Exenatide Affects Circulating Cardiovascular Risk Biomarkers Independently of Changes in Body Composition

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OBJECTIVE — To study the effect of exenatide on body composition and circulating cardio-vascular risk biomarkers.

RESEARCH DESIGN AND METHODS — Metformin-treated patients with type 2 diabetes (*N* = 69) were randomized to exenatide or insulin glargine and treated for 1 year. Body composition was evaluated by dual-energy X-ray absorptiometry. Additionally, body weight, waist circumference, and cardiovascular biomarkers were measured.

RESULTS — Treatment with exenatide for 1 year significantly reduced body weight, waist circumference, and total body and trunkal fat mass by 6, 5, 11, and 13%, respectively. In addition, exenatide increased total adiponectin by 12% and reduced high-sensitivity C-reactive protein by 61%. Insulin glargine significantly reduced endothelin-1 by 7%. These changes were statistically independent of the change in total body fat mass and body weight.

CONCLUSIONS — Exenatide treatment for 1 year reduced body fat mass and improved the profile of circulating biomarkers of cardiovascular risk. No significant changes were seen with insulin glargine except a trend for reduced endothelin-1 levels.

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A bdominal obesity is associated with both type 2 diabetes and metabolic complications (1), including elevations in several circulating biomarkers of cardiovascular risk (2). Most pharmacological glucose-lowering treatments increase body weight (3). Therefore, treatments that not only reduce A1C, but also improve other associated changes such as abdominal obesity are urgently needed (4).

We previously reported in *Diabetes Care* that exenatide improves glycemic control to the same extent as insulin glargine, although exenatide decreased and insulin glargine raised body weight (5). Herein we present additional data on associated changes in body composition and circulating levels of biomarkers of cardiovascular risk after 1 year of treatment.

RESEARCH DESIGN AND METHODS — Details on study design were reported previously (5). Patients were randomized to exenatide (*n* = 36) or

insulin glargine (*n* = 33) added to their ongoing metformin therapy (baseline characteristics and patient disposition are shown in supplemental Fig. 1 in the online appendix available at http://care. diabetesjournals.org/cgi/content/full/ dc09-2361/DC1). The study protocol was approved by each site's ethics review committee and was in accordance with the principles described in the Declaration of Helsinki. All participating patients gave their written informed consent prior to screening.

Dual-energy X-ray absorptiometry scan
Lean body and fat mass was assessed using dual-energy X-ray absorptiometry (DEXA) scans (Delphi A; Hologic, Waltham, MA) at baseline and after treatment. Trunk (abdominal) and limb (hip/leg) regions of interest were determined from a total body scan. Waist circumference was measured at the midline of the interval between the iliac crest and the lowest rib using the mean of two measurements prior to the DEXA scan.

Biochemical analyses
Cardiovascular risk biomarkers were collected at baseline and after 1 year of treatment. Serum was separated by centrifugation and stored at −80°C until analysis. All serum samples were analyzed in the Lundberg Laboratory for Diabetes Research using a single batch. Total adiponectin, high molecular weight (HMW) adiponectin, resistin, leptin, high-sensitive C-reactive protein (hs-CRP), interleukin (IL)-6, monocyte chemotactic protein (MCP)-1, and endothelin-1 were determined by commercial ELISAs (R&D Systems, Abingdon, U.K.).

Statistical analysis
Non-normally distributed data were log-transformed prior to statistical analysis, after which they approximated the normal distribution. All outcome measures are compared between the two treatment groups using an ANCOVA model including factors for treatment, investigative

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Table 1—*Body composition, circulating cardiovascular risk biomarkers and percentage change from baseline*

	n	Baseline	Endpoint	Percentage change from baseline		
				LS mean	Between-treatment group difference	P
Total fat mass (kg)						
Insulin glargine	28	29.9 ± 1.6	28.5 ± 1.9	−1% (−7% to +5%)		
Exenatide	29	27.8 ± 1.4	25.4 ± 1.6	−11% (−18% to −5%)	−10% (−16% to −4%)	0.003
Total lean mass (kg)						
Insulin glargine	28	60.1 ± 1.7	60.6 ± 1.8	0% (−1% to +2%)		
Exenatide	29	57.8 ± 2.1	58.1 ± 2.4	0% (−2% to +1%)	−1% (−3% to +1%)	0.480
Trunk fat mass (kg)						
Insulin glargine	28	17.8 ± 0.9	16.6 ± 1.1	−1% (−8% to +5%)		
Exenatide	29	16.3 ± 0.8	14.8 ± 1.0	−13% (−18 to −7%)	−11% (−18% to −4%)	0.002
Body weight (kg)						
Insulin glargine	29	94.1 ± 2.5	93.8 ± 2.7	−1% (−3% to +1%)		
Exenatide	30	90.3 ± 2.4	86.4 ± 2.6	−6% (−8% to −3%)	−5% (−7% to −2%)	0.001
Waist circumference (cm)						
Insulin glargine	29	106.9 ± 1.9	107.4 ± 2.0	+1% (−1% to +3%)		
Exenatide	30	106.1 ± 1.9	100.6 ± 2.1	−5% (−7% to −3%)	−6% (−8% to −4%)	<0.001
Leptin (μg/l)						
Insulin glargine	29	7.79 ± 1.29	8.41 ± 1.53	+7% (−11% to +29%)		
Exenatide	30	8.50 ± 1.32	7.45 ± 1.17	−14% (−27% to +2%)	−19% (−34% to −1%)	0.045
Total adiponectin (ng/ml)						
Insulin glargine	29	4,648 ± 461	4,508 ± 436	−5% (−13% to +5%)		
Exenatide	30	4,848 ± 432	5,314 ± 466	+12% (+3% to +21%)	+17% (+6% to +30%)	0.004
HMW adiponectin (ng/ml)						
Insulin glargine	29	1,277 ± 221	1,321 ± 236	−0% (−24% to +31%)		
Exenatide	30	1,571 ± 255	1,850 ± 273	+19% (−6% to +51%)	+19% (−12% to +61%)	0.253
hs-CRP (mg/l)						
Insulin glargine	29	1.42 ± 0.27	1.38 ± 0.35	−20% (−50% to +27%)		
Exenatide	30	1.81 ± 0.25	1.30 ± 0.22	−61% (−74% to −42%)	−52% (−71% to −19%)	0.008
IL-6 (pg/ml)						
Insulin glargine	29	1.96 ± 0.21	2.17 ± 0.20	−4% (−26% to +25%)		
Exenatide	30	2.11 ± 0.22	2.10 ± 0.25	−10% (−28% to +14%)	−6% (−30% to +26%)	0.670
MCP-1 (pg/ml)						
Insulin glargine	29	1.22 ± 0.07	1.24 ± 0.07	−1% (−12% to +11%)		
Exenatide	30	1.18 ± 0.09	1.21 ± 0.11	−4% (−13% to +7%)	−2% (−14% to +12%)	0.728
Resistin (ng/ml)						
Insulin glargine	29	330 ± 15	329 ± 20	−3% (−13% to +7%)		
Exenatide	30	316 ± 14	311 ± 16	−0% (−9% to +9%)	+3% (−8% to +16%)	0.577
Endothelin-1 (ng/ml)						
Insulin glargine	29	2.57 ± 0.18	2.46 ± 0.19	−7% (−11% to −2%)		
Exenatide	30	2.53 ± 0.19	2.53 ± 0.19	−1% (−5% to +3%)	+6% (−1% to +12%)	0.045

Data are means ± SEM (body composition measures) or geometric means ± SEM (cardiovascular biomarkers) and body weight change–adjusted least-squares mean percentage change (95% CI) from baseline. LS, least-squares.

site, and baseline A1C stratum (≤8.5% or >8.5%), and baseline values of corresponding outcome measure as a covariate (5). Statistical analysis was performed using SPSS 16.0 for Mac OS X (SPSS, Chicago, IL). All inferential statistical tests were conducted at a significance level of 0.05 (two-sided).

RESULTS — Treatment for 1 year with exenatide resulted in a statistically significant reduction in total body fat mass (Table 1), mainly in the abdominal region, as illustrated by the decrease in trunk fat mass and waist circumference, in contrast to insulin glargine. Neither treatment significantly affected lean body mass.

In univariate analysis, the reduction in body weight in the exenatide arm was significantly correlated with the changes in leptin ($r = 0.580$, $P = 0.001$) and hs-CRP ($r = −0.590$, $P = 0.001$). No statistically significant univariate correlation was found between changes in body weight and other biomarkers. Interestingly, changes in all circulating biomark-ers did not correlate with the changes in total body fat mass (total adiponectin: Pearson χ^2 test, $r = −0.224$, $P = 0.106$; HMW adiponectin: $r = 0.057$, $P = 0.694$; leptin: $r = 0.229$, $P = 0.106$; hs-CRP: $r = −0.023$, $P = 0.872$).

After multivariate analysis and statistical adjustment for body weight change, exenatide increased total adiponectin and decreased hs-CRP concentrations, whereas insulin glargine did not (Table 1). Insulin glargine reduced endothelin-1 concentrations, whereas exenatide did

not. No statistically significant effect of either treatment on HMW adiponectin, IL-6, MCP-1, and resistin was observed.

The crude between–treatment group differences remained statistically significant after additional multivariate adjustment for total body fat mass change: total adiponectin +16% (95% CI: +5% to +28%), $P = 0.004$; leptin −20% (−34% to −2%), $P = 0.028$; hs-CRP −48% (−69% to −13%), $P = 0.015$; and body weight change (Table 1): total adiponectin +17% (95% CI +6% to +30%), $P = 0.004$; leptin −19% (−34% to 0%), $P = 0.045$; hs-CRP −52% (−71% to −19%), $P = 0.008$.

CONCLUSIONS — This study showed that exenatide reduced body fat mass and improved the profile of circulating cardiovascular biomarkers. The changes in the different biomarkers could not be fully attributed to the observed changes in body fat mass and body weight. Direct effects of glucagon-like peptide 1 (GLP-1) receptor agonists on adipocyte function have been described in both animal experimental studies and in vitro studies in normal human adipocytes (rev. in 6); however, as a significant univariate correlation between change in body weight (not with fat mass) and cardiovascular biomarkers was present, our relatively small population may influence the statistical power of our study.

Animal studies have also demonstrated beneficial effects of exenatide on visceral fat mass (7) and circulating adiponectin (8), leptin (9), and CRP (10) concentrations. However, to the best of our knowledge, controlled clinical studies on the long-term effects of GLP-1 receptor agonists on body composition and biomarkers of cardiovascular risk have not previously been reported.

A recent 3-month study comparing exenatide to insulin glargine in 56 patients with type 2 diabetes has a design comparable to our 1-year study. Similar to our findings, this study showed that exenatide treatment was associated with reduced hs-CRP, without affecting the IL-6 levels (11).

Subanalysis of the Liraglutide Effect and Action in Diabetes (LEAD)-3 study data reported that liraglutide treatment for 52 weeks compared with treatment with glimiperide reduced DEXA-measured total fat tissue mass (12). Lean tissue mass was also reduced after 1 year of treatment, but as glimiperide also reduced lean tissue mass, this reduction was not statistically significantly different between the groups. Twenty-six-week data from the LEAD-2 study was used to show that the observed reduction in fat mass was mainly a result of a reduction in visceral fat (12). Unfortunately, this study did not report the effects of body composition on circulating biomarkers. Serum leptin, hs-CRP, and IL-6 concentrations did not change in a 14-week placebo-controlled study with liraglutide 1.9 mg (13).

Of particular interest in our study was the finding that the changes in biomarkers of cardiovascular risk appeared to be independent of the changes in body fat mass. Recently, Chung et al. reported exendin-4 directly increased adiponectin mRNA levels and secretion in 3T3-L1 adipocytes (14). In that study, exendin-4 also decreased mRNA levels of IL-6 and MCP-1 (14). Additionally, we (15) and others (10) have previously reported beneficial effects of exenatide on hepatic steatosis, which also may contribute to a reduction in CRP.

In conclusion, we found that exenatide treatment for 1 year led to a reduced total fat mass, including visceral fat, while lean body mass was not significantly altered. Additionally, the circulating levels of adiponectin, leptin, and hs-CRP showed an improved profile that appeared to be independent of the changes in fat mass. In contrast, no significant changes in body composition or circulating biomarkers were seen with insulin glargine.

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