/L

Data are presented as percent.
### TABLE 3. Multivariable Log-Linear Regression Models Performed Separately for Lipase and Amylase*

| Effect | 95% Confidence Limits | P     | Percentage Change |
|--------|-----------------------|-------|-------------------|
| **A. Lipase** | | | |
| eGFR, per 10 mL/min per 1.73 m² | | | |
| <30 | 1.540 | 1.413–1.678 | <0.0001 | 54.0 |
| 30–59 | 1.287 | 1.243–1.334 | <0.0001 | 28.7 |
| 60–89 | 1.121 | 1.093–1.150 | <0.0001 | 12.1 |
| ≥90† | 1.000 | | | |
| Sex | | | |
| Female | 0.955 | 0.931–0.980 | 0.0005 | −4.5 |
| Male† | 1.000 | | | |
| Smoking | 0.972 | 0.939–1.006 | 0.1052 | −2.8 |
| Race | | | |
| Asian | 1.018 | 0.979–1.059 | 0.3659 | 1.8 |
| Black | 0.951 | 0.913–0.991 | 0.0178 | −4.9 |
| Other | 1.026 | 0.972–1.084 | 0.3487 | 2.6 |
| White† | 1.000 | | | |
| Prior cardiovascular disease | | | |
| No | 1.011 | 0.979–1.044 | 0.5227 | 1.1 |
| Yes† | 1.000 | | | |
| Age, per 10-year increment | 0.942 | 0.925–0.958 | <0.0001 | −5.8 |
| BMI, per 10-kg/m² increment | 0.942 | 0.924–0.960 | <0.0001 | −5.8 |
| HbA1c, per % increment | 1.018 | 1.011–1.026 | <0.0001 | 1.8 |
| Diabetes duration, per year increment | 0.999 | 0.998–1.001 | 0.2060 | −0.1 |
| Autoimmune disorder | 1.040 | 0.974–1.111 | 0.2417 | 4.0 |
| Thyroid disease | 0.971 | 0.940–1.003 | 0.0792 | −2.9 |
| β-Blocker use | 1.015 | 0.991–1.039 | 0.1289 | 1.5 |
| H2 receptor antagonist use | 0.980 | 0.921–1.043 | 0.5254 | −2.0 |
| Proton pump inhibitor use | 0.896 | 0.871–0.921 | <0.0001 | −10.4 |
| Glucocorticoid use | 0.947 | 0.883–1.017 | 0.1346 | −5.3 |
| LDL cholesterol, per mmol/L increment | 0.967 | 0.955–0.979 | <0.0001 | −3.3 |
| HDL cholesterol, per mmol/L increment | 1.018 | 0.978–1.059 | 0.3933 | 1.8 |
| Triglycerides, per mmol/L increment | 1.048 | 1.033–1.063 | <0.0001 | 4.8 |
| History of pancreatitis | 1.000 | 0.932–1.073 | 0.9940 | −0.0 |
| History of cholecystitis | 0.965 | 0.921–1.012 | 0.1397 | −3.5 |
| History of diabetic nephropathy | 1.040 | 1.014–1.066 | 0.0025 | 4.0 |
| History of gallstones | 1.032 | 0.995–1.071 | 0.0938 | 3.2 |
| Diabetes pretreatment | | | |
| Insulin + oral antihyperglycemics | 1.024 | 0.972–1.078 | 0.3776 | 2.4 |
| Insulin only | 0.869 | 0.816–0.926 | <0.0001 | −13.1 |
| Oral antihyperglycemics only | 1.114 | 1.059–1.171 | <0.0001 | 11.4 |
| None/diet† | 1.000 | | | |
| **B. Amylase** | | | |
| eGFR, per 10 mL/min per 1.73 m² | | | |
| <30 | 1.466 | 1.368–1.572 | <0.0001 | 46.6 |
| 30–59 | 1.261 | 1.225–1.297 | <0.0001 | 26.1 |
| 60–89 | 1.114 | 1.091–1.137 | <0.0001 | 11.4 |
| ≥90† | 1.000 | | | |
| Sex | | | |
| Female | 0.891 | 0.873–0.910 | <0.0001 | −10.9 |
| Male† | 1.000 | | | |
| Smoking | 0.968 | 0.941–0.995 | 0.0201 | −3.2 |
| Race | | | |
| Asian | 1.158 | 1.122–1.196 | <0.0001 | 15.8 |
| Black | 1.358 | 1.314–1.404 | <0.0001 | 35.8 |

(continued on next page)
in 2 isoenzyme forms: p-amylase (mostly originating from the pancreas) and s-amylase (mostly originating from the salivary glands). However, other sources in the body can contain various mixtures of both forms, including the fallopian tubes, thyroid, small intestine, liver, placenta, testis, skeletal muscle, and spleen, as well as various tumors.24–29 In the asymptomatic subject, serum amylase is made up of a mixture of p-amylase (approximately 40%) and s-amylase (approximately 60%). In acute pancreatitis, the predominant isoenzyme in the serum is p-amylase. However, p-amylase level has been reported to be markedly elevated in other nonpancreatic acute conditions such as ovarian cyst rupture.30 Several types of lipase are also present in the human body, including gastric, pancreatic, hepatic, endothelial, and muscle.31–33 The pancreas itself contains several different lipases, including pancreatic triglyceride lipase, pancreatic lipase-related proteins 1 and 2, carboxyl ester lipase, and phospholipase A2.34

**TABLE 3. (Continued)**

| B. Amylase | Effect | 95% Confidence Limits | P | Percentage Change |
|------------|--------|------------------------|---|------------------|
| Race       |        |                        |   |                  |
| Other      | 1.168  | 1.118–1.221             | <0.0001 | 16.8 |
| White†     | 1.000  |                        |   |                  |
| Prior cardiovascular disease |        |                        |   |                  |
| No         | 0.989  | 0.964–1.015             | 0.4094 | −1.1 |
| Yes†       | 1.000  |                        |   |                  |
| Age, per 10-year increment | 0.981  | 0.967–0.995             | 0.0071 | −1.9 |
| BMI, per 10-kg/m² increment | 0.853  | 0.840–0.866             | <0.0001 | −14.7 |
| HbA1c, per % increment | 0.984  | 0.978–0.990             | <0.0001 | −1.6 |
| Diabetes duration, per year increment | 1.000  | 0.999–1.001             | 0.7723 | 0.0 |
| Autoimmune disorder | 1.012  | 0.959–1.067             | 0.6742 | 1.2 |
| Thyroid disease | 0.980  | 0.955–1.006             | 0.1390 | −2.0 |
| β-Blocker use | 0.967  | 0.949–0.985             | 0.0005 | −3.3 |
| H2 receptor antagonist use | 0.945  | 0.899–0.994             | 0.0283 | −5.5 |
| Proton pump inhibitor use | 0.934  | 0.913–0.955             | <0.0001 | −6.6 |
| Glucocorticoid use | 0.976  | 0.921–1.033             | 0.4004 | −2.4 |
| LDL cholesterol, per mmol/L increment | 0.988  | 0.978–0.998             | 0.0201 | −1.2 |
| HDL cholesterol, per mmol/L increment | 1.079  | 1.045–1.115             | <0.0001 | 7.9 |
| Triglycerides, per mmol/L increment | 0.994  | 0.983–1.006             | 0.3214 | −0.6 |
| History of pancreatitis | 0.970  | 0.916–1.027             | 0.2923 | −3.0 |
| History of cholecystitis | 0.937  | 0.902–0.974             | 0.0009 | −6.3 |
| History of diabetic nephropathy | 1.044  | 1.023–1.066             | <0.0001 | 4.4 |
| History of gallstones | 1.006  | 0.976–1.036             | 0.7160 | 0.6 |
| Diabetes pretreatment |        |                        |   |                  |
| Insulin + oral antihyperglycemics | 1.033  | 0.991–1.077             | 0.1245 | 3.3 |
| Insulin only | 0.966  | 0.918–1.017             | 0.1848 | −3.4 |
| Orally antihyperglycemics only | 1.040  | 0.998–1.083             | 0.0591 | 4.0 |
| None/diet† | 1.000  |                        |   |                  |

*Effective number of subjects contributing to analysis: n = 8824 (no missing values allowed).
†Reference.

in 2 isoenzyme forms: p-amylase (mostly originating from the pancreas) and s-amylase (mostly originating from the salivary glands). However, other sources in the body can contain various mixtures of both forms, including the fallopian tubes, thyroid, small intestine, liver, placenta, testis, skeletal muscle, and spleen, as well as various tumors.24–29 In the asymptomatic subject, serum amylase is made up of a mixture of p-amylase (approximately 40%) and s-amylase (approximately 60%). In acute pancreatitis, the predominant isoenzyme in the serum is p-amylase. However, p-amylase level has been reported to be markedly elevated in other nonpancreatic acute conditions such as ovarian cyst rupture.30 Several types of lipase are also present in the human body, including gastric, pancreatic, hepatic, endothelial, and muscle.31–33 The pancreas itself contains several different lipases, including pancreatic triglyceride lipase, pancreatic lipase-related proteins 1 and 2, carboxyl ester lipase, and phospholipase A2.34 It is generally

**TABLE 4. Distribution of Lipase and Amylase Activity According to eGFR (mL/min per 1.73 m²)**

| eGFR     | Lipase Normal, Amylase Normal | Lipase Normal, Amylase >ULN | Amylase Normal, Lipase >ULN | Amylase >ULN, Lipase >ULN |
|----------|--------------------------------|----------------------------|-----------------------------|---------------------------|
| <30 (n = 177) | 95 (53.7)                     | 20 (11.3)                  | 35 (19.8)                  | 27 (15.3)                  |
| 30–59 (n = 1840) | 1221 (66.4)                  | 177 (9.6)                 | 275 (15.0)                 | 167 (9.1)                 |
| 60–89 (n = 3837) | 2998 (78.1)                  | 220 (5.7)                 | 405 (10.6)                 | 214 (5.6)                 |
| 290 (n = 3417) | 2852 (83.5)                   | 149 (4.4)                 | 301 (8.8)                  | 115 (3.4)                 |
| Total (n = 9271) | 7166 (77.3)                   | 566 (6.1)                 | 1016 (11.0)                | 523 (5.6)                 |

*Data are presented as n (%).
†ULN = 63 U/L
‡ULN = 100 U/L
assumed that basal serum lipase is of pancreatic origin. However, the actual proportion of basal serum lipase from other sources is unknown. The lipase assay in LEADER uses colipase and bile salts and hence is thought to be specific for pancreatic lipase activity. However, some experts question whether these assays can truly distinguish pancreatic from nonpancreatic lipases (personal communication: Mark Lowe, MD, PhD, vice-chairman and professor of pediatrics at University of Pittsburgh School of Medicine).

Lipase and amylase are synthesized in the pancreatic acinar cell, packaged in zymogen granules, and thought to reach the blood stream by leakage of these enzymes from the basal lateral membrane of the acinus into the surrounding capillaries or via the pancreatic lymph with drainage through the thoracic duct.31,35 Secretory studies in animals and humans demonstrate an effect of a high-carbohydrate or high-fat diet on the composition of amylase and lipase in these granules.36,37 However, it is unknown whether fasting or food consumption has an effect on levels of serum activity for either enzyme. Amylase is filtered by the renal glomerulus and excreted into urine where it can be measured. Lipase is also filtered at the glomerulus but almost completely reabsorbed by the renal tubules, leaving negligible amounts of lipase in the urine.38–40 Rat studies suggest that amylase, and particularly lipase, is degraded by the renal tubules; however, there are no data on this process in humans.40

Given the limited knowledge on the origins and excretion/degradation of serum amylase and lipase, there are several possible explanations for elevated activity in the diabetic population. Our data confirm prior observations that reduced eGFR raises serum lipase and amylase activity. Possible mechanisms include reduced glomerular filtration, a change in tubular reabsorption, degradation of the enzymes, or all of these factors. It should be noted, however, that reduced eGFR does not affect both enzymes equally. In the group with eGFR less than 30 mL/min per 1.73 m² (CKD stage 4–5), amylase activity alone was elevated in 11%, lipase activity alone was elevated in 19.8%, and both were elevated in 15.3% (Table 4). Neither of these findings nor our finding that more than one half of the subjects with eGFR less than 65 mL/min per 1.73 m² had normal lipase and amylase activity can be explained on the basis of known mechanisms.

It is unclear what is driving elevated lipase and amylase activity unrelated to kidney function (12% and 7% of the entire cohort, respectively). Subclinical pancreatic inflammation may be one explanation. Prior studies suggest that subclinical chronic pancreatitis may be increased in subjects with type 2 diabetes. In an asymptomatic diabetes population, Hardt and colleagues41 showed that 35% (compared with 18% in controls) of subjects with diabetes had a low fecal elastase concentration, which is thought to be a marker of pancreatic insufficiency or chronic pancreatitis. One autopsy study revealed that subjects with diabetes had a greater level of pancreatic fibrosis and evidence of chronic pancreatitis than controls.42 However, if pancreatic inflammation explained the enzyme elevations, it might be expected that lipase and amylase activity would be elevated to a similar degree. Nevertheless, our data, as well as that from others,15–18 indicate that lipase activity elevations predominate over amylase in the percentage of subjects affected. In addition, amylase activity is elevated about 25% of the time without an elevation in lipase (Table 4).

Because amylase and lipase come from other potential sites (gastric, hepatic, endothelial, muscle), it is possible that these serum enzymes in diabetes may originate in areas other than the pancreas. It is also possible that diabetes—or the drugs that subjects with type 2 diabetes take—may cause changes in the secretion or excretion/degradation of these enzymes by the kidney that may also lead to elevations. An increased glucose concentration, as found in poorly controlled diabetes or ketoacidosis, has previously been reported to affect enzyme activity43; however, this finding in relation to HbA1c could not be confirmed.16 In our population, a higher HbA1c was related to higher lipase but lower amylase activity. The impact of confounding risk factors that may be present and more common in a type 2 diabetes population, such as cholelithiasis or hypertriglyceridemia, also does not provide a clear picture. Although some of these factors showed significant associations due to the large sample size in LEADER, the effect sizes were small and presented no consistent trend (eg, HbA1c).

The present study is strengthened by its robust sample size, consisting of the largest study to date of lipase and amylase in a type 2 diabetes population. On the other hand, LEADER specifically enrolled subjects at high cardiovascular risk, potentially limiting the generalizability of our findings. However, there is no reason to expect that cardiovascular disease per se would change pancreatic enzyme activity. Furthermore, similar elevations in lipase and amylase were noted in other studies that did not specifically include subjects at high cardiovascular risk.15,16,18 Our study measured total amylase and not pancreatic (p-) amylase; thus different findings may have resulted from use of this assay. Others have in fact measured p-amylase in subjects with diabetes and found that 6% had an elevation, approximately one half of our results.16

In conclusion, our study shows that among subjects with type 2 diabetes, 16.6% had elevated lipase (including 1.2% that

| TABLE 5. Other Studies Reporting Elevated Lipase and Amylase Levels in Subjects With Type 2 Diabetes |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Lipase                          | Amylase         | Lipase          | Amylase         |
| >ULN                            | >3× ULN         | >ULN            | >3× ULN         |
|---------------------------------|-----------------|-----------------|-----------------|
| Lando et al15 (n = 33)           | 18.0            | NR              | 3.0             | NR              |
| Bastyr et al17 (n = 440)         | 13.0            | NR              | 5.0             | NR              |
| Malloy et al16 (n = 514)         | 13.0            | 1.0             | 6.0             | 0.5             |
| Steinberg et al18 (n = 987)      | 20.4            | 2.1             | 8.5             | 0.1             |
| Exenatide LAR13                  | 11.4†           | NR              | 4.8†            | NR              |
| LEADER (present study) (n = 9273)| 16.6            | 1.2             | 11.7            | 0.17            |

*Subjects with lipase measurements (n = 2153).
†Subjects with amylase measurements (n = 1199).
NR, not recorded.
have a greater than 3-fold elevation) and 11.8% had elevated amylase activity (0.2% >3-fold elevated). Although it may be the single most important parameter responsible for some of the elevations, reduced kidney function is not responsible for most. Other significant causes remain unknown. Because pancreatic enzyme elevations (especially a 3-fold elevation) are crucial for the diagnosis of acute pancreatitis (along with significant upper abdominal pain and positive imaging with computed tomography, magnetic resonance imaging, or abdominal ultrasound),16 it is important for the gastroenterologist to be aware of the pitfalls of these laboratory studies in subjects with diabetes. In addition, certain antidiabetic drugs such as GLP-1 receptor agonists may raise pancreatic enzymes above baseline, further confusing the enzyme picture.18 Therefore, it is important for the clinician to pay extra attention to clinical symptoms and imaging in the diabetic population when considering the diagnosis of acute pancreatitis.

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REFERENCES
1. Noel RA, Braun DK, Patterson RE, et al. Increased risk of acute pancreatitis and biliary disease observed in patients with type 2 diabetes: a retrospective cohort study. Diabetes Care. 2009;32:834–838.
2. Girman CJ, Kou TD, Cai B, et al. Patients with type 2 diabetes mellitus have higher risk for acute pancreatitis compared with those without diabetes. Diabetes Obes Metab. 2010;12:766–771.
3. Garg R, Chen W, Pendergrass M. Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. Diabetes Care. 2010;33:2349–2354.
4. Gonzalez-Perez A, Schlienger RG, Rodriguez LA. Acute pancreatitis in association with type 2 diabetes and anti-diabetic drugs: a population-based cohort study. Diabetes Care. 2010;33:2580–2585.
5. Daner PS, Dimarco PE. Exenatide (exendin-4)-induced pancreatitis: a case report. Diabetes Care. 2006;29:471.
6. Lee PH, Stockton MD, Franks AS. Acute pancreatitis associated with liraglutide. Am Pharmacother. 2011;45:e22.
7. Tripathy NR, Basha S, Jain R, et al. Exenatide and acute pancreatitis. J Assoc Physicians India. 2008;56:978–988.
8. Singh S, Chang HY, Richards TM, et al. Glucagonlike peptide 1-based therapies and risk of hospitalization for acute pancreatitis in type 2 diabetes mellitus: a population-based matched case-control study. JAMA Intern Med. 2013;173(7):534–539.
9. Elashoff M, Matveyenko AV, Gier B, et al. Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1–based therapies. Gastroenterology. 2011;141:150–156.
10. Dore DD, Seeger JD, Arnold Chan K. Use of a claims-based active drug safety surveillance system to assess the risk of acute pancreatitis with exenatide or sitagliptin compared to metformin or glyburide. Curr Med Res Opin. 2009;25:1019–1027.
11. Alves C, Batel-Marques F, Macedo AF. A meta-analysis of serious adverse events reported with exenatide and liraglutide: acute pancreatitis and cancer. Diabetes Res Clin Pract. 2012;98:271–284.
12. Dore DD, Bloomen GL, Wienten M, et al. A cohort study of acute pancreatitis in relation to exenatide use. Diabetes Obes Metab. 2011;13:559–566.
13. Exenatide LAR New Drug Application Clinical Review. 2012. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/022205Orig1s000MedR.pdf. Accessed November 19, 2013.
14. DeVries JH, Bain SC, Rodbard HW, et al. Sequential intensification of metformin treatment in type 2 diabetes with liraglutide followed by randomized addition of basal insulin prompted by A1C targets. Diabetes Care. 2012;35:1446–1454.
15. Lando HM, Attar M, Dua AP. Elevated amylase and lipase levels in patients using glucagon-like peptide-1 receptor agonists or dipeptidyl-peptidase-4 inhibitors in the outpatient setting. Endocr Pract. 2012;18:472–477.
16. Malloy J, Gurney K, Shan K, et al. Increased variability and abnormalities in pancreatic enzyme concentrations in otherwise asymptomatic subjects with type 2 diabetes. Diabetes Metab Syndr Obes. 2012;5:419–424.
17. Baxtry EJ, Barkin J, Botros FT, et al. High incidence of elevated lipase and amylase in type 2 diabetes patients (T2DM) (abstract). Pancreas. 2009;38:980.
18. Steinberg WM, Rosenstock J, DeVries JH, et al. Elevated serum lipase activity in adults with type 2 diabetes and no gastrointestinal symptoms (abstract). Gastroenterology. 2012;142(suppl 1):S93–S94.
19. Steinberg WM, DeVries JH, Wadden TA, et al. Longitudinal monitoring of lipase and amylase in adults with type 2 diabetes and obesity: evidence from two phase 3 randomized clinical trials with once daily GLP-1 analog liraglutide. Gastroenterology. 2012;142(suppl 1):S850–S851.
20. Banks PA, Bollen TL, Dervenis C, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62:102–111.
21. Marso SP, Poulter NR, Nissen SE, et al. Design of the liraglutide effect and action in diabetes: evaluation of cardiovascular outcome results (LEADER) trial. Am Heart J. 2013;166:823–830 e825.
22. Kimmel PL, Tenner S, Halwe VQ, et al. Trypsinogen and other pancreatic enzymes in patients with renal disease: a comparison of high-efficiency hemodialysis and continuous ambulatory peritoneal dialysis. Pancreas. 1995;10:325–330.
23. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144:1252–1261.
24. Green CL. Identification of alpha-amylase as a secretion of the human fallopian tube and tubelike epithelium of müllerian and mesonephric duct origin. Am J Obstet Gynecol. 1957;73:402–408.
25. Berk JE, Shimamura J, Fridhandler L. Tumor-associated hyperamylasemia. Am J Gastroenterol. 1977;68:572–577.
26. Whitten RO, Chandler WL, Thomas MG, et al. Survey of alpha-amylase activity and isoamylases in autopsy tissue. Clin Chem. 1988;34:1552–1555.
27. Apple F, Benson P, Preese L, et al. Lipase and pancreatic enzyme activities in tissues and in patients with hyperamylasemia. Am J Clin Pathol. 1991;96:610–614.
28. Shimamura J, Fridhandler L, Berk JE. Does human pancreas contain salivary-type isoamylase? Gut. 1975;16:1006–1009.
29. Fridhandler L, Berk JE, Ueda M. Isolation and measurement of pancreatic amylase in autopsy tissue. Am J Gastroenterol. 1995;88:770–777.
30. Sinha S, Khan H, Timms PM, et al. Pancreatic-type hyperamylasemia and hyperlipasemia secondary to ruptured ovarian cyst: a case report and review of the literature. J Emerg Med. 2010;38:463–466.
31. Tietz NW, Shuey DF. Lipase in serum. Clin Chem. 1988;34:1552–1555.
32. Jocken JW, Moro C, Goossens GH, et al. Skeletal muscle lipase content and activity in obesity and type 2 diabetes. J Clin Endocrinol Metab. 2010;95:5449–5453.
33. Langin D, Dicker A, Tavernier G, et al. Adipocyte lipases and defect of lipolysis in human obesity. *Diabetes*. 2005;54:3190–3197.
34. Whitcomb DC, Lowe ME. Human pancreatic digestive enzymes. *Dig Dis Sci*. 2007;52:1–17.
35. Wanke M. Pathogenese und morphologisches Bild akuter Pankreaserkrankungen. In: Forell MM, ed. *Pankreas. Hdb Inn Med, 5. Aufl, Bd 3. Teil 6*. Berlin-Heidelberg-New York: Springer; 1976:S520–S615.
36. Boivin M, Lanspa SJ, Zinsmeister AR, et al. Are diets associated with different rates of human interdigestive and postprandial pancreatic enzyme secretion? *Gastroenterology*. 1990;99:1763–1771.
37. Ricketts J, Brannon PM. Amount and type of dietary fat regulate pancreatic lipase gene expression in rats. *J Nutr*. 1994;124:1166–1171.
38. Junge W, Malyusz M, Ehrens HJ. The role of the kidney in the elimination of pancreatic lipase and amylase from blood. *J Clin Chem Clin Biochem*. 1985;23:387–392.
39. Møller-Peterson J, Dati F. Renal handling of pancreatic lipase. *Clin Chem*. 1984;30:343–344.
40. Malyusz M, Wrigge P, Caliebe D, et al. Renal handling of 125I-labelled homologous pancreatic lipase and amylase in the rat. *J Clin Chem Clin Biochem*. 1988;26:611–615.
41. Hardt PD, Hauenschild A, Jaeger C, et al. High prevalence of steatorrhea in 101 diabetic patients likely to suffer from exocrine pancreatic insufficiency according to low fecal elastase 1 concentrations: a prospective multicenter study. *Dig Dis Sci*. 2003;48:1688–1692.
42. Lazarus SS, Volk BW. Pancreas in maturity-onset diabetes. Pathogenetic considerations. *Arch Pathol*. 1961;71:44–59.
43. Butler AE, Campbell-Thompson M, Gurlo T, et al. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes*. 2013;62:2595–2604.