

# Liraglutide Treatment Is Associated with a Low Frequency and Magnitude of Antibody Formation with No Apparent Impact on Glycemic Response or Increased Frequency of Adverse Events: Results from the Liraglutide Effect and Action in Diabetes (LEAD) Trials

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**Context:** Therapeutic proteins/peptides can produce immunogenic responses that may increase the risk of adverse events or reduce efficacy.

**Objective:** The objectives were to measure and characterize antibody formation to liraglutide, a glucagon-like peptide-1 receptor agonist, to investigate the impact on glycemic control and safety, and to compare it with exenatide, an agent in the same class.

**Design:** Antibody data were collected during six Liraglutide Effect and Action in Diabetes (LEAD) trials (26–104 wk duration).

**Setting:** Samples for determination of antibody formation were collected at LEAD trial sites and analyzed at central laboratories.

**Participants:** Antibodies were measured in LEAD trial participants with type 2 diabetes.

**Interventions:** Interventions included once-daily liraglutide (1.2 or 1.8 mg) or twice-daily exenatide (10 µg).

**Main Outcome Measures:** The main outcome measures included the proportion of patients positive for anti-liraglutide or anti-exenatide antibodies, a glucagon-like peptide-1 cross-reacting effect, and an *in vitro* liraglutide- or exenatide-neutralizing effect. Change in glycosylated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) by antibody status and magnitude [negative, positive (high or low level)].

**Results:** After 26 wk, 32 of 369 (8.7%) and 49 of 587 (8.3%) patients had low-level antibodies to liraglutide 1.2 and 1.8 mg, respectively [mean 3.3% antibody-bound radioactivity out of total radioactivity (%B/T), range 1.6–10.7%B/T], which did not attenuate glycemic efficacy (HbA<sub>1c</sub> reductions 1.1–1.3% in antibody-positive vs. 1.2% in antibody-negative patients). In LEAD-6, 113 of 185 extension patients (61%) had anti-exenatide antibodies at wk 26 (range 2.4–60.2%B/T). High levels of anti-exenatide antibodies were correlated with significantly smaller HbA<sub>1c</sub> reductions ( $P = 0.0022$ ). After switching from exenatide to liraglutide, anti-exenatide antibodies did not compromise a further glycemic response to liraglutide (additional 0.4% HbA<sub>1c</sub> reduction).

**Conclusions:** Liraglutide was less immunogenic than exenatide; the frequency and levels of anti-liraglutide antibodies were low and did not impact glycemic efficacy or safety. (*J Clin Endocrinol Metab* 96: 1695–1702, 2011)

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Abbreviations: AE, Adverse events; %B/T, antibody-bound radioactivity out of total radioactivity; GLP-1, glucagon-like peptide-1; HbA<sub>1c</sub>, glycosylated hemoglobin A<sub>1c</sub>; LEAD, Liraglutide Effect and Action in Diabetes; LOCF, last observation carried forward.

**T**herapeutic proteins/peptides are used to treat many diseases but have the potential to produce immunogenic responses that may adversely affect clinical outcomes by increasing the risk of adverse events (AE), reducing efficacy, or both. AE associated with an unwanted immune response include injection site reactions, allergic reactions, anaphylaxis, hypersensitivity, and autoimmunity and can range from mild to life threatening (1). In addition, neutralizing antibodies can compromise efficacy by binding and blocking the active site of the therapeutic protein or by changing the conformation of the protein. For example, neutralizing antibodies can inhibit the ability of factor VIII and IX replacement products to stop bleeding episodes in hemophilia A and B patients (2), interferon- $\beta$  to modify disease progression in multiple sclerosis patients (3, 4), and animal insulin preparations to provide glycemic control in some diabetes patients (5, 6). However, often the level and affinity of antibodies to therapeutic proteins/peptides may be too low to have clinical consequences.

In general, therapeutic proteins/peptides with high structural similarity to endogenous proteins/peptides have lower risk for unwanted immunogenicity (<http://www.ema.europa.eu/pdfs/human/biosimilar/1432706en.pdf>). Liraglutide and exenatide are two therapeutic peptides in the glucagon-like peptide-1 (GLP-1) receptor agonist class indicated for type 2 diabetes. Liraglutide is a recombinant once-daily human GLP-1 analog with 97% amino acid sequence identity to endogenous human GLP-1 ([http://www.victoza.com/pdf/Victoza\\_CombiPI\\_1-4-11.pdf](http://www.victoza.com/pdf/Victoza_CombiPI_1-4-11.pdf)), and exenatide is synthetic exendin-4, a xenopeptide originally identified in the saliva of the Gila monster (*Heloderma suspectum*), with 53% amino acid sequence identity to human GLP-1 with respect to the first 31 amino acids; the sequence of the remaining eight amino acids has no resemblance to any known mammalian peptide (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>) (9, 10). In exenatide's development program, 38 and 6% of patients developed low- and high-titer anti-exenatide antibodies at 30 wk, respectively. Half the patients with high-titer antibodies (3% of the total population) had an attenuated glycemic response to exenatide treatment (9).

The six Liraglutide Effect and Action in Diabetes (LEAD) trials were designed to evaluate the safety and efficacy of liraglutide as monotherapy or in combination with one or two oral antidiabetes drugs *vs.* standard antidiabetes therapies (11–16). In all six trials, immunogenicity to liraglutide was assessed by measuring and characterizing anti-liraglutide antibodies using antibody assays. The objectives of these analyses were to measure and characterize antibody formation to liraglutide and investigate the impact on glycemic control and safety; compare the frequency of antibody formation to liraglutide and exenatide and assess the impact of

antibody formation on clinical efficacy of each of the peptides; and determine the effect of persistent anti-exenatide antibodies on the glycemic response to liraglutide for patients switching from exenatide to liraglutide therapy.

## Materials and Methods

The LEAD trial designs have been reported previously (11–16). The LEAD 1–5 trials that tested liraglutide in combination with oral agents or as monotherapy had 26- and 52-wk double-blind treatment periods, respectively. Two of these trials (combination with metformin and monotherapy trials) had additional open-label extensions of 78 and 52 wk duration, respectively, totaling 2 yr treatment. LEAD-6 was an open-label trial comprising a 26-wk head-to-head comparison of liraglutide with exenatide, both in combination with metformin and/or sulfonylurea, followed by an additional 52-wk liraglutide extension.

Anti-liraglutide antibodies were to be measured for all patients in the LEAD trials, including those in comparator arms. In LEAD-6, anti-exenatide antibodies were measured throughout the trial for the group of patients originally randomized to exenatide; in addition, anti-liraglutide antibodies were measured after switching from exenatide to liraglutide treatment at wk 26. Fasting blood samples were used for determination of anti-liraglutide or anti-exenatide antibodies. At a minimum, antibodies were to be measured at wk 0 (randomization) and 1 wk after completing treatment. This was because liraglutide's long half-life required that samples be obtained 5 d or longer (approximately nine half-lives) after drug discontinuation to avoid interference in the assay from unlabeled liraglutide in the blood. Patients who withdrew were to have an end-of-trial antibody measurement 1 wk after discontinuing the trial drug.

## Screening and GLP-1 cross-reacting antibody assays

Patient serum samples were screened for anti-liraglutide/anti-exenatide antibodies and positive samples characterized further for cross-reactivity *in vitro* to native GLP-1 using RIA with a [ $^{125}$ I]-labeled liraglutide or exenatide tracer and polyethylene glycol precipitation. RIA were performed by Celerion (formerly MDS Pharma Services Switzerland AG, Fehraltorf, Switzerland). Liraglutide antibody RIA had 50 ng/ml sensitivity, less than 12% interassay variation, and less than 5% intraassay variation, whereas exenatide antibody RIA had 70 ng/ml sensitivity, less than 10.2% interassay variation, and less than 8.5% intraassay variation; both were validated in compliance with internationally recognized recommendations for antibody assays in clinical trials (<http://www.ema.europa.eu/pdfs/human/biosimilar/1432706en.pdf>) (17, 18). Sensitivity concentrations refer to the lowest concentration of reference antibody detectable in the assay. See Supplemental Materials for further RIA details.

Results were expressed as the percentage of antibody-bound radioactivity out of total radioactivity (%B/T). Samples were considered antibody positive if %B/T was greater than 1.55 for liraglutide or greater than 2.23 for exenatide. Samples greater than 15%B/T were diluted and the titer determined as the dilution factor needed to obtain 15%B/T. See Supplemental Fig. 2 for correlation of antibody titer *vs.* %B/T of the anti-liraglutide/anti-exenatide antibody-positive samples in LEAD-5 and -6, re-

spectively. The correlation between antibody titer and %B/T was not linear above 50%B/T; hence, samples with %B/T above this value contained even higher antibody levels than reflected in the %B/T value. This was relevant only for anti-exenatide antibody-positive samples.

### ***In vitro* cell-based neutralizing assay**

Samples testing positive for anti-liraglutide/anti-exenatide antibodies at the off-drug visits, and at wk 26 for exenatide in LEAD-6, were tested for an *in vitro* liraglutide/exenatide-neutralizing effect by Novo Nordisk A/S (Måløv, Denmark) using a cell-based assay that measured cAMP signaling and downstream luciferase activity as a marker for GLP-1 receptor activation (see Supplemental Materials for assay methodology). Neutralizing antibodies block the binding of the GLP-1 receptor agonist to the GLP-1 receptor, preventing cAMP induction and leading to a decrease in luminescence compared with a pretreatment sample (*i.e.* without antidrug antibodies). The liraglutide/exenatide-neutralizing antibody assays had 60 and 200 ng/ml sensitivity, less than 7.6 and less than 7.9% interassay variation and less than 8.5 and less than 5.4% intraassay variation, respectively, and were validated according to recommended guidelines for cell-based neutralizing assays (19).

### **Assay interference**

Both the RIA and *in vitro* neutralizing antibody assays were sensitive to the presence of liraglutide in the plasma samples (see Supplemental Materials), meaning low-titer anti-liraglutide antibodies may not be detected when sampling is done during liraglutide treatment. In contrast, the low concentrations of plasma exenatide did not interfere with the anti-exenatide antibody assays. Exenatide concentrations in plasma have been shown to be approximately 100-fold lower than liraglutide (20) due to lack of albumin binding and the lower drug dose per injection (10  $\mu$ g *vs.* 1.2 or 1.8 mg). In addition, exenatide has a

shorter half-life (2.4 h), leading to low plasma levels at the time of antibody sample collection (fasting before administration). To avoid false-negative samples due to assay interference by plasma liraglutide, only anti-liraglutide antibody values from the end of the trial after a liraglutide washout period of 5 d or longer [corresponding to approximately nine half-lives (see Supplemental Materials)] were used for the evaluation of effect on efficacy and safety and comparison with exenatide.

### **Statistical methods**

Antibody data from the safety population from the 26-wk double-blind treatment periods were pooled for the liraglutide 1.2- and 1.8-mg dose groups. Mean glycosylated hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) or body weight changes for anti-liraglutide, antibody-positive and -negative patients were calculated for intent to treat patients with antibody measurements off drug for at least 5 d. Missing values were handled using a last observation carried forward (LOCF) approach, unless noted otherwise. The extension safety population of LEAD-6 was analyzed to determine antibody formation in patients who continued liraglutide treatment or who switched from exenatide to liraglutide treatment at wk 26. High antibody levels were defined as greater than 20%B/T and very high-titer antibodies greater than 50%B/T. A regression analysis was conducted to determine the relationship between %B/T and change in HbA<sub>1c</sub> or body weight. Fisher's exact test was used to compare the proportion of antibody-positive patients.

### **Results**

#### **Anti-liraglutide antibody results from double-blind trial periods**

In the 26-wk, double-blind treatment periods, 8.7 and 8.3% of patients with antibody measurements after a washout period of 5 d or longer (approximately nine half-

**TABLE 1.** Anti-liraglutide antibodies at the end of treatment (off drug for at least 5 d)

|                                                   | Liraglutide 1.2 mg,<br>n (%) | Liraglutide 1.8 mg,<br>n (%) |
|---------------------------------------------------|------------------------------|------------------------------|
| Pooled 26-wk data from double-blind trial periods |                              |                              |
| Antibody measurements after $\geq$ 5-d washout    | 369                          | 587                          |
| Anti-liraglutide antibody negative                | 337 (91.3)                   | 538 (91.7)                   |
| Anti-liraglutide antibody positive                | 32 (8.7)                     | 49 (8.3)                     |
| GLP-1 cross-reacting effect                       | 18 (4.9)                     | 25 (4.3)                     |
| <i>In vitro</i> neutralizing effect               | 4 (1.1)                      | 5 (0.9)                      |
| 52-wk data from double-blind trial period         |                              |                              |
| Antibody measurements after $\geq$ 5-d washout    | 42                           | 37                           |
| Anti-liraglutide antibody negative                | 39 (92.9)                    | 35 (94.6)                    |
| Anti-liraglutide antibody positive                | 3 (7.1)                      | 2 (5.4)                      |
| GLP-1 cross-reacting effect                       | 3 (7.1)                      | 1 (2.7)                      |
| <i>In vitro</i> neutralizing effect               | 1 (2.4)                      | 1 (2.7)                      |
| Pooled 2-yr data from open-label extensions       |                              |                              |
| Antibody measurements after $\geq$ 5-d washout    | 160                          | 143                          |
| Anti-liraglutide antibody negative                | 155 (96.9)                   | 138 (96.5)                   |
| Anti-liraglutide antibody positive                | 5 (3.1)                      | 5 (3.5)                      |
| GLP-1 cross-reacting effect                       | 4 (2.5)                      | 3 (2.1)                      |
| <i>In vitro</i> neutralizing effect               | 2 (1.3)                      | 0                            |

Twenty-six-week data are from the double-blind periods of LEAD-1, -2, -4, and -5 pooled by treatment group, LOCF. Fifty-two-wk data are from the double-blind period of LEAD-3, LOCF. Two-year data are from the open-label extension periods of LEAD-2 and -3 pooled by treatment group for patients completing 2 yr of liraglutide treatment. Data are number (percent) patients.

**TABLE 2.** Change in HbA<sub>1c</sub> (%) from baseline to wk 26 (LOCF) by anti-liraglutide antibody status (positive/negative) in liraglutide-treated patients off drug for at least 5 d

|                                                                             | Liraglutide 1.2 mg |                            |                     | Liraglutide 1.8 mg |                            |                     |
|-----------------------------------------------------------------------------|--------------------|----------------------------|---------------------|--------------------|----------------------------|---------------------|
|                                                                             | n                  | Baseline HbA <sub>1c</sub> | Δ HbA <sub>1c</sub> | n                  | Baseline HbA <sub>1c</sub> | Δ HbA <sub>1c</sub> |
| Anti-liraglutide antibody negative                                          | 337                | 8.5 (1.1)                  | −1.2 (1.1)          | 534                | 8.4 (1.0)                  | −1.2 (1.1)          |
| Anti-liraglutide antibody positive                                          | 32                 | 8.9 (1.2)                  | −1.3 (1.2)          | 48                 | 8.3 (1.1)                  | −1.1 (0.9)          |
| Anti-liraglutide antibody positive with GLP-1 cross-reacting effect         | 18                 | 8.7 (0.9)                  | −1.2 (1.2)          | 25                 | 8.4 (1.0)                  | −1.2 (0.8)          |
| Anti-liraglutide antibody positive with <i>in vitro</i> neutralizing effect | 4                  | 8.8 (0.9)                  | −1.6 (1.7)          | 5                  | 8.3 (1.0)                  | −1.6 (0.7)          |

Data are from the 26-wk double-blind periods of LEAD-1, -2, -4, and -5 pooled by treatment group for intent to treat patients with antibody measurements 5 d or longer off liraglutide. Changes from baseline to wk 26 (LOCF) are mean (SD).

lives) had low-level anti-liraglutide antibodies to liraglutide 1.2 and 1.8 mg, respectively (mean 3.3%B/T, range 1.6–10.7%B/T) (Table 1). A low proportion of patients had antibodies that cross-reacted with native GLP-1 or had an *in vitro* liraglutide-neutralizing effect (Table 1). The frequency of comparator-treated patients testing positive for anti-liraglutide antibodies (*i.e.* false positives) was one of 316 (0.3%) with placebo and one of 433 (0.2%) with active comparator; none cross-reacted with GLP-1 or had an *in vitro* neutralizing effect.

Anti-liraglutide antibody formation did not attenuate the glycemic response to liraglutide, as shown by the similar mean reductions in HbA<sub>1c</sub> between antibody-positive and antibody-negative patients (Table 2). Likewise, in the small number of patients with anti-liraglutide antibodies that cross-reacted with GLP-1 or had an *in vitro* neutralizing effect, antibodies did not compromise the mean glycemic response to liraglutide (Table 2). The relationship between antibody level and glycemic response showed similar glycemic responses between liraglutide antibody-positive patients in the top %B/T quartile and the other liraglutide antibody-positive patients (Fig. 1), and a regression analysis showed no significant correlation between antibody level and change in HbA<sub>1c</sub> ( $r^2 = 0.0251$ ,  $P = 0.1604$ ). The liraglutide 1.2 mg patient with the 10.7%B/T antibody level had HbA<sub>1c</sub> values of 7.0, 6.5, 6.8, 6.9, and 7.0% at wk 0, 8, 12, 18, and 26, respectively. Similarly, the relationship between anti-liraglutide antibody level and change in body weight was not significant (see Supplemental Materials for results).

Consistent with the 26-wk data, at the end of the 52-wk, double-blind period of the monotherapy trial, of the few patients who withdrew early or who did not continue into the open-label extension period and were off the drug for 5 d or longer, 7.1 and 5.4% had low-titer antibodies to liraglutide 1.2 and 1.8 mg, respectively (mean 4.6%B/T, range 1.9–9.9%B/T) (Table 1). No samples from the comparator (glimepiride) patients tested positive for anti-liraglutide antibodies.

During the blinded periods, eight patients with anti-liraglutide antibodies reported 11 immunogenicity/injection site reaction AE (see Supplemental Table 1 for details). All of these AE were nonserious, and all were of mild severity, with the exception of one moderate skin allergy AE; none led to trial discontinuation. No patients with anti-liraglutide antibodies had serious or systemic immunologic AE.

#### Anti-liraglutide antibody results from open-label extension periods

After 2 yr treatment, 3.1 and 3.5% of patients off drug for 5 d or longer had anti-liraglutide antibodies to liraglutide 1.2 and 1.8 mg, respectively (Table 1). No samples from 31 placebo and 150 glimepiride comparator patients completing 2 yr tested positive for anti-liraglutide antibodies. Levels and frequencies of anti-liraglutide antibodies over 2 yr from the metformin combination trial are shown in Supplemen-

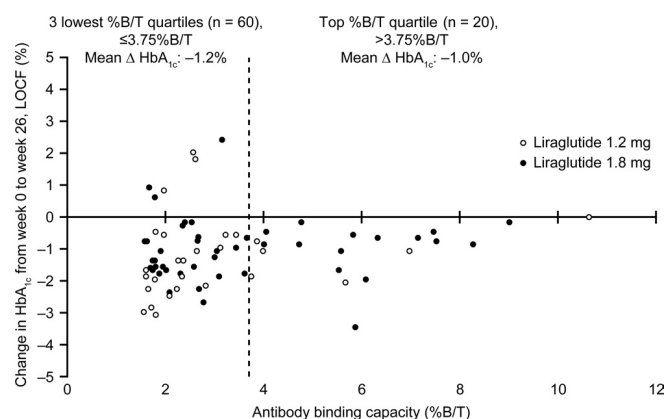


**FIG. 1.** Correlation between anti-liraglutide antibody levels and change in HbA<sub>1c</sub>. The change in HbA<sub>1c</sub> from wk 0 to wk 26 was plotted vs. the antibody binding capacity (%B/T) at the end of the study after a washout period of 5 d or longer for 80 of 951 patients treated with liraglutide 1.2 or 1.8 mg (8.4% who were positive for anti-liraglutide antibodies (%B/T > 1.55%). White and black circles denote patients treated with liraglutide 1.2 and 1.8 mg, respectively. The dotted line separates the top quartile of antibody-positive patients according to antibody levels; however, all anti-liraglutide antibody-positive patients had values less than 15%B/T, corresponding to antibody titers of 1. A regression analysis of these data showed no significant correlation between antibody level and change in HbA<sub>1c</sub> ( $r^2 = 0.0251$ ,  $P = 0.1604$ ).

tal Fig. 3. The increase in antibody levels and frequency at wk 105, when patients were washed off drug, compared with wk 104 was consistent with the known interference of plasma liraglutide with the assay. Nevertheless, anti-liraglutide antibody formation and levels remained low over 2 yr.

### Anti-liraglutide and anti-exenatide antibody results from LEAD-6

In LEAD-6, after the 26-wk, head-to-head comparison of liraglutide to exenatide, all exenatide-treated patients were switched to receive liraglutide for an additional 52 wk. Of those patients initially randomized to exenatide who entered the liraglutide extension, 61% had anti-exenatide antibodies at wk 26, ranging from 2.4 to 60.2%B/T (Fig. 2), five partially cross-reacted with GLP-1, and 12 had an *in vitro* exenatide-neutralizing effect. High levels of anti-exenatide antibodies were associated with smaller mean HbA<sub>1c</sub> reductions from wk 0 to wk 26 (Fig. 3); this was significant ( $r^2 = 0.0811$ ,  $P = 0.0022$ ). The relationship between anti-exenatide antibody level and change in body weight was not significant (see Supplemental Materials).

After switching from exenatide to liraglutide at wk 26, 50 and 17% of patients had persistent anti-exenatide antibodies

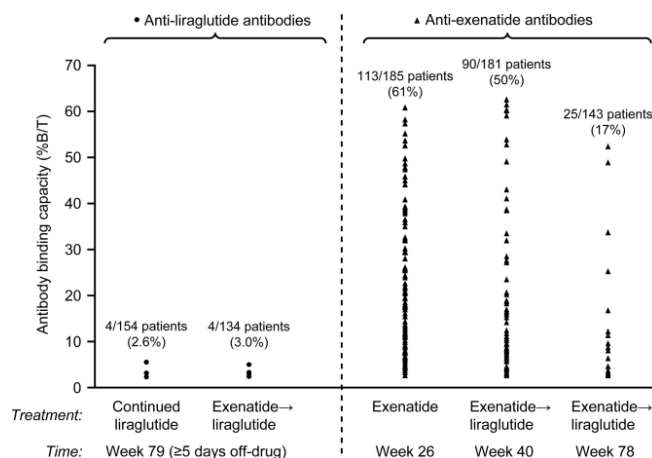


**FIG. 2.** Anti-liraglutide and anti-exenatide antibody levels in LEAD-6. Antibody levels [antibody binding capacity (%B/T)] are shown for patients positive for anti-liraglutide antibodies ( $>1.55\%B/T$ ) 5 d or longer off drug and patients positive for anti-exenatide antibodies ( $>2.23\%B/T$ ). In LEAD-6, patients were treated with either once-daily liraglutide 1.8 mg or twice-daily exenatide 10  $\mu$ g for 26 wk. At wk 26, all exenatide-treated patients were switched to receive liraglutide 1.8 mg treatment for an additional year (wk 26 to wk 78, exenatide→liraglutide group). Patients originally randomized to receive liraglutide 1.8 mg continued treatment up until wk 78. Liraglutide antibody levels measured after a washout period of 5 d or longer (i.e. at wk 79) are presented for both groups. Exenatide antibody levels were measured over time for the patients originally randomized to exenatide treatment, even after switching to liraglutide. Circles and triangles denote anti-liraglutide and anti-exenatide antibody-positive patients, respectively. Above the data points, percentages of antidrug-positive patients of all patients with antibody measurements at that time point (no imputation) are provided.

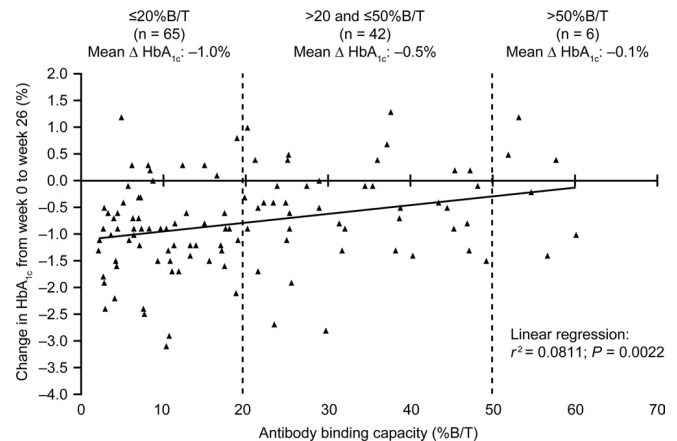


**FIG. 3.** Correlation between anti-exenatide antibody levels and change in HbA<sub>1c</sub>. The change in HbA<sub>1c</sub> from wk 0 to wk 26 was plotted vs. the antibody binding capacity (%B/T) in LEAD-6 for 113 of 185 (61%) extension patients treated with twice-daily exenatide 10  $\mu$ g who were positive for anti-exenatide antibodies (%B/T  $>2.23\%$ ) at wk 26. The dotted lines separate those with high anti-exenatide antibody levels ( $>20$  to  $\leq 50\%B/T$ ) and those with the highest levels of anti-exenatide antibodies ( $>50\%B/T$ ), corresponding to much higher titers. The solid line is the trend line from a regression analysis of these data, showing a significant positive correlation between anti-exenatide antibody level and change in HbA<sub>1c</sub>.

at wk 40 and 78, respectively (Fig. 2). However, persistent anti-exenatide antibodies did not compromise the further glycemic response to liraglutide, and patients with anti-exenatide antibody levels greater than 20%B/T had the greatest glycemic response after switching to liraglutide (Fig. 4). Five

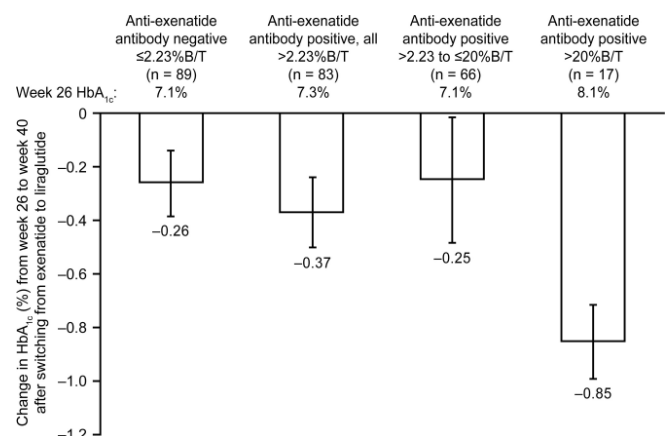


**FIG. 4.** Change in HbA<sub>1c</sub> after switching from exenatide to liraglutide by anti-exenatide antibody status. In LEAD-6, patients randomized to treatment with twice-daily exenatide 10  $\mu$ g were switched to once-daily liraglutide 1.8 mg at wk 26. anti-exenatide antibodies continued to be measured after switching from exenatide to liraglutide treatment. After 14 wk of additional treatment with liraglutide, the observed mean ( $\pm 2$  SE) change in HbA<sub>1c</sub> from wk 26 to wk 40 was presented for extension patients grouped according to their anti-exenatide antibody status at wk 40 [negative ( $\leq 2.23\%B/T$ ), all positive ( $>2.23\%B/T$ ), positive with lower levels ( $>2.23$  to  $\leq 20\%B/T$ ), or higher levels ( $>20\%B/T$ )]. Data for all patients with anti-exenatide antibody and HbA<sub>1c</sub> values at wk 40 are presented (n = 172). HbA<sub>1c</sub> values above each bar are the values at the end of the exenatide treatment period (i.e. wk 26) before switching to liraglutide treatment for each group.

of six patients with the highest anti-exenatide antibody levels ( $>50\%$ B/T) at wk 26 had HbA<sub>1c</sub> reductions ranging from  $-0.5$  to  $-1.2\%$  after switching to liraglutide, despite anti-exenatide antibody levels remaining greater than  $50\%$ B/T at wk 40 after the switch in four of six patients.

In patients switched from exenatide to liraglutide at wk 26 with antibody measurements off drug for 5 d or longer,  $3.0\%$  of extension completers [and four of 165 ( $2.4\%$ ) of extension patients (LOCF)] had low-level anti-liraglutide antibodies ranging from  $2.2$  to  $4.8\%$ B/T (Fig. 2). Similar results for anti-liraglutide antibody formation were obtained for patients originally randomized to liraglutide treatment who continued liraglutide during the extension (total liraglutide treatment: 78 wk). Of patients off drug for 5 d or longer who completed 78 wk of continued liraglutide treatment,  $2.6\%$  had low-level anti-liraglutide antibodies from  $1.9$  to  $5.3\%$ B/T (Fig. 2). Four samples cross-reacted with GLP-1 and one had an *in vitro* neutralizing effect. End-of-trial LOCF results were similar: five of 177 ( $2.8\%$ ) of extension patients in the continued liraglutide group off drug for 5 d or longer had anti-liraglutide antibodies. The difference in the proportion of exenatide antibody-positive patients at the end of exenatide treatment (wk 26) ( $61\%$ ) *vs.* liraglutide antibody-positive patients at the end of liraglutide treatment, off drug 5 d or longer (wk 79) ( $2.6\%$ ) was significant ( $P < 0.0001$ ).

In LEAD-6, two of 202 continued liraglutide patients ( $1.0\%$ ) had injection-site reactions: one was antibody positive later in the trial. Four of 187 patients in the group that switched from exenatide to liraglutide ( $2.1\%$ ) had injection site reactions (three during exenatide treatment and one during liraglutide treatment): three were exenatide antibody positive. All AE were mild and the patients recovered and continued in the trial. No systemic immunologic AE such as anaphylaxis occurred. See Supplemental Table 2 for details.

## Discussion

Generally, high-titer antibodies directed against therapeutic peptides and proteins may be associated with adverse clinical consequences such as attenuated efficacy or safety concerns. Liraglutide treatment resulted in a low frequency ( $<10\%$ ) and magnitude ( $<11\%$ B/T in undiluted serum) of anti-liraglutide antibody formation, demonstrating a low immunogenic potential. Results from open-label extensions show that the frequency and magnitude of anti-liraglutide antibody formation does not increase with more prolonged exposure to liraglutide. Anti-liraglutide antibodies (even GLP-1 cross-reacting or liraglutide neutralizing) did not attenuate glycemic control, most likely due to

their low levels (21). Anti-liraglutide antibodies were not associated with an increased frequency of AE, and no serious reactions were reported.

One factor that prohibited a more detailed time-course comparison of the development of anti-liraglutide antibodies was the impact of plasma drug on the assay sensitivity. Because the presence of plasma liraglutide reduced assay sensitivity, the most accurate measurements were obtained after a drug washout period. After washout, the level of anti-liraglutide antibodies did not increase radically, but additional samples with low antibody levels were detected (Supplemental Fig. 3, wk 104 and 105). In contrast, plasma exenatide concentrations were low and thus did not interfere with the exenatide antibody RIA, enabling the accurate measurement of antibody levels and frequencies over the entire trial. Although time courses of antibody development, levels and persistence for individual patients treated with liraglutide could not be established, end-of-trial antibody results from trials of differing liraglutide exposure (26, 52, 78, and 104 wk) demonstrated no substantial differences in antibody incidence or levels over time. This was further supported by analyzing samples of patients who discontinued liraglutide treatment at various points before the scheduled end of the individual trials. For example, in LEAD-6, at wk 26, none of the 12 patients with anti-liraglutide antibody measurements off drug for 5 d or longer were antibody positive.

In a head-to-head comparison, exenatide resulted in a much higher frequency and magnitude of antibody formation than liraglutide, perhaps due to greater differences in amino acid sequence between exenatide and human GLP-1 (22–24). The proportion of patients with anti-exenatide antibodies after 26 wk of exenatide treatment ( $61\%$  of extension patients) was slightly higher than reported during the 30-wk exenatide clinical trials ( $41$ ,  $43$ , and  $49\%$ ) (22–24). This possibly resulted from the earlier timing of antibody collection because the frequency of anti-exenatide antibody formation is highest after 4–5 months and declines thereafter (25), or from differences in antibody assay methodology. Here a RIA was used to measure antidrug antibodies *vs.* an ELISA in the exenatide trials (26). Using RIA, five of 185 exenatide antibody-positive samples ( $2.7\%$ ) partially cross-reacted with GLP-1, whereas this was not observed with the ELISA (27). These assay differences could result from different criteria (specificity cut points) for categorizing a sample as cross-reacting. In the RIA, intraassay variation was used to set the criteria, meaning partial cross-reactivity would be detected. Cross-reactivity between exenatide and native GLP-1 is not unexpected, given the fact that exenatide shares  $53\%$  amino acid sequence identity with native GLP-1, and the N-terminal part is even more homologous.

Anti-exenatide antibody-positive samples greater than 50% B/T contained high concentrations of anti-exenatide antibodies (Supplemental Fig. 2). The highest levels of anti-exenatide antibodies were associated with a higher propensity for an attenuated glycemic response to exenatide treatment, similar to results from exenatide trials (9). After switching from exenatide to liraglutide, anti-exenatide antibodies often persisted but did not reduce the glycemic response to liraglutide. Although the patient numbers were small, those with high anti-exenatide antibody levels actually experienced the greatest HbA<sub>1c</sub> reductions after switching to liraglutide, with all antibody groups benefiting, consistent with the further significant HbA<sub>1c</sub> reductions reported after switching from exenatide to liraglutide (28). These data suggest that the small subset of patients not adequately responding to exenatide, or who exhibit loss of glycemic control over time, may benefit from switching to liraglutide.

Switching also allowed us to assess the risk of antibody formation to two GLP-1 receptor agonists in the same group of patients, thus controlling for patient-specific factors (*e.g.* age, genetics, immune status) known to influence immunogenicity (<http://www.ema.europa.eu/pdfs/human/biosimilar/1432706en.pdf>). The low incidence of anti-liraglutide antibody formation in patients who previously developed anti-exenatide antibodies reaffirms the lower immunogenicity of liraglutide *vs.* exenatide and the safety of switching from exenatide to liraglutide.

Although not directly compared with liraglutide in head-to-head trials using a common assay, antidrug antibodies to other GLP-1 receptor agonists in development have been reported. The proportion of the patients positive for anti-exenatide antibodies in the patients treated with the once-weekly formulation was even higher than with the twice-daily formulation (7, 29–32): 67% over 15 wk (29), up to 74% at 30 wk (30), and 45% at 52 wk (7). Anti-albiglutide antibodies developed in 2.5% of patients in a 16-wk trial (8). The frequency of anti-taspoglutide antibody formation is unknown, but rare, serious hypersensitivity reactions have been reported ([http://www.roche.com/investors/ir\\_update/inv-update-2010-06-18b.htm](http://www.roche.com/investors/ir_update/inv-update-2010-06-18b.htm)).

In conclusion, liraglutide was less immunogenic than exenatide; less than 10% of liraglutide-treated patients developed antibodies to liraglutide. Overall, anti-liraglutide antibody levels were low and did not impact the glycemic efficacy or safety of liraglutide treatment.

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