

LEADER 2: baseline calcitonin in 9340 people with type 2 diabetes enrolled in the Liraglutide Effect and Action in Diabetes: Evaluation of cardiovascular outcome Results (LEADER) trial: preliminary observations

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Aims: To report preliminary data on baseline serum calcitonin concentrations and associated clinical characteristics in a global population with type 2 diabetes before liraglutide or placebo randomization.

Methods: The ongoing LEADER trial has enrolled 9340 people with type 2 diabetes and at high risk of cardiovascular disease at 410 centres worldwide. People with baseline serum calcitonin ≤ 50 ng/l were randomized to liraglutide once daily or placebo and will be followed for up to 5 years. Serum calcitonin was measured at baseline and will be measured annually thereafter. An independent committee of thyroid experts will oversee calcitonin monitoring throughout the trial and will review all calcitonin concentrations ≥ 20 ng/l.

Results: The mean age of participants was 64.3 ± 7.2 years, 64.3% were men, and mean the body mass index was 32.5 ± 6.3 kg/m². The median (interquartile range) baseline serum calcitonin values were 3.9 (1.0 to >7.6) ng/l in men and 1.0 (1.0 to >1) ng/l in women. Serum calcitonin was > 10 ng/l in 14.6% of men and in 0.96% of women. In sex-specific multivariable linear analysis of covariance models, a reduced glomerular filtration rate (GFR) was associated with higher serum calcitonin concentrations that were statistically significant. A 20 ml/min/1.73 m² decrease in estimated GFR (eGFR) was associated with a 14% increase in serum calcitonin in women and an 11% increase in men.

Conclusions: In the LEADER population, the prevalence of elevated serum calcitonin concentrations at baseline was high, and there was an inverse association between eGFR and serum calcitonin concentrations.

Keywords: calcitonin, c-cell disease, diabetes, incretins

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Introduction

Liraglutide is a glucagon-like peptide 1 (GLP-1) analogue approved for use in adults with type 2 diabetes mellitus [1,2]. In preclinical rodent studies, liraglutide and other GLP-1 receptor agonists were associated with hyperplasia of the calcitonin-producing C-cells [C-cell hyperplasia (CCH)], C-cell adenomas and C-cell carcinomas. C-cell pathology was absent in liraglutide-treated GLP-1 receptor knockout animals [3,4],

supporting a role for GLP-1 receptors in these pathological findings. C-cell pathology did not develop in cynomolgus monkeys treated with liraglutide for >18 months and at doses up to 64-fold greater than those calculated for human exposure [3].

In humans, long-term exposure to liraglutide does not significantly affect serum calcitonin concentrations [5]. In combined data from nine studies of ≥ 20 weeks' duration, including >5000 subjects with either type 2 diabetes or obesity without diabetes, there was no significant difference in the proportion of subjects with calcitonin concentrations > 20 ng/l in the liraglutide versus the placebo groups [5]. Nonetheless, based on non-clinical studies, labelling information in the USA includes a boxed warning that liraglutide causes thyroid C-cell tumours in rodents, and its use is contraindicated in people with a personal or family history of medullary thyroid carcinoma (MTC).

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The reference range of serum calcitonin is generally accepted to be <10 ng/l [6]. Serum calcitonin is higher in healthy men (<8.4 ng/l) than in healthy women (<5.0 ng/l). Serum calcitonin >10 ng/l without known C-cell pathology has been reported in people who consume tobacco and alcohol, as well as in people with elevated serum gastrin levels, reduced kidney function, autoimmune thyroid disease, sepsis, heterophilic antibodies and with calcitonin production from non-MTC malignancies [7].

In people with thyroid nodules, a serum calcitonin concentration >100 ng/l is generally associated with MTC; concentrations between 10 and 100 ng/l are considered indeterminate and associated with C-cell pathology in only a minority of subjects; a higher serum calcitonin concentration is associated with a higher the likelihood of MTC [8]. In patients with thyroid nodules, the sensitivity, specificity and positive predictive values for detection of MTC depend on cut-off values for serum calcitonin [9]. For these reasons the role of serum calcitonin measurements in screening for MTC in the thyroid nodule population is controversial, and uncertainty is even greater concerning the specificity of indeterminate calcitonin concentrations in people without known thyroid abnormalities and with other medical conditions such as type 2 diabetes [10–14].

The Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial, is an international, double-blind, placebo-controlled trial, currently evaluating the cardiovascular safety of liraglutide (www.clinicaltrials.gov NCT01179048). A total of 9340 people with type 2 diabetes and at high risk of cardiovascular events (with or without existing cardiovascular disease) have been enrolled at 410 centres worldwide and randomized 1:1 to liraglutide or placebo. Participants will be followed for up to 5 years. To monitor any potential effects of liraglutide on calcitonin concentrations, serum calcitonin samples are collected at baseline and then at various time points.

The present study is a preliminary report of baseline measurements from the LEADER population. Specifically, we report the baseline serum calcitonin concentrations in the LEADER population, evaluate the influence of various clinical characteristics on baseline calcitonin concentrations, and describe the calcitonin monitoring programme developed for the LEADER trial.

Materials and Methods

Study Design

The design of the LEADER trial has been described previously [15]. People with type 2 diabetes and elevated cardiovascular risk, who were either drug-naïve or treated with one or more antihyperglycaemic drug (including basal and premix insulins) were included. Initial exclusion criteria included basal calcitonin concentration >100 ng/l that was subsequently lowered to ≥ 50 ng/l, as requested by regulators in the USA. People with a personal or family history of multiple endocrine neoplasia type 2 or familial MTC were also excluded. As the target population was at high risk of cardiovascular disease, we encouraged the

enrolment of people with reduced kidney function. We did not exclude people using medication thought to increase serum calcitonin levels. Each enrolling centre's institutional review board approved the study.

Calcitonin Screening and Monitoring

Serum calcitonin was measured in the fasting state in all participants using a chemiluminescent immunometric assay (Siemens Healthcare Diagnostics, Malvern, PA, USA) performed by a central laboratory (ICON PLC, Dublin, Ireland). A serum calcitonin concentration of 2.0 ng/l was defined as the lower limit of quantification (LLQ). For this particular assay, the upper limit of normal (ULN) was defined as 8.4 ng/l for men and 5.0 ng/l for women.

An independent calcitonin monitoring committee (CMC) of thyroid experts (L.H., R.M.T. and Steven Sherman, MD, University of Texas MD Anderson Cancer Center, Houston, TX, USA) was appointed to oversee serum calcitonin monitoring and to provide guidance for trial investigators. CMC clinicians are not otherwise involved in the LEADER trial. Calcitonin concentrations ≥ 20 ng/l will be reported to the CMC for review along with relevant supplementary data (demographics, diabetes history, concomitant medical history, concomitant medications, smoking status, supplemental laboratory tests, and relevant adverse event data reported during the trial). Upon review of all relevant materials, the CMC will provide recommendations to the investigators for individual subjects, including diagnostic procedures, treatment and continuation of the study drug according to a pre-specified algorithm (Appendix). Furthermore, both calcitonin concentrations ≥ 20 ng/l and diagnoses of thyroid disease (whether related to the recommendations from the CMC or not) will be reported as medical events of special interest.

After randomization, study visits occur at months 1, 3 and 6, and every 6 months thereafter until termination of the trial. Participants will be followed for up to 5 years. During each study visit, clinical events, study drug compliance and concomitant medication usage are documented. Calcitonin concentrations are measured annually.

Statistical Analysis

Serum calcitonin concentrations were not normally distributed, partly because of the substantial number of baseline calcitonin values below the LLQ (87.3% of women, 27.8% of men). The high prevalence of baseline calcitonin values <2.0 ng/l warranted the logarithmical transformation before analysis, with all values <2.0 ng/l arbitrarily set as half the LLQ value (2 ng/l) for descriptive purposes. Multivariable linear analysis of covariance models were developed separately for men and women because of the major sex-dependent differences in the calcitonin distribution, with the log-transformed calcitonin value as the dependent variable. Independent covariates in the analysis of covariance models were prespecified and included age, body mass index (BMI), smoking status, ethnicity, diabetes, medical history, glycated haemoglobin, estimated glomerular filtration rate (eGFR), autoimmune disease, thyroid disease (not otherwise specified), lipid concentrations and

medication use (proton pump inhibitors, β -blocker agents, H2 receptor antagonists and glucocorticoids). These variables were selected before analysis by consideration of the potential impact on calcitonin levels, based on an extensive review of the literature and availability in the LEADER database. The effect of each covariate on baseline calcitonin was first derived on the log-calcitonin scale and then transformed back to obtain a relative effect expressed as a percentage. All analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). Two-way interactions between the covariates eGFR kidney function and BMI and thyroid disease and smoking status (yes/no) as factors were evaluated in both gender models. The main effects of all covariates/factors were retained in the model, as the ample sample size of the LEADER study implied no need for arbitrary variable selection.

Results

Baseline Demographics

The baseline demographic profile of the men, women and all randomized LEADER trial participants ($n = 9340$) is shown in Table 1. Of the 12 094 screened subjects, 2754 (23%) failed to meet the inclusion criteria. Among the latter group, 42 subjects (0.35% of the population screened) were excluded for having calcitonin concentrations >50 ng/l. Because of the nature of the study, no further information about these subjects is available. Two subjects with a calcitonin value of 115 ng/l were erroneously randomized. As soon as this error was discovered, trial treatment was discontinued.

Serum Calcitonin

Baseline serum calcitonin concentrations were available for 6003 men and 3337 women. The distribution of serum calcitonin concentrations in study participants by sex is shown in Figure 1 and Table 2. The median (interquartile range) baseline serum calcitonin values were 3.9 (1.0 to >7.6) ng/l in men and 1.0 (1.0 to >1) ng/l in women. Overall, 2.8% of women and 21.3% of men had serum calcitonin concentrations above their normal sex-specific reference values of 5.0 and 8.4 ng/l, respectively. Sex was the most important determinant of elevated serum calcitonin concentrations. Serum calcitonin concentrations in men were <2.0 ng/l (LLQ) in 27.8%, between 2.0 and 8.4 ng/l (ULN) in 50.3%, and >8.4 ng/l in 21.1%. Serum calcitonin concentrations in men were between >10 and ≤ 20 ng/l in 7.3% and between >20 and <50 ng/l in 3.8%. In contrast, 87.3% of women had serum calcitonin concentrations below 2.0 ng/l (LLQ), 9.9% had calcitonin concentrations between 2.0 and 5.0 ng/l (ULN), and 2.8% had calcitonin concentrations >5.0 ng/l. Only 0.75% of women had serum calcitonin concentrations >10 and <20 ng/l and 0.2% had calcitonin concentrations >20 and ≤ 50 ng/l.

The relationships between baseline calcitonin concentrations, expressed in relation to the LLQ and sex-specific ULN in men and women, and eGFR, BMI and smoking are shown in Table 2. Among women, serum calcitonin concentrations >10 ng/l were present in 0.9% with eGFR 60–89 ml/min/1.73 m² (corresponding to The National Kidney

Foundation – Kidney Disease Outcomes Quality Initiative chronic kidney disease (CKD) stage 2), 1.7% with eGFR 30–59 ml/min/1.73 m² (CKD stage 3), and 3.9% in women with eGFR <30 ml/min/1.73 m² (CKD stage 4 and 5). In men, serum calcitonin concentrations >10 ng/l increased as eGFR declined from normal (≥ 90 ml/min/1.73 m²) to eGFR values corresponding to CKD stages 2, 3 and 4/5 (13.4, 14.9, 23.2 and 38.0%, respectively). Among participants with serum calcitonin concentrations >10 ng/l, 21 of 32 women (65.6%) and 678 of 979 men (69.3%) had an eGFR below normal (<90 ml/min/1.73 m²).

Results of the log-linear multivariate models, by sex, are shown in Figure 2A, B. In both men and women, eGFR, smoking and higher BMI were clearly associated with higher serum calcitonin concentrations. A 20-ml/min/1.73 m² decrease in eGFR was associated with a 14% increase in serum calcitonin in women and an 11% increase in men. Smoking and each 5-kg/m² increase in BMI were associated with, respectively, 21.9 and 11.1% increases in serum calcitonin in women, and 16.3 and 9.9% increases in men. There was a statistically significant association between absence of cardiovascular disease and calcitonin concentrations in men. In women there was insufficient information to draw a conclusion (Figure 2B). Two-way interactions between eGFR kidney function and BMI as covariates and thyroid disease and current smoking status (yes/no) were not identifiable (non-significant) for either gender. Increasing age had a statistically significant negative correlation with calcitonin concentrations in women but not in men, a finding of uncertain clinical significance and mechanism.

Discussion

This present report, in which the baseline calcitonin concentrations in a global high cardiovascular risk type 2 diabetes population [15] were analysed, is believed to be the largest study of baseline calcitonin concentrations in a population not specifically selected for the presence of thyroid nodules. In the thyroid nodule population, a calcitonin concentration >10 ng/l is often regarded as a threshold above which pathological C-cell disorders should be investigated [8]. In our LEADER trial population, 10.8% of participants had serum calcitonin concentrations >10 ng/l, and 2.6% had concentrations >20 ng/l. We analysed several possible explanations for the elevated calcitonin concentration in this population. Because of the nature of the baseline information and because the effect of liraglutide on calcitonin is subject to ongoing monitoring throughout the trial, information concerning baseline thyroid palpation, thyroid ultrasonography and thyroid pathology is not available in this population, nor are follow-up data available for patients excluded for having baseline calcitonin concentrations >50 ng/l.

The maximum C-cell surface area in the adult thyroid gland is twice as high in men as in women [16], which could account for the differences in baseline and stimulated calcitonin in men compared with women [7]. In the present study, sex was highly determinative of elevated calcitonin concentrations: $>21\%$ of men and nearly 3% of women had serum calcitonin concentrations above their

Table 1. Baseline demographic characteristics of the study participants.

	Total (n = 9340)	Female (n = 3337)	Male (n = 6003)
Age, years			
Mean ± s.d.	64.3 ± 7.2	64.4 ± 7.2	64.2 ± 7.3
Median	64.0	64.0	64.0
Body weight, kg			
Mean ± s.d.	91.8 ± 21.0	84.7 ± 19.5	95.7 ± 20.8
Median	89.9	82.4	93.2
Body mass index, kg/m ²			
Mean ± s.d.	32.5 ± 6.3	33.6 ± 6.8	31.9 ± 5.9
Median	31.7	32.8	31.2
HbA1c, %			
Mean ± s.d.	8.7 ± 1.5	8.8 ± 1.6	8.6 ± 1.5
Median	8.3	8.4	8.2
Diabetes duration, years			
Mean ± s.d.	12.7 ± 8.0	13.3 ± 8.3	12.4 ± 7.8
Median	11.3	11.8	11.1
Blood pressure, mmHg			
Systolic			
Mean ± s.d.	137.7 ± 18.6	139.1 ± 19.2	136.9 ± 18.2
Median	137.0	138.0	136.0
Diastolic			
Mean ± s.d.	77.9 ± 10.5	78.1 ± 10.7	77.8 ± 10.3
Median	78.5	79.0	78.5
Heart rate, beats per min			
Mean ± s.d.	73.2 ± 11.4	74.4 ± 10.9	72.5 ± 11.6
Median	72.0	74.0	72.0
eGFR, n (%)			
≥90 ml/min/1.73 m ²	3447 (36.9)	1207 (36.2)	2240 (37.3)
60–89 ml/min/1.73 m ²	3860 (41.3)	1353 (40.5)	2507 (41.8)
30–59 ml/min/1.73 m ²	1854 (19.9)	700 (21.0)	1154 (19.2)
<30 ml/min/1.73 m ²	177 (1.9)	76 (2.3)	101 (1.7)
Glucose-lowering treatment, n (%)			
Diet or no treatment	504 (5.4)	128 (5.3)	376 (5.5)
Insulin	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, n (%)	7592 (81.3)	2544 (76.2)	5048 (84.1)
Number of oral antihyperglycaemic 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)
Yes	7592 (81.3)	2594 (76.2)	5048 (84.1)
No	1748 (18.7)	793 (23.8)	955 (15.9)
Smoking, n (%)			
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)
Proton pump inhibitor use, n (%)	1963 (21.0)	753 (22.6)	1210 (20.2)
H2 receptor antagonist use, n (%)	303 (3.2)	106 (3.2)	197 (3.3)

BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; s.d., standard deviation.

*Not used in combination with insulin.

sex-specific normal reference values of 8.4 and 5.0 ng/l, respectively. Differences between men and women persisted at the higher serum calcitonin thresholds of 10 ng/l (16.3 vs 0.96%, respectively) and 20 ng/l (4.0 vs 0.21%, respectively). As a consequence of our inclusion criteria, two-thirds of our participants had an eGFR <90 ml/min/1.73 m² at baseline [15]. We found a significant inverse association between baseline serum calcitonin concentration and eGFR in both men and

women, similarly to other investigators. Lissak et al. [17], reported significantly higher basal calcitonin concentrations ($p < 0.05$) in 30 subjects with moderately reduced kidney function (eGFR <58 ml/min) compared with controls (3.55 vs 2.00 ng/l). Specifically, 20% had basal calcitonin >10 ng/l (maximum: 51 ng/l), including six out of 18 men and none out of 12 women. Kanbay et al. [18] reported mean serum calcitonin concentrations of 11.5 ± 7.8 ng/l in a population

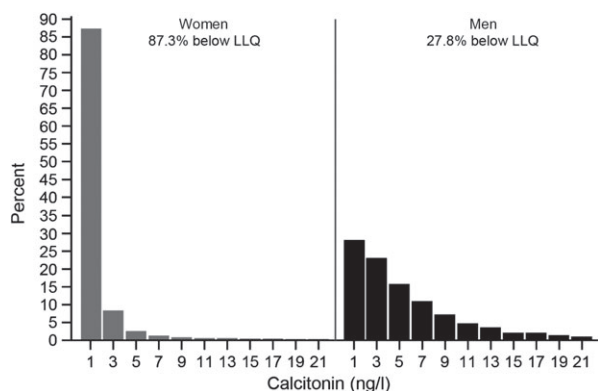


Figure 1. Distribution of calcitonin measurements by sex. The lower limit of quantification (LLQ) for both men and women was 2.0 ng/l. The upper limit of normal was 5.0 ng/l for women and 8.4 ng/l for men.

of 88 subjects with moderately-to-mildly reduced kidney function (eGFR 46–87 ml/min/1.73 m²). Niccoli et al. [19] studied 154 people undergoing haemodialysis and found basal calcitonin concentrations >20 ng/l in 25.3% and >90 ng/l in 7.8%. Akan et al. [20] studied 283 people undergoing dialysis and found median calcitonin concentrations of 12 ng/l in men and 2 ng/l in women, while 58% of men and 10% of women had concentrations >10 ng/l.

In women, the prevalence of a serum calcitonin concentration >10 ng/l was similar across eGFR categories (eGFR ≥90 ml/min/1.73 m²: 0.9%; eGFR 60–89 ml/min/1.73 m²: 1.0%; eGFR 30–59 ml/min/1.73 m²: 0.7%; and eGFR <30 ml/min/1.73 m²: 3.9%). In men, the prevalence of a serum calcitonin concentration >10 ng/l increased with declining eGFR, from 13.4% for men with eGFR ≥90 ml/min/1.73 m², 14.9% for those with eGFR 60–89 ml/min/1.73 m², 23.2% for those with eGFR 30–59 ml/min/1.73 m² to 38.0% for those with eGFR <30 ml/min/1.73 m². In people with serum calcitonin concentrations >10 ng/l, 21 of 32 women (65.6%) and 678 of 978 men (69.3%) had an eGFR <90 ml/min/1.73 m².

In most studies the ULN for serum calcitonin is higher in smokers than in non-smokers [21], but the mechanism for this difference is unknown. In the present study, 1.8% of women who smoked had serum calcitonin concentrations >10 ng/l, twice the rate of female non-smokers (0.9%). In men, these figures were 19.2% for smokers and 15.7% for non-smokers, respectively. Among participants with serum calcitonin concentrations >10 ng/l, 5 out of 27 (18.5%) women and 163 out of 978 (16.7%) men were current smokers.

In multivariable analysis performed separately for men and women, factors common to both sexes that were associated with statistically significant changes in serum calcitonin concentrations were reduced eGFR, smoking, increased BMI, and presence of thyroid disease, but not use of proton pump inhibitors. There was an inverse relationship between serum calcitonin and both eGFR and presence of thyroid disease, and a direct relationship between serum calcitonin and both BMI and smoking. Serum calcitonin concentration has been reported to be higher when serum gastrin is elevated, including individuals with pernicious anaemia, intake of drugs inhibiting gastric acid secretion, or gastrinomas. In work by Erdogan

et al. [22], individuals taking proton pump inhibitors or H2 blockers had a mean serum calcitonin of 11.9 ng/l, whereas the concentration was 6.0 ng/l in those not taking these agents. We found no association between serum calcitonin and H2 receptor antagonist or proton pump inhibitor use. Although the explanation for our lack of correlation is uncertain, it might be attributable to reporting bias in that non-prescription use of these agents was not recorded. Information on the types of thyroid disease was not collected as part of the trial, and more detailed baseline information is not available. It is possible that the lower calcitonin concentrations might be related to previous thyroidectomy.

Food intake has been reported to elevate serum calcitonin concentration [23], but we measured serum calcitonin concentrations in the fasting state. Heterophilic antibodies may falsely elevate serum calcitonin concentrations in up to 1.3% of the population [24], however, these were not assessed in the present study. Other potential factors leading to elevated serum calcitonin concentrations unrelated to C-cell pathology include calcitonin production from non-MTC malignancies, autoimmune thyroid disease, alcohol consumption and sepsis [7]. Previous studies have not confirmed an association between autoimmune thyroid disease and elevated serum calcitonin concentrations [25]. The present study did not find a significant association between autoimmune thyroid disease and serum calcitonin concentrations; however, there was no systematic effort to identify autoimmune thyroid disease. A calcitonin concentration >10 ng/l is often regarded as a threshold above which pathological C-cell disorders should be investigated [8]. For this reason, it is important to compare the present study with several other population studies. Only 25 of 5000 (0.5%) people with type 2 diabetes mellitus who were enrolled in previous trials of liraglutide had a baseline serum calcitonin concentration >20 ng/l [5]. Costante et al. [8] studied 5817 subjects with thyroid nodules, a population thought to have a high prevalence of MTC, and found that only 5% had a serum calcitonin concentration >10 ng/l. d'Herbomez et al. [7] measured serum calcitonin in 375 clinically euthyroid subjects using five different calcitonin assays and found 2.5–9.8% had serum calcitonin concentrations >10 ng/l, most of whom were male smokers, although there was considerable variability between assays used.

In the present study, 64% of participants were men compared with only 19% in the study by Costante et al. [8]. Although there was a statistically significant association between smoking status and serum calcitonin in both men and women, it is unlikely that smoking alone accounts for the differences between the present study and others. It is unlikely that the particular calcitonin assay used in the present study explains the higher prevalence of elevated serum calcitonin concentrations, given that 95% of normal healthy people tested with this assay have serum calcitonin concentrations ≤8.4 ng/l for men and ≤5.0 ng/l for women. It is likely that our high-risk population included a higher prevalence of subjects with elevated BMI. Screening calcitonin studies in thyroid nodule populations have typically not reported BMI data.

For the present study, we intentionally selected a population with type 2 diabetes at high risk of cardiovascular events. The reported higher proportion of both men and women with

Table 2. Distribution of baseline calcitonin values stratified by sex and clinically relevant risk factors.

Women*							
	Calcitonin (ng/l)						
	≤2.0 (n = 2911)	>2.0 to ≤5.0 (n = 329)	>5.0 to ≤10.0 (n = 61)	>10.0 to ≤20.0 (n = 25)	>20.0 to ≤50.0 (n = 7)		
eGFR, ml/min/1.73 m ²							
≥90	1091 (90.5)	87 (7.2)	17 (1.4)	10 (0.8)	1 (0.1)		
60–89	1195 (88.5)	119 (8.8)	23 (1.7)	9 (0.7)	4 (0.3)		
30–59	570 (81.4)	105 (15.0)	20 (2.9)	4 (0.6)	1 (0.1)		
<30	54 (71.1)	18 (23.7)	1 (1.3)	2 (2.6)	1 (1.3)		
BMI, kg/m ²							
<25 kg/m ²	357 (92.5)	24 (6.2)	3 (0.8)	0	2 (0.5)		
25–30 kg/m ²	791 (90.2)	69 (7.9)	7 (0.8)	9 (1.0)	1 (0.1)		
30–35 kg/m ²	855 (86.0)	113 (11.4)	17 (1.7)	8 (0.8)	1 (0.1)		
>35 kg/m ²	902 (84.3)	123 (11.5)	34 (3.2)	8 (0.7)	3 (0.3)		
Current smoking							
No	2676 (87.7)	296 (9.7)	53 (1.7)	22 (0.7)	5 (0.2)		
Yes	235 (83.6)	33 (11.7)	8 (2.8)	3 (1.1)	2 (0.7)		
Cardiovascular disease							
Age ≤60 years	761 (84.6)	103 (11.5)	22 (2.4)	10 (1.1)	3 (0.3)		
Age >60 years	1430 (87.0)	168 (10.2)	32 (1.9)	10 (0.6)	3 (0.2)		
Thyroid disease							
	730 (89.6)	59 (7.2)	13 (1.6)	11 (1.3)	2 (0.2)		
Men†							
	Calcitonin (ng/l)						
	≤2.0 (n = 1669)	>2.0 to ≤8.4 (n = 3048)	>8.4 to ≤10.0 (n = 301)	>10.0 to ≤20.0 (n = 738)	>20.0 to ≤50.0 (n = 230)	>50.0 to ≤100.0 (n = 8)	>100.0 (n = 2)
eGFR							
≥90 ml/min/1.73 m ²	732 (32.7)	1104 (49.3)	103 (4.6)	234 (10.5)	62 (2.8)	4 (0.2)	0
60–89 ml/min/1.73 m ²	685 (27.4)	1318 (52.7)	127 (5.1)	301 (12.0)	68 (2.7)	3 (0.1)	0
30–59 ml/min/1.73 m ²	240 (20.8)	578 (50.1)	68 (5.9)	181 (15.7)	84 (7.3)	1 (0.1)	2 (0.2)
<30 ml/min/1.73 m ²	11 (11.0)	48 (48.0)	3 (3.0)	22 (22.0)	16 (16.0)	0	0
BMI							
<25 kg/m ²	270 (31.7)	44 (52.4)	29 (3.4)	91 (10.7)	16 (1.9)	0	0
25–30 kg/m ²	584 (28.6)	1077 (52.8)	92 (4.5)	220 (10.8)	66 (3.2)	2 (0.1)	0
30–35 kg/m ²	503 (27.3)	926 (50.3)	95 (5.2)	237 (12.9)	77 (4.2)	2 (0.1)	1 (0.1)
>35 kg/m ²	312 (24.8)	594 (47.3)	85 (6.8)	190 (15.1)	71 (5.6)	4 (0.3)	1 (0.1)
Current smoking							
No	1452 (28.2)	51.0 (253)	253 (4.9)	619 (12.0)	188 (3.7)	6 (0.1)	2 (0.0)
Yes	217 (25.6)	49.5 (58.0)	48 (5.7)	119 (14.0)	42 (5.0)	2 (0.2)	0
Cardiovascular disease							
Age ≤60 years	514 (29.1)	870 (49.2)	87 (4.9)	224 (12.7)	67 (3.8)	5 (0.3)	2 (0.1)
Age >60 years	858 (26.2)	1705 (52.1)	164 (5.0)	413 (12.6)	130 (4.0)	3 (0.1)	0
Thyroid disease							
	207 (39.3)	215 (40.8)	18 (3.4)	63 (12.0)	21 (4.0)	2 (0.4)	1 (0.2)

BMI, body mass index; eGFR, estimated glomerular filtration rate.

Data are n (%).

*Lower limit of quantification = 2.0 ng/l; upper limit of normal = 5.0 ng/l.

†Lower limit of quantification = 2.0 ng/l; upper limit of normal = 8.4 ng/l.

calcitonin concentrations above the ULN in the present study is probably related to the specific population under investigation. The present study also had a greater proportion of men, a higher prevalence of current smokers and a high mean BMI, as well as other unknown factors that could potentially affect calcitonin levels; however, in the absence of clinical correlations, we cannot completely exclude an increased prevalence of C-cell pathology in the present population.

In the general population, an elevated serum calcitonin level is a sensitive biomarker for pathological diseases of the C cells,

MTC and CCH. Given the high prevalence of elevated serum calcitonin concentrations in the present study it is important to consider the specificity of serum calcitonin concentrations between 10 and 100 ng/l for MTC or CCH. In a study of 5817 subjects with thyroid nodules, a population thought to be at higher risk of MTC than the general population, 216 (3.7%) had a serum calcitonin concentration between >10 and ≤20 ng/l [8]. Only 1 patient eventually had surgery because of rising serum calcitonin concentration, and CCH was found. There were 49 subjects (0.84%) with serum calcitonin concentrations

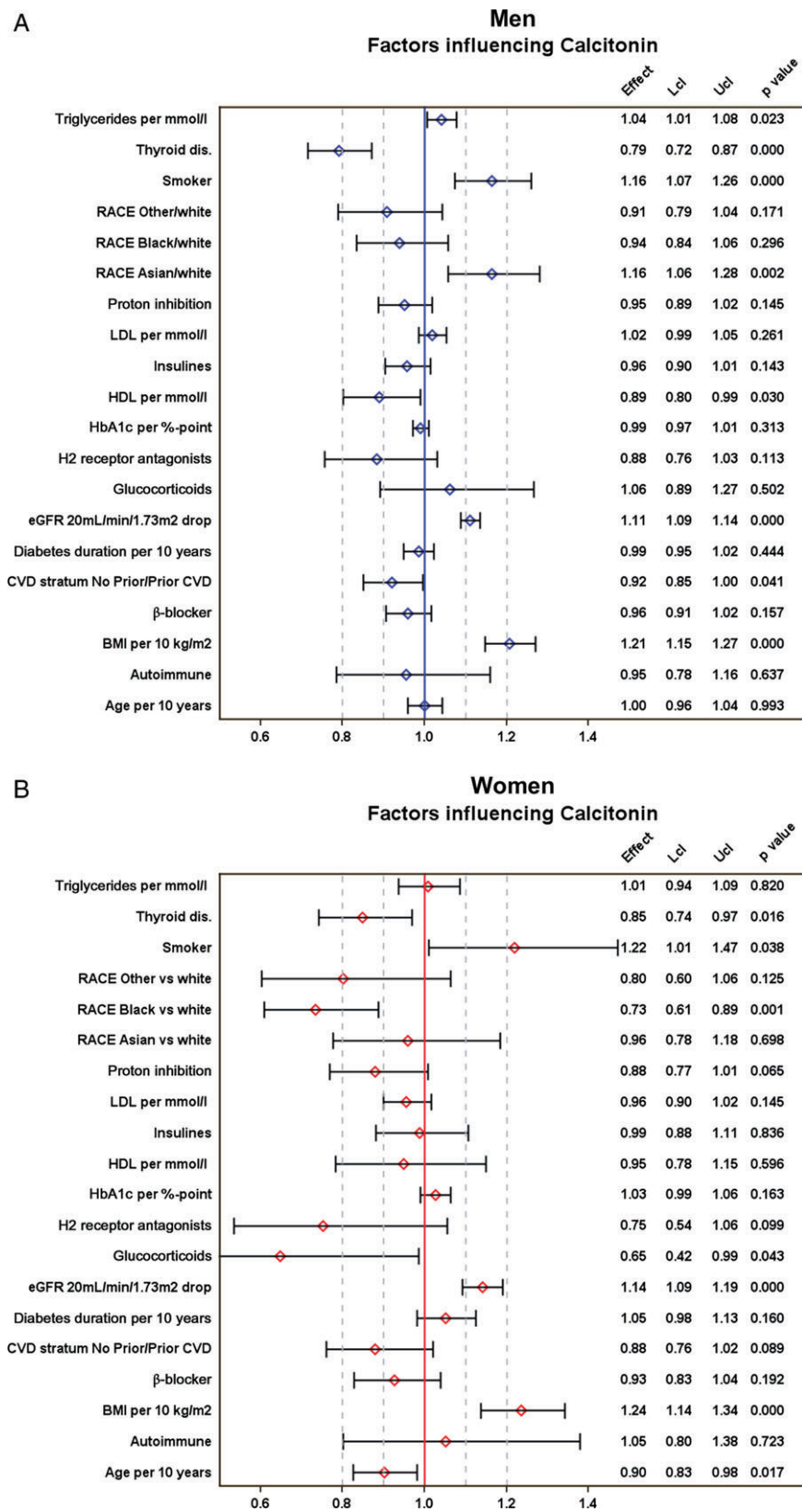


Figure 2. Forest plots showing the results of multivariate log-linear analysis of calcitonin concentrations in (A) men and (B) women. For each explanatory variable, the relative effect (1 is the neutral value) is depicted together with the corresponding 95% confidence interval. The right panel cites the numbers together with the p value derived from the test of 'no difference'. HbA1c, glycated haemoglobin; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease.

between >20 and <50 ng/l. Of these, 12 had abnormal penta-gastrin stimulation tests and 10 agreed to surgery; 4 of these subjects had MTC, and 4 had CCH. All nine subjects with basal calcitonin >100 ng/l had MTC. These findings indicate that only a small percentage of individuals with thyroid nodules and serum calcitonin concentrations between 10 and <50 ng/l will have MTC. Based on our observations, serum calcitonin concentrations between 20 and 50 ng/l may have even lower specificity for C-cell disease in men with type 2 diabetes at high risk of atherosclerotic disease, such as that in the present patient population. For this baseline assessment of the LEADER population, we do not have additional information about thyroid investigations in any of these subjects. Information about subjects with persistent serum calcitonin concentrations >20 ng/l will be collected and will be the subject of future publications.

In summary, the LEADER trial population had a higher than anticipated prevalence of elevated serum calcitonin concentrations. The population was obese (mean BMI 32.5 kg/m²), and nearly 25% of participants had reductions in eGFR <60 ml/min/1.73 m². Lower eGFR and higher BMI had a significant association with serum calcitonin concentrations. Other factors probably contributing to the distribution of calcitonin concentrations in the present study were the high prevalence of men and smokers. Further studies are needed to determine the serum calcitonin threshold that should trigger a search for C-cell pathology in a population such as the present one, particularly in light of the additional 42 subjects with serum calcitonin concentrations >50 ng/l excluded before our study (0.35% of subjects screened). Notably, a thyroid nodule population thought to be at higher risk of C-cell disorders [6] found 17 patients (0.29%) with a basal calcitonin ≥50 ng/l; therefore, the reference range for serum calcitonin concentrations in the normal population may not be applicable to subjects with cardiovascular disease and type 2 diabetes mellitus.

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Conflict of Interest

R. M. B. has served on a scientific advisory board, consulted on or performed 479 clinical research studies with Abbott Diabetes Care, Amylin, Bayer, Becton Dickinson, Boehringer Ingelheim, Bristol-Myers Squibb/AstraZeneca, Intuity, Calibra, DexCom, Eli Lilly, Halozyme, Helmsley Trust, Hygieia, Johnson & Johnson, Medtronic, Merck, NIH, Novo Nordisk, ResMed, Roche, Sanofi, and Takeda. His employer, non-profit Park Nicollet Institute, contracts for his services, and no personal income goes to him. He has inherited stock in Merck. J. B. B. is an investigator and/or consultant without any direct financial benefit under contracts between his employer (the

University of North Carolina) and the following companies: Andromeda; Astellas; Astra-Zeneca; Bayhill Therapeutics, Inc.; Boehringer Ingelheim GmbH & Co. KG; Bristol-Myers Squibb; Dance Pharmaceuticals; Elcelyx Therapeutics, Inc.; Eli Lilly and Company; GI Dynamics; GlaxoSmithKline; Halozyme Therapeutics; F. Hoffmann-La Roche, Ltd.; Intarcia Therapeutics; Johnson & Johnson; Lexicon; LipoScience; Medtronic; Merck; Metabolic Solutions Development Co.; Metabolon, Inc.; Metavention; Novartis; Novo Nordisk A/S; Orexigen Therapeutics, Inc.; Osiris Therapeutics, Inc.; Pfizer, Inc.; Rhythm Pharmaceuticals; Sanofi; Spherix, Inc.; Takeda; Tolerx; TransTech Pharma; Veritas; Verva. J.B.B. is a consultant for and will receive stock options from PhaseBio. G. H. D. is a consultant for Genzyme (Sanofi), Exilixis, and Novo Nordisk. J. D. K. is employed by Novo Nordisk. L. H. is a consultant for Novo Nordisk, heads the Novo Nordisk Calcitonin Steering Committee, and has received research support as well as unrestricted research grants from the Novo Nordisk Foundation. J. F. E. M. is an investigator and/or consultant receiving honoraria from Abbott, Bayer, Boehringer-Ingelheim, Novo-Nordisk, Roche, and Vifor. S. P. M. has received research grants and or consulting fees from Amylin, Novo Nordisk, St. Jude Medical, Terumo, The Medicines Company, and Volcano Corp. A. C. M. is a full-time employee of and holds stock in Novo Nordisk A/S.

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All authors reviewed and approved the final manuscript and participated in the conception, planning and execution of the calcitonin-monitoring portion of the LEADER trial.

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Appendix

Algorithm for calcitonin screening and monitoring

- Calcitonin ≥ 50 ng/l
 - A calcitonin measurement >50 ng/l is a screening failure, and the patient shall be referred to a thyroid specialist for further evaluation.
- Calcitonin <20 ng/l
 - Investigator shall evaluate factors potentially leading to calcitonin elevation.
 - Calcitonin sampling shall be performed at intervals indicated in the study protocol with no further action unless levels rise to ≥ 20 ng/l.
 - If the value is the last one taken in the trial, the subject shall be referred to a thyroid specialist for further evaluation.
- Calcitonin ≥ 20 and <50 ng/l
 - Calcitonin sampling shall be performed at intervals indicated in the study protocol with no further action unless levels rise to ≥ 50 ng/l.
 - No impact on continuation of trial treatment.
 - If the value is the last one taken in the trial, the subject shall be referred to a thyroid specialist for further evaluation.
- Calcitonin ≥ 50 and <100 ng/l
 - The subject shall be referred to a thyroid specialist for further evaluation.
 - Recommendations about a diagnostic evaluation shall include ultrasound examination and, if available and not contraindicated, a pentagastrin stimulation test (Europe). Subjects with positive pentagastrin stimulation tests will be considered for surgery. In the US, where pentagastrin is not available, thyroid ultrasound and fine needle aspiration biopsy may add important clinical information informing the need for surgery.
 - If calcitonin levels fluctuate around 50 ng/l without demonstrating a progressive rise, then subjects may continue on the drug.
 - If calcitonin level is confirmed to be above 50 ng/l, then the CMC shall recommend the study investigator remove the subject from study treatment. Study treatment may be re-introduced if the reason for discontinuation no longer exists (e.g., suspicion of potential C-cell disease is excluded).

- Calcitonin ≥ 100 ng/l

- The subject shall be referred to a thyroid specialist for further evaluation.

- Recommendations about a diagnostic evaluation shall include ultrasound examination and, if available and not contraindicated, a pentagastrin stimulation test (Europe). Subjects with positive pentagastrin stimulation tests will be considered for surgery. In the US, where pentagastrin is not available, thyroid ultrasound and fine needle aspiration

biopsy may add important clinical information informing the need for surgery.

- If C-cell neoplasia is diagnosed, family history of MTC or multiple endocrine neoplasia type 2 will be evoked, and a RET protooncogene analysis should be undertaken. The CMC shall provide recommendations to the investigator regarding interpretation of genetic test results.

- If calcitonin level rises above 100 ng/l, then the CMC shall recommend the study investigator remove the subject from study treatment.