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## ORIGINAL ARTICLE

# Subcutaneous adipose tissue composition and function are unaffected by liraglutide-induced weight loss in adults with type 1 diabetes

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## Abstract

Adipose tissue is the primary energy reservoir of the human body, which also possesses endocrine functions. The glucagon-like peptide agonist liraglutide produces weight loss, although the specific effects on adipose tissue are unknown. We aimed to characterize the white adipose tissue composition and pericellular fibrosis of subcutaneous adipose tissue in response to liraglutide treatment. Furthermore, we explored the level of circulating free fatty acids, cluster of differentiation 163 (CD163) macrophage marker, leptin and adiponectin. Thirty-nine adults with type 1 diabetes and polyneuropathy were randomly assigned to 26 weeks of liraglutide or placebo treatment. Biopsies of subcutaneous tissue were formalin-fixed stained with picrosirius red to visualize collagen or immunohistochemically stained for CD163. Serum concentrations of free fatty acids, CD163, leptin and adiponectin were assessed with immunoassays or multiplex panels. In comparison with placebo, liraglutide induced weight loss (3.38 kg, 95% CI −5.29; −1.48,  $P < 0.001$ ), but did not cause any differences in cell size, distribution of CD163-positive cells, pericellular fibrosis and serum levels of free fatty acids, CD163, leptin or adiponectin (all  $P < 0.1$ ). Additionally, no associations between weight loss, cell size and serum markers were found (all  $P > 0.08$ ). In conclusion, despite liraglutide's effect on weight loss, sustained alterations in subcutaneous adipose tissue did not seem to appear.

## KEYWORDS

adipose, anti-inflammatory drugs, diabetes mellitus, liraglutide, weight loss drugs

## 1 | INTRODUCTION AND BACKGROUND

Adipose tissue is a highly dynamic tissue that functions as the primary energy reservoir in the human body and can be

viewed as an “organ,” dispersed at discrete locations in the body referred to as depots. Adipocytes comprise approximately 90% of the tissue volume and are traditionally divided into two morphologically and functionally distinct subtypes referred to as white and brown adipocytes.<sup>1</sup> White adipose

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tissue possesses endocrine functions and secretes adipokines, including leptin and adiponectin, which regulate appetite and energy metabolism. Furthermore, they are involved in systemic metabolic homeostasis and redistribution of adipose tissue, while the less abundant brown adipocytes are rich in mitochondria and thus metabolically active.<sup>2,3</sup>

Liraglutide is a GLP-1 agonist that shares the same pharmacological effects as native GLP-1.<sup>4</sup> GLP-1's primary effect is reducing plasma glucose concentrations by stimulating insulin secretion from pancreatic  $\beta$ -cells and depresses glucagon secretion. Liraglutide is known to improve glycaemic control, induce body-weight loss and decrease exogenous insulin requirement when used as an adjunct to insulin in type 1 diabetes.<sup>5</sup> In addition, *in vitro* and *in vivo* studies have consistently shown that GLP-1 analogues possess antioxidative and anti-inflammatory effects, putatively mediated alterations in monocyte function, diminished macrophage infiltrations and consequently inhibition of pro-inflammatory pathways.<sup>6,7</sup>

Finally, GLP-1 receptors are expressed in adipose tissues<sup>8</sup> and are thus believed to play a role in lipid metabolism. Liraglutide-induced weight loss is known to affect all adipose depots in the body,<sup>9-12</sup> though the underlying mechanisms are not fully elucidated.<sup>10</sup> Rodent studies suggest that liraglutide and similar GLP-1 agonists may not only induce weight loss, with a reduction in whole-body fat mass but concomitantly reduce white adipocyte cell size, suggestive of improved metabolic function.<sup>13,14</sup> Taken together, activation of the GLP-1 axis impacts the composition of the white adipose tissue by inducing changes in (a) cell size, (b) the relative degree of connective tissue to cell size and (c) the presence of inflammatory cells. There are, however, no reports on the GLP-1-induced fat-reducing mechanisms in human beings.

Thus, we hypothesize that weight loss caused by liraglutide administration will influence the composition and function of subcutaneous adipose tissue independent of glucose metabolism. We aimed to characterize the white adipose cell size and grade the relative pericellular fibrosis in the subcutaneous adipose tissue in response to liraglutide/placebo treatment. Secondly, we explored the level of circulating free fatty acids, the macrophage marker sCD163, the hormones leptin and adiponectin in response to liraglutide/placebo treatment, and if they were associated with weight loss and cell size.

## 2 | MATERIAL AND METHODS

### 2.1 | Study design and patient selection

These data are secondary analysis deriving from a larger, randomized, double-blinded single-centre, parallel-group, placebo-controlled prospective clinical trial investigating the neuroprotective and anti-inflammatory effect of liraglutide for the treatment of distal symmetrical polyneuropathy.<sup>15</sup>

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.<sup>16</sup> Ethical approval was granted by The North Denmark Region Committee on Health Research Ethics, Denmark (N-20130077), and all participants gave written informed consent before entry. The study was registered in public databases (EUDRA CT (ref 2013-004375-12) and clinicaltrials.gov (ref NCT02138045) and performed in accordance with the International Council for Harmonization Good Clinical Practice and the Declaration of Helsinki. The experiment was conducted between June 2014 and January 2017 at Aalborg University Hospital.

Potential participants with type 1 diabetes were recruited at the Department of Endocrinology. Due to the primary outcome of the RCT, participants were included based on nerve conduction tests to diagnose distal symmetrical polyneuropathy (DSPN) according to the Toronto criteria.<sup>17</sup> Additional inclusion criteria were age above 18 years, a verified diagnosis of type 1 diabetes for a minimum of 2 years: (glycated haemoglobin [HbA1c]  $\geq 6.5\%$  [ $>48$  mmol/mol]), stable hyperglycaemic medication ensuring that participants as a minimum had received the given treatment (long-acting and fast-acting insulin or insulin pump with dosing adjustments according to regimens) for at least 3 months before study entry, body mass index  $>22$  kg/m<sup>2</sup> and written consent. Exclusion criteria included type 2 diabetes, other neurological disorders than DSPN, estimated glomerular filtration rate  $< 60$  mL/min/1.73 m<sup>2</sup>; calcitonin  $> 25$  ng/L, HbA1c level  $< 6.5\%$ , use of GLP-1 agonist, or DPP-4 inhibitors.

Treatments appeared identical and were randomly assigned 1:1 liraglutide or placebo in blocks of eight from a randomization list generated by the drug supplier. The intervention was titrated over six weeks to a dose between 1.2 and 1.8 mg/d, depending on individual tolerability, and continued for further 26 weeks. In case of drop-outs or withdrawals, a new participant was recruited to receive the same treatment as the drop-out in a mirrored randomized fashion. The subcutaneous biopsies were collected at baseline and after 26 weeks of intervention, and a venous fasting serum sample was obtained from an antecubital vein.

### 2.2 | Biopsies and histological techniques

Local anaesthetic (xylocaine, lidocaine HCl 20 mg/mL) was injected into the subcutaneous paraumbilical abdominal area. When the analgesic effect was obtained after 10-15 minutes, the adipose tissue biopsies were obtained by moderate vacuum suction performed with a syringe (14G\*31/8", 2.1\*80 mm) and plunger (50 mL). The adipose tissue was flushed three times with 0.9% NaCl to remove erythrocytes before snap-freezing in liquid N<sub>2</sub>. Frozen adipose tissue was transferred to 1-mL cryo tubes and stored at  $-80^{\circ}\text{C}$  until all samples collectively could be processed further. Frozen biopsies were fixed in 4% formalin

and embedded in paraffin, and 5- $\mu\text{m}$  sections were cut according to standard procedures. Immunohistochemical staining with antibody against CD163 was performed to evaluate adipose tissue inflammation as assessed by the presence of CD163-positive macrophages. For visualization of collagen in tissue sections, picrosirius red staining was performed. Preparation of biopsies for histological assessment was performed at the Institute of Pathology, Aalborg University Hospital. The sections were blinded with regard to patient and treatment status.

## 2.3 | Image acquisition

An automated slide scanner (Hamamatsu Photonics) was used to acquire full-slide light microscopy 20 $\times$  magnification scans (ndpi files) of the biopsies. Images (TIFF files) of the biopsies were generated using the export function in NanoZoomer viewer (Hamamatsu Photonics). The open-source platform for biological image analysis (Fiji is Just) ImageJ 2.2.0 was applied for image analysis. Non-tissue areas were cropped from images using the freehand selection tool in preparation for image analysis. Furthermore, blood vessels and streaks of connective tissue were cropped from images applied for the assessment of pericellular fibrosis.

## 2.4 | Image analysis

### 2.4.1 | Quantification of adipocyte size

For assessment of cell size ( $\mu\text{m}^2$ ), cropped images of picrosirius red-stained tissue were analysed with the ImageJ plugin Adiposoft 1.14 (Adiposoft, CIMA).<sup>18</sup> Settings: manual mode; output units: microns; one image analysis. Calibration: micrometre per pixel: 0.457 (calculated from measurements of scalebar in un-cropped 20 $\times$  images); minimum diameter: 20  $\mu\text{m}$ ; maximum diameter: 150  $\mu\text{m}$ . Using the manual edition mode, outputs were cleaned by removing faulty counts such as damaged cells, structures resembling cells and cells with  $\geq 2$  counts inside or shape covering less than approximately 80%

of the area. Prior to statistical analysis, biopsies were scored from 0 to 3 concerning quality, and those with a score of 0 were excluded to ensure data quality. Representative images of biopsies before and after treatment are shown in Figure 1.

### 2.4.2 | Assessment of inflammation and CD163-positive cells

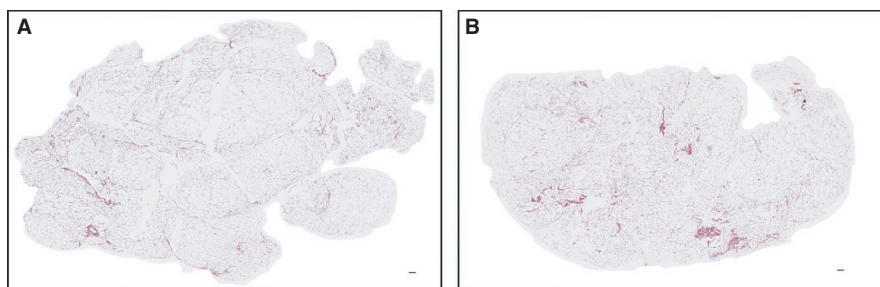
Tissue sections were immunohistochemically stained with antibodies specific to the surface marker CD163 to identify cells of monocyte/macrophage lineage,<sup>19</sup> and images were acquired. An image of a representative area at 10 $\times$  magnification of anti-CD163 stained sections for each biopsy was assessed by visual inspection. Each biopsy was assigned a score for the frequency of CD163-positive cells from the following arbitrary scale (score: estimated number of positive cells): 0:  $\leq 50$ ; 1: 50 > and < 130; 2:  $\geq 130$ .

### 2.4.3 | Quantification of pericellular fibrosis

The red stain level in cropped images of picrosirius red-stained tissue was quantified by running a segmentation macro in batch mode in ImageJ to assess pericellular fibrosis. The macro is based on a protocol for quantification of stained liver tissue<sup>20</sup> and involves six steps. The ratio of the red-stained area (step 4) to the full tissue area (step 6) was calculated.

## 2.5 | Clinical biochemistry

Serum concentrations of non-esterified free fatty acids (FFA) were assessed by the enzymatic colorimetric assay NEFA-HR(2) (Wako Chemicals). Serum concentrations of the specific macrophage marker sCD163 were analysed by enzyme-linked immunosorbent assay at the Department of Clinical Biochemistry, Aarhus University Hospital. Serum concentrations of adiponectin and leptin were determined by Multiplex Luminex assay using the Abundant Serum



**FIGURE 1** Composition of white adipose tissue: depicts representative images of abdominal subcutaneous adipose biopsies before (A) and after (B) treatment with liraglutide. Collagen surrounding the adipocytes is stained with picrosirius red staining and analysed under non-polarized light. Scale bar (—) represents 100  $\mu\text{m}$

Markers 26-Plex Human ProcartaPlex™ Panel at Steno Diabetes Center Copenhagen. Serum levels of triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol were analysed according to standard procedures by the Department of Biochemistry, Aarhus University Hospital.

## 2.6 | Statistical analysis

Normality of the data was assessed using the Shapiro-Wilk test of normality. Normally distributed data are expressed as mean  $\pm$  SD or  $\pm$ SEM, and non-normally distributed data are expressed as median (IQR). Analyses were performed using Stata Statistical Software: Release 15. (StataCorp. 2017: StataCorp LLC) with a significance level of  $P < 0.05$ . Differences in treatment groups were assessed using a two-sample  $t$  test for continuous data, a chi-squared test for categorical variables and a Mann-Whitney  $U$  test for non-parametric data. Associations between weight or cell area and serum markers were calculated using simple linear regressions and presented as a coefficients plot.

## 3 | RESULTS

### 3.1 | Demographics

Forty-eight participants were randomized, 9 of whom withdrew from the study due to adverse effects (7/9: severe nausea; 5/9: vomiting; 3/9: reflux; 3/9: decreased appetite). Consequently, 19 participants randomized to liraglutide and 20 to placebo completed the trial. Demographics are shown in Table 1. There were no differences in the demographics, except that the number of participants who received long-acting insulin was higher in the liraglutide group ( $P = 0.04$ ); however, the doses were similar.

### 3.2 | Weight loss

Liraglutide treatment resulted in a mean weight reduction of 3.38 kg (95% CI  $-5.29$ ;  $-1.48$ ,  $P < 0.001$ ) compared to placebo.<sup>15</sup> The absolute weight loss for the liraglutide and placebo group were 3.95 kg (95%CI  $-5.31$ ;  $-2.59$ ) and 0.55 kg (95%CI  $-2.17$ ;  $1.07$ ), respectively. Median (Q1-Q4) weight pre- and post-intervention are shown in Table 2.

### 3.3 | Subcutaneous adipose cell size and pericellular fibrosis

Liraglutide did not induce a difference in the median cell size in comparison with placebo ( $\Delta 900 \mu\text{m}^2$  vs  $\Delta 441 \mu\text{m}^2$ ;

**TABLE 1** Baseline characteristics

	Liraglutide (n = 19)	Placebo (n = 20)	P-value
Demographics			
Sex (male)	17/89%	14/70%	0.13
Age (years)	51 (10)	50 (8)	0.66
Body mass index (kg/m <sup>2</sup> )	30 (5)	29 (4)	0.62
Diabetes duration (years)	32 (11)	32 (7)	0.97
Haemoglobin A1c (mmol/mol)	69 (12)	64 (10)	0.14
Regular smoking (yes)	4/21%	4/20%	0.94
Heart rate (beats/min)	75 (68-87)	73 (69-78)	0.44
Systolic blood pressure (mmHg)	144 (16)	138 (12)	0.21
Diastolic blood pressure (mmHg)	78 (9)	79 (9)	0.70
Medication			
Fast-acting insulin (yes)	17/89%	13/65%	0.07
Dose (IE)	32 (26;50)	33 (24;47)	1.00
Long-acting insulin (yes)	17/89%	12/60%	0.04
Dose (IE)	24 (22;30)	33 (20;40)	0.48
Insulin pump (yes)	2/11%	7/35%	0.07
Statins (yes)	8/42%	9/47%	0.74
Diuretics (yes)	2/11%	3/15%	0.68
Analgesics (yes)	5/26%	4/20%	0.64
Beta-blockers (yes)	4/21%	2/10%	0.34
Antihypertensives (yes)			
Angiotensin-converting enzyme inhibitors	10/53%	9/45%	0.63
Angiotensin II-receptor agonist	8/42%	6/30%	0.43
Calcium antagonists	7/37%	4/20%	0.24

Note: Data are presented as mean (standard deviation), median (Q2-Q3) or number/percentage.

Data have previously reported by Brock et al 2019.<sup>15</sup>

Italic indicates significant value.

$P = 0.42$ ) (Table 2). Adipocyte size in the upper (Q4) and lower (Q1) quartiles was assessed separately. Liraglutide did not induce a difference in cell size of the upper quartile Q4 ( $\Delta 629 \mu\text{m}^2$  vs  $\Delta 409 \mu\text{m}^2$ ;  $P = 0.56$ ), but it slightly increased cell size in the lower quartile Q1 ( $\Delta 682 \mu\text{m}^2$  vs  $\Delta 81 \mu\text{m}^2$ ;  $P = 0.04$ ) in comparison with placebo. Representative images



TABLE 2 Compositional and biochemical changes

	Liraglutide n (19)			Placebo n(20)			$\Delta$ P-value
	Pre	Post	$\Delta$	Pre	Post	$\Delta$	
Weight (kg)	91 (83;99)	85 (77;96)	-3.9 (-5.3;-2.6)	86 (78;103)	86 (79;99)	-0.6 (-2.2;1.0)	0.001
Body mass index (kg/m <sup>2</sup> )	29 (25;32)	27.1 (25.1;29.2)	-2.0 (-16.0;0.1)	28 (26;31.5)	27.8 (26;31.4)	0.3 (-6.3;13.9)	0.004
Composition							
Cell size total (µm <sup>2</sup> )	2495 (1568;3133)	3846 (1917;4331)	900 (321;1479)	2451 (1735;3237)	2993 (2184;3813)	441 (-185;1067)	0.415
Cell size Q1 (µm <sup>2</sup> )	680 (580;1761)	1746 (963;1974)	682 (331;1034)	848 (576;1427)	1186 (888;1776)	81 (-383;545)	0.038
Cell size Q4 (µm <sup>2</sup> )	5862 (2275)	6183 (2780)	629 (-380;1638)	5561 (1811)	5725 (1544)	-409 (-1663;846)	0.555
Pericellular fibrosis (%)	6.0 (4.1-7.8)	5.4 (4.1-7.1)	-0.1 (-1.5;1.4)	5.2 (3.6-7.8)	5.5 (2.8-7.5)	-0.2 (-2.2;1.9)	0.944
Biochemistry							
CD163 (mg/L)	2.0 (1.6;2.8)	1.9 (1.6;2.6)	-0.1 (-0.2;0.0)	1.9 (1.5;2.5)	1.9 (1.6;2.4)	-0.0 (-0.1;0.1)	0.173
Adiponectin (ng/mL)	127 (89;235)	157 (88;220)	2.0 (-14.6;18.7)	131 (92;357)	149 (101;214)	0.2 (-17.8;18.2)	0.987
Leptin (ng/mL)	5.0 (2.7;10.5)	4.2 (2.3;10.8)	-0.4 (-1.9;1.2)	6.2 (3.4;12.6)	5.8 (2.8;13.5)	-1.8 (-5.6;2.0)	0.593
Free fatty acids (mmol/L)	0.5 (0.3)	0.4 (0.2)	-0.1 (-0.3;0.0)	0.4 (0.2)	0.4 (0.3)	0.0 (-0.2;0.2)	0.387
Triglycerides (mmol/L)	0.8 (0.7;1.2)	0.9 (0.6;1.1)	-0.1 (-0.2;0.1)	0.9 (0.7;1.1)	0.9 (0.7;1.3)	0.1 (-0.1;0.2)	0.427
Total cholesterol (mmol/L)	4.4 (0.8)	4.2 (0.9)	-0.2 (-0.5;0.1)	4.6 (0.6)	4.7 (0.6)	0.1 (-0.1;0.3)	0.412
HDL cholesterol (mmol/L)	1.5 (0.5)	1.5 (0.5)	0.0 (-0.1;0.1)	1.6 (0.4)	1.6 (0.5)	0.0 (-0.1;0.1)	0.836
LDL cholesterol (mmol/L)	2.4 (0.6)	2.3 (0.6)	-0.2 (-0.5;0.2)	2.5 (0.5)	2.6 (0.5)	0.1 (-0.1;0.3)	0.803

Note: Data are presented as mean (standard deviation) or median (Q2-Q3). Delta values (Δ) are expressed as delta mean and 95% CI intervals. Data on weight have previously reported by Brock et al 2019.<sup>15</sup> HDL, high-density lipoproteins; LDL, low-density lipoprotein; Post, after 26 weeks of treatment; Pre, at baseline (before treatment); Q1, 1st quartile; Q4, 4th quartile.

of abdominal subcutaneous adipose tissue biopsies before and after treatment are depicted in Figure 1.

Liraglutide did not induce any differences in the relative pericellular fibrosis ( $\Delta$ -0.1% vs  $\Delta$ -0.2%;  $P = 0.94$ ).

### 3.4 | Biochemical and hormonal effects

Liraglutide did not induce any differences in the abundance of CD163-positive cells ( $\Delta$ -4.7  $\pm$  32.8 vs  $\Delta$ -10  $\pm$  32.5,  $P = 0.19$ ) in comparison with placebo. Representative images of sections from before and after liraglutide treatment are presented in Figure 2A and B, respectively.

Liraglutide did not induce any differences in the serum level of free fatty acids ( $\Delta$ -0.1 vs  $\Delta$ 0.0,  $P = 0.38$ ), CD163 ( $\Delta$ -0.1 vs  $\Delta$ 0.0 mg/L,  $P = 0.17$ ), leptin ( $\Delta$ -0.4 vs  $\Delta$ -1.8 ng/mL,  $P = 0.59$ ) or adiponectin ( $\Delta$ 2.0 vs  $\Delta$ 0.2 ng/mL;  $P = 0.99$ ), see Table 2.

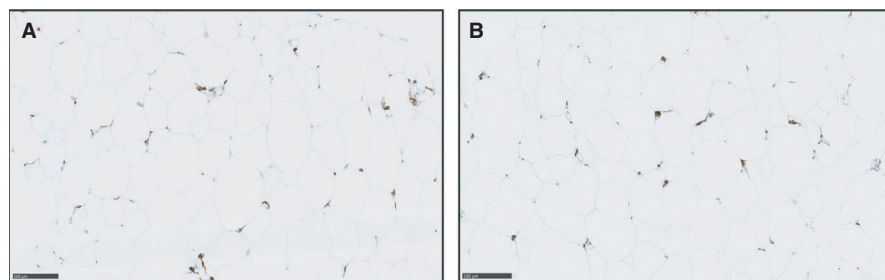
There were no associations between weight loss or cell size and serum markers ( $P > 0.08$ ). A representation of the associations has been plotted as coefficient plots in Figure 3.

## 4 | DISCUSSION

Twenty-six weeks of treatment with liraglutide induced weight loss, which confirms compliance to the protocol. The weight loss was not associated with subcutaneous adipose cell size, pericellular fibrosis or CD163-positive macrophage infiltration. Neither did liraglutide induce changes in the systemic level of circulating free fatty acids, the macrophage marker sCD163, or the adipokines leptin and adiponectin involved in systemic metabolic homeostasis. Mechanism-related parameters such as insulin utility were not affected,<sup>15</sup> despite the fact that glucagon induces gluconeogenesis. We believe that this exposes the complexity of different pathways involved in glucose kinetics.<sup>21</sup>

The abundant white adipocytes primarily store and mobilize fatty acids, such as triglycerides, as energy supply, and can broadly be classified as subcutaneous or visceral depots. The subcutaneous depots are the largest and account for approximately 80% of the body fat in normal weight individuals.<sup>22</sup> Several human studies have documented the weight loss effect of liraglutide based on its suppression of

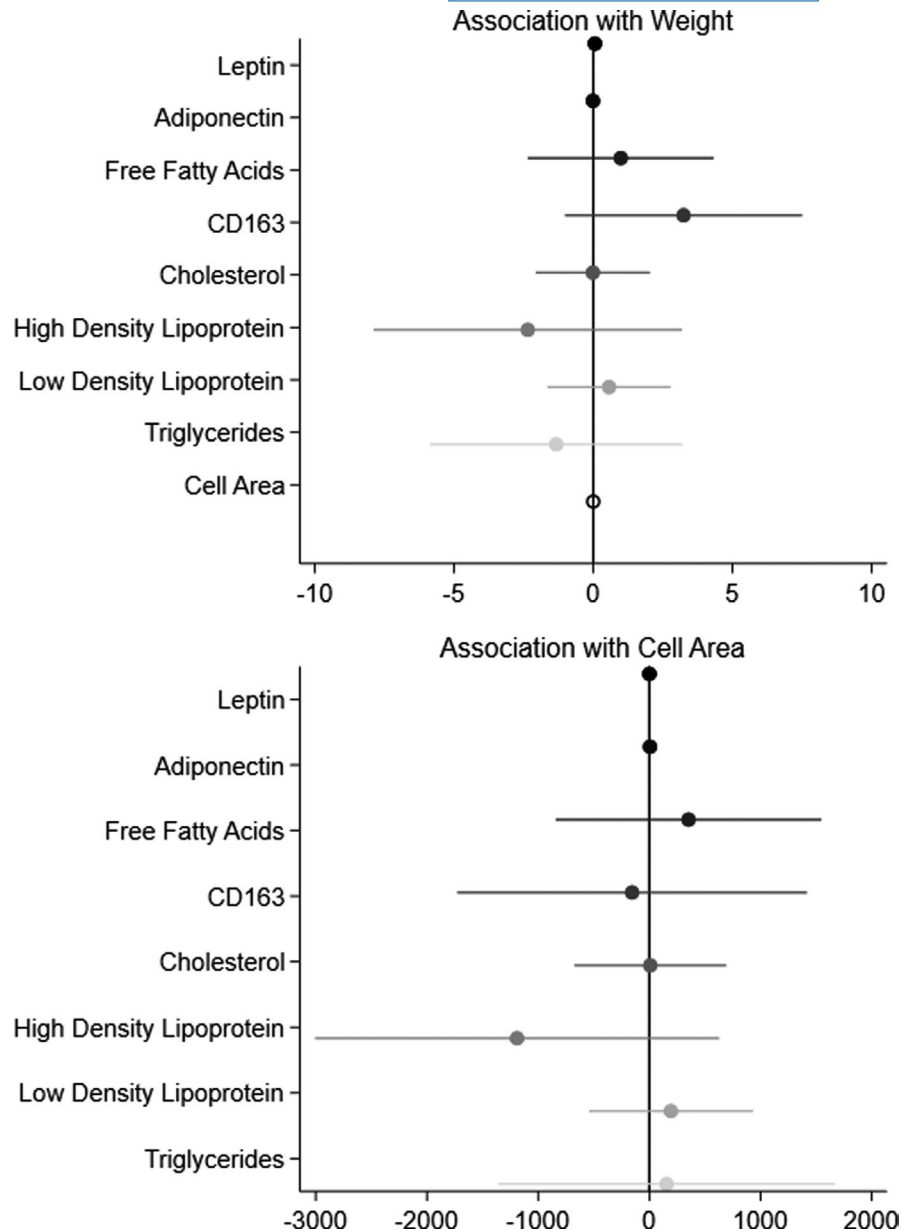
appetite<sup>23-25</sup>; for example, Vilsbøl et al reported in a meta-analysis<sup>26</sup> an estimated weighted mean reduction of 2.9 kg after GLP-1 administration. The induced weight loss in the current study of 3.4 kg confirms patient compliance to the protocol. Multiple studies<sup>9-11</sup> have quantified the mass and size of different adipose tissues following liraglutide administration, using imaging methods like computerized tomography, ultrasound or magnetic resonance imaging, to assess the adipose thickness of the area. However, these studies have reported contradictory results, including reductions in either of or both the subcutaneous and visceral adipose tissues. One of the potential mechanisms involved in white adipose redistribution could be mediated through the autonomic nervous system. In support of this, Kreier et al<sup>27</sup> showed that the sympathetic and parasympathetic branches, respectively, exert a catabolic and anabolic effect on adipose tissues. Thus, it has been suggested that liraglutide alters adipose morphology through its action on the autonomic nervous system.<sup>11</sup> Moreover, it has been suggested that liraglutide affects the lipogenesis: lipolysis ratio through GLP-1 receptors in the adipose tissue.<sup>8,11</sup> In rats, such a reduction in fat mass in response to GLP-1 activation was associated with reduced adipocyte size.<sup>28</sup> Therefore, we anticipated a similar decrease in subcutaneous adipocytes cell size, but no differences in cellular composition were shown between liraglutide and placebo administration in the current study. This finding could be explained by a more substantial loss of adipose mass in visceral adipose tissues<sup>29</sup> or related to the methodology, where the biopsy—due to its limited location and vulnerable nature—may not represent the subcutaneous layer as a whole. In a cohort of overweight or obese adults with type 1 diabetes, systemic effects of 26 weeks of liraglutide treatments showed increased lipolysis in the subcutaneous tissue sample, albeit MRI scans showed a primary fat mass reduction in the visceral adipose tissues.<sup>30</sup> Though no changes in adipocyte sizes were shown in this study, changes in the underlying metabolic mechanisms, for example lipolysis, may still have been present. Administration of GLP-1 in an ob/ob mouse model of diabetes reduced the inflammatory level and oxidative stress in adipocytes and macrophages, which collectively improved insulin sensitivity.<sup>14</sup> Furthermore, studies in obese mice have shown that GLP-1 inhibits M1 and M2 macrophage infiltration and inflammation.<sup>14,31</sup>



**FIGURE 2** Assessment of systemic inflammation in adipose tissue: depicts immunohistochemical stained CD163-positive cells in subcutaneous adipose tissue before (A) and after (B) treatment with liraglutide. Scale bar (—) represents 100  $\mu$ m



**FIGURE 3** Association between weight or cell size and serum markers represented as coefficients plots. The plot depicts the associations between weight (upper) or cell area (lower) serum markers. The different serum markers (coefficients) are placed on the y-axis, and the estimates and their confidence intervals are plotted along the x-axis



The relative presence of pericellular fibrosis was negatively associated with adipocyte cell size and commonly associated with adipose tissue inflammation and fat mass loss.<sup>32</sup> Moreover, an *in vitro* study showed that the GLP-1 agonist exendin-4 has promoted the up-regulation of adiponectin levels in adipocytes by preventing the production of inflammatory adipokines.<sup>33</sup> However, we could not document differences in numbers of CD163<sup>+</sup> M2 macrophages or the relative pericellular fibrosis in our human subcutaneous adipose biopsies in response to 26 weeks of liraglutide treatment. Research within metabolism research has established that the adipocytes are central in regulation of systemic nutrient and energy homeostasis. In line with that, white adipose tissue possesses endocrine functions and secretes adipokines, including leptin and adiponectin. Previous reports have shown that systemic levels of leptin

were independently associated with fat cell size and adipose tissue mass.<sup>34</sup> In addition, a reduction in cell size, which occurs during weight loss, has been associated with increases in circulating adiponectin.<sup>35-37</sup> Our relatively small dataset could not support this. Leptin interacts with pathways in the central nervous system and through direct peripheral mechanisms involved in energy expenditure. Moreover, it regulates energy status and metabolism through interactions with insulin, glucagon, the insulin-like growth factors, growth hormone and glucocorticoids.<sup>38</sup> The role of leptin in hepatic gluconeogenesis shows controversies, and both promotion and suppression are shown.<sup>39-41</sup>

In contrast, adiponectin inhibits hepatic gluconeogenesis and thereby depresses hepatic glucose production and improves glycaemia.<sup>42,43</sup> Taken together, the induced weight loss did not influence the “adipose function.” Measures of serum

levels of free fatty acids, triglycerides, sCD163, leptin and adiponectin could potentially have served as biomarkers for changes in the subcutaneous adipose composition, as leptin and adiponectin are known to associate with subcutaneous adipose morphology, and CD163 is a marker of inflammation.

The lack of effect of liraglutide on the subcutaneous white adipose cell size and macrophage infiltration may result from multiple factors. The full effect of liraglutide demands regulation through the autonomic nervous system. The fact that all our participants suffered from polyneuropathy may have prevented this mechanism fully or partially.<sup>11</sup> However, as GLP-1 receptors are expressed within the adipose tissue, we expected a direct effect of the activated GLP-1 axis<sup>8,11</sup> mediated through neuronal control. Thus, diabetic neuropathy may provide a possible explanation of denervated adipose tissue, which could serve as a model for adipose function, including measurement of adipokines.

The strength of this human study is the randomized, double-blinded design; however, this study is based on secondary analysis and is not without limitations. Firstly, the fragile nature of adipose tissue in the biopsies has made the work challenging due to the variable quality of the biopsies, and only one slide per biopsy was analysed. Secondly, the white adipose tissue composition analysis provides novel insight as fat biopsies hitherto primarily have been used for protein expression in, for example, Western blotting. However, interpretation of CD163-positive cells must be done with caution because evaluation may have been prone to subjective bias due to the non-automated method. Additionally, we investigated most of the relating factors in the serum, which may provide a proxy for actual effects. Thirdly, these subcutaneous adipose-specific results should be interpreted cautiously as other adipose depots, for example visceral fat, vary in cellular composition, metabolism, innervation, vascularisation and endocrine output. Thus, the obtained weight loss in response to liraglutide must have induced more significant effects in other adipose tissue depots, which may not have been detectable with imaging methods. Additionally, the evaluation of fibrosis with picrosirius red staining was analysed under non-polarized light, a method that may be suboptimal, and thus, these results should be interpreted with caution. Finally, it is essential to emphasize that this study was carried out in type 1 diabetes. Therefore, the analysed anti-inflammatory effect was independent of glucose metabolism, in contrast to models, including metabolically induced inflammation in obesity.

## 5 | CONCLUSION

We could not demonstrate sustained alterations in the subcutaneous adipose tissue composition or characteristics in

response to 26 weeks of liraglutide intervention resulting in weight loss. Consequently, the effect of liraglutide on weight loss is believed to be more pronounced in the visceral fat mass. These results provide the basis for further studies into the mechanistic effects of liraglutide on weight loss.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest associated with this manuscript. No funding source had any role in study design, data collection, data analysis, data interpretation or the preparation of this article. The authors had full access to all data in the study and final responsibility for the decision to submit for publication.

## AUTHOR CONTRIBUTION

CB, AMD and BB provided study design and original idea. CB collected data. TM, AB, HZ, MV, NJ and ALW analysed the data, and all authors interpreted the data. TM wrote the first draft, but all authors contributed to the final manuscript and critically reviewed the manuscript. CB is the guarantor of the work, with full access to all the data in the study, and takes responsibility for the data integrity and data analysis accuracy.

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