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Research Article

GLP-1 analogue-induced weight loss does not improve obesity-induced AT dysfunction

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Glucagon-like peptide-1 (GLP-1) analogues aid weight loss that improves obesity-associated adipose tissue (AT) dysfunction. GLP-1 treatment may however also directly influence AT that expresses the GLP-1 receptor (GLP-1R). The present study aimed to assess the impact of GLP-1 analogue treatment on subcutaneous AT (SCAT) inflammatory and fibrotic responses, compared with weight loss by calorie reduction (control). Among the 39 participants with Type 2 diabetes recruited, 30 age-matched participants were randomized to 4 months treatment with Liraglutide ($n = 22$) or calorie restriction based on dietetic counselling ($n = 8$). Assessments included clinical characteristics and repeated subcutaneous abdominal AT biopsies. Liraglutide resulted in weight loss in most participants (-3.12 ± 1.72 kg, $P = 0.007$) and significant reduction in visceral AT (VAT). It was more effective in lowering fasting glucose, in comparison with weight loss by dieting. However, tumour necrosis factor-α (*TNFA*) AT-expression ($P = 0.0005$), macrophage chemoattractant protein-1 (*MCP-1*) expression ($P = 0.027$) and its serum levels ($P = 0.048$) increased with Liraglutide, suggestive of an inflammatory response unlike in the diet arm in which a trend of lower cluster of differentiation 14 (*CD14*) expression ($P = 0.09$) was found. Liraglutide treatment also increased expression of factors involved in extracellular matrix (ECM) deposition, transforming growth factor-β (*TGFB*) and collagen type 1 alpha 1 chain (*COL1A1*) (*TGFB1*: before 0.73 ± 0.09 arbitrary units (AU), after 1.00 ± 0.13 AU, $P = 0.006$; *COL1A1*: 0.84 ± 0.09 AU compared with 1.49 ± 0.26 AU, $P = 0.026$). Liraglutide thus appears to induce an inflammatory response in AT and influences ECM remodelling. Despite its superior effect on glycaemia, Liraglutide does not improve obesity-associated AT dysfunction in subcutaneous tissue. It is yet unclear whether this limits AT storage capacity for lipids. This may be of importance in patients being re-exposed to positive energy balance such as post GLP-1 discontinuation.

Introduction

Nutrient-induced secretion of glucagon-like peptide-1 (GLP-1) from intestinal L-cells stimulates insulin release by pancreatic β-cells and decreases glucagon secretion by pancreatic α-cells [1]. GLP-1's beneficial effects on pancreatic islets led to clinical use of GLP-1 analogues in Type 2 diabetes. Other beneficial effects of GLP-1 analogue treatments include blood pressure (BP) lowering [2] and improvement of fatty liver disease [3]. Among its physiological effects, GLP-1 also increases satiety and slows gastric emptying, generally promoting weight loss [1] that makes it also suitable for obesity treatment [2]. Due to the associated weight loss, however, it remains difficult to distinguish direct effects of GLP-1 on improvement of obesity-associated co-morbidities from effects that may result from weight loss. GLP-1 acts through its receptor GLP-1 receptor (GLP-1R), expressed in various organs including adipose tissue (AT) [4]. The expression of GLP-1R in human abdominal AT as shown by us recently [5] suggests that GLP-1 may affect

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AT directly and independently of weight loss, as observed in *in vitro* studies [6]. These *in vitro* studies are however an insufficient model for complex chronic GLP-1 therapy effects including its influence on obesity-associated AT dysfunction. This necessitates human *in vivo* studies.

Characteristic features of AT dysfunction, as found with increasing obesity and in patients with Type 2 diabetes, include inflammation and accumulation of extracellular matrix (ECM) components that lead to fibrosis. Positive energy imbalance causes a rapid expansion of AT characterized by adipocyte hypertrophy, which seems to play a key role in AT dysfunction [7] and insufficient angiogenesis leading to local hypoxia. These induce AT infiltration by macrophages, inflammatory cytokine production and increased synthesis of collagens that limit adipogenesis, decrease AT's expandability and thus reduce its storage capacity [8]. Consequently, as described by the overspill theory, this predisposes to storage of surplus triacylglycerols in organs such as the liver, heart, muscle and pancreas as well as around blood vessels [9]. This ectopic lipid accumulation contributes to the development of several pathologies associated with obesity such as non-alcoholic fatty liver diseases, cardiovascular disease and insulin-resistance that can progress to Type 2 diabetes [10]. Weight loss improves AT dysfunction and its adipogenic potential [11]. Indeed, profound weight loss by very low calorie dieting or bariatric surgery was shown to decrease macrophage infiltration and both expression and release of inflammatory cytokines [12–14]. Bariatric surgery-induced weight loss may also decrease pericellular and total fibrosis [15].

The mechanism by which GLP-1 analogue treatment leads to weight loss has been clearly established [16]; however, weight loss has not sufficiently been taken into account when analysing changes in adipokine levels and systemic inflammation. Direct effects of GLP-1 analogues on human AT in a randomized controlled manner in comparison with a weight loss arm have so far not been explored, which is important in view of determining the progression of obesity-induced AT dysfunction. In the present study, we examined if Liraglutide (a GLP-1R agonist) improves AT dysfunction in individuals with Type 2 diabetes independent of weight loss. Although we expected superior improvements to compared with those of diet-induced weight loss, we found evidence of increased inflammation and ECM remodelling after Liraglutide.

Materials and Methods

Experimental design

This clinical trial (NCT01740921, ClinicalTrials.gov) to study the independent effect of Liraglutide on AT function was approved by the National Research Ethics Service, South West Reference Number: 10/H0106/29. Subjects gave consent through the NIHR Exeter Clinical Research Facility and the study was conducted in accordance with the Declaration of Helsinki. A total of 39 subjects were recruited of whom 30 met inclusion criteria and were randomized on a basis of 1 to 3 to Liraglutide or diet group (for CONSORT diagram including inclusion criteria see Supplemental Figure S1) with particular attention to exclusion of subjects with an inflammatory condition and on anti-inflammatory medication.

For the diet control arm, subjects received counselling in face to face visits and phone consultations with the dietician. The aim was to lose weight by reduction in total calorie intake with a balanced diet (without use of meal replacements, very low calorie diets or encouragement of activity change). In the Liraglutide arm, subjects received a once-daily injection of the GLP-1 analogue, Liraglutide (Victoza, Novo Nordisk), started at a dose of 0.6 mg for 2 weeks and continued with 1.2 mg to week 16 with the exception of two patients who required a longer stabilization period on 0.6 mg (3 and 4 weeks).

Baseline and 4 month's study assessments are described in Supplemental Figure S1. Briefly, during visits, participants received clinical assessments, blood tests, OGTT, MRI scans and had AT biopsies taken as recently described [17].

Serum biochemistry

Glucose, insulin and glycated haemoglobin (HbA1c) were measured as a part of the routine hospital biochemistry at the Royal Devon and Exeter Hospital (Exeter, U.K.). ELISAs were performed to assess active GLP-1 (which does not cross-react with Liraglutide according to the manufacturer, Millipore), non-esterified fatty acid (NEFA) (Abcam), macrophage chemoattractant protein-1 (MCP-1), adiponectin, high-sensitivity C-reactive protein (hs-CRP) and tumour necrosis factor-α (TNFA) (all from Life Technologies).

Gene expression analysis

Isolated RNA using TRIreagent was treated with DNase I and converted into cDNA using the SuperScript VILO cDNA Synthesis Kit (all reagents from Life Technologies). Gene expression analysis was carried out using TaqMan

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low-density array (TLDA) cards (Life Technologies; probes listed in Supplemental Table S1). Relative gene expression was determined using the $\Delta\Delta C_T$ method with expression levels normalized to the geometric mean of three housekeeping genes (*GAPDH*, *PPIA* and *UBC*), as previously described [17]. Data were presented as arbitrary units (AU).

Statistical analysis

All data were presented as the mean ± S.E.M. unless otherwise stated. The Wilcoxon matched-pairs signed-rank test was used for paired data analysis and the Mann–Whitney for unpaired data. Statistics were performed using GraphPad Prism 5.04 software, P values <0.05 were considered significant. The hypothesis and primary endpoints were to find differences in inflammatory (*MCP-1*) and fibrotic (transforming growth factor-β1, *TGFB1*) gene expression.

Results

Anthropometric and clinical parameters

Data were analysed from the 22 participants who completed this 16 weeks study (diet group, $n = 7$ and Liraglutide group, $n = 15$) and attended both assessments. Baseline anthropometric and clinical parameters are shown in Table 1. At baseline, all participants were on metformin. During the study, three participants of the Liraglutide group stopped metformin, two reduced its dose and two stopped gliclazide. There was no change in diabetes medication in the diet group.

Following 16 weeks of dietary advice, all patients in the diet group lost weight (mean loss of 6.07 ± 3.27 kg). In comparison, the Liraglutide group showed a mean loss of 2.39 ± 2.57 kg ($P = 0.04$). After excluding two subjects who gained weight (respectively 1 and 3.8 kg), the weight loss was 3.12 ± 1.72 kg, n=13, $P = 0.08$. A concomitant body mass index (BMI) change of −1.94 ± 1.03 kg/m^2 in the diet and −0.81 ± 0.84 kg/m^2 in the Liraglutide group ($P = 0.07$) was observed, which was not significant when considering only subjects with weight loss in the Liraglutide group ($P = 0.13$). The observed weight change in both groups was characterized by a decrease in fat mass percentage and waist circumference whereas the waist/hip ratio was only significantly reduced in the Liraglutide arm (Table 1). Liraglutide-treated participants also displayed a significant decrease in the amount of visceral AT (VAT), assessed by MRI. The visceral/subcutaneous ratio showed a tendency to decrease in response to Liraglutide (baseline: 0.93 ± 0.50, 4 months: 0.83 ± 0.4581, $P = 0.065$), though as mentioned above, not all subjects lost weight. Morphological changes were not attributable to a modification of spontaneous physical activity (diet group: baseline 14143 ± 4638 steps/48 h, after 4 months 14068 ± 4965 steps/48 h; Liraglutide group: baseline 13976 ± 6169 steps/48 h compared with 18370 ± 10710 steps/48 h; ns).

Patients in the Liraglutide arm had lower systolic BP after follow up ($P = 0.013$). In both groups, we observed a comparable decrease in HbA1c (change in HbA1c by −0.50 ± 0.43% i.e. −5.71 ± 4.57 mmol/mol in the diet group; Liraglutide group: −0.72 ± 0.65% i.e. −8.07 ± 7.10 mmol/mol; $P = 0.62$) associated with an improvement of fasting glucose concentrations only significant in the Liraglutide group (Table 1 and Figures 1A and 1B). We did not observe any significant change in fasting insulin levels in either of the groups. Reductions in anti-diabetic medications in the Liraglutide arm but not diet arm (see above) have to be taken into account in interpretation of the effect on glycaemia. Glucose-stimulated circulating GLP-1 levels were higher at follow up after Liraglutide treatment but not dietary weight loss (Figure 1C). We did not find any changes in circulating NEFA or the related index of adipose tissue insulin resistance (ADIPO-IR) (ADIPO-IR = NEFA concentration × insulin levels), which reflect AT insulin resistance (Table 1) [18]. We did not observe any modifications in hs-CRP levels, a marker of low-grade chronic inflammation between groups (Table 1) [19].

Adipokines

Adiponectin, a hormone predominantly secreted by adipocytes, has insulin-sensitizing, cardioprotective and anti-inflammatory properties [20]. We did not observe any significant modification of *ADIPOQ* expression in subcutaneous AT (SCAT) with Liraglutide and a tendency of an increase with dieting (Table 2). Adiponectin plasma levels were not affected, unless we only account for participants who lost weight with Liraglutide, in whom circulating adiponectin increased (baseline: 7190 ± 2192 ng/ml; after 4 months: 7740 ± 2344 ng/ml; $P = 0.04$).

Leptin is secreted by adipocytes and its concentrations correlate with BMI. It is involved in the regulation of glucose and lipid metabolism, energy expenditure and satiety [20]. *LEP* expression only decreased insignificantly in response to Liraglutide treatment (Table 2) and did not change with diet-induced weight loss (Table 2).

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Table 1 Patient characteristics before and after 4 months of diet-induced weight loss or treatment with Liraglutide

Data were presented as mean ± S.D., except for gender that is the percentage of participants. [a]These results are from a subgroup of paired participants assessments (pre- and post-treatment); diet group: $n = 7$, Liraglutide group: $n = 13$ (two haemolysed). [b]These results are from a subgroup of paired participants assessments (pre- and post-treatment); diet group: $n = 3$, Liraglutide group: $n = 10$; *$P < 0.05$, **$P < 0.01$. Wilcoxon matched-pairs signed rank test, comparison of baseline measurements with those taken after 4 months, in each group; †$P < 0.05$, Mann–Whitney test comparing changes between groups.

	Diet ($n = 7$)		Liraglutide ($n = 15$)	
	Baseline	**After 4 months**	**Baseline**	**After 4 months**
Groups characteristics				
Male sex (%)	71.43		53.33	
Age (years)	59.71 ± 9.21		63.67 ± 5.60	
Medications				
Metformin (mg/day)	1925 ± 793 (7/7)	1842 ± 904 (7/7)	1653 ± 509 (15/15)	1133 ± 609 (12/15)
Gliclazide (mg/day)	267 ± 75 (3/7)	187 ± 38 (3/7)	67 ± 19 (3/15)	80 ± 0 (1/15)
Anthropometric measurements				
Weight (kg)	97.61 ± 17.18	91.54 ± 18.05*	89.48 ± 21.93	87.09 ± 22.90**,†
BMI (kg/m^2)	31.40 ± 3.47	29.47 ± 4.12*	30.06 ± 4.85	29.25 ± 5.20**
Waist measure (cm)	111.09 ± 8.88	106.21 ± 9.95*	104.48 ± 16.31	102.77 ± 15.97*
Hip measure (cm)	106.50 ± 6.05	104.80 ± 6.61	104.93 ± 8.24	105.27 ± 8.39
Waist/Hip ratio	1.04 ± 0.07	1.01 ± 0.07	0.99 ± 0.11	0.97 ± 0.10*
Clinical characteristics				
Heart rate (bpm)	69.43 ± 12.49	59.20 ± 10.94	67.93 ± 11.84	67.20 ± 10.04
Systolic BP (mmHg)	145.12 ± 15.73	138.42 ± 18.32	141.07 ± 11.58	133.49 ± 12.04*
Diastolic BP (mmHg)	81.90 ± 7.50	78.14 ± 9.62	80.68 ± 8.42	77.04 ± 8.97
HbA1c (%)	7.34 ± 0.66	6.84 ± 0.60*	7.43 ± 0.65	6.71 ± 0.36**
HbA1c (mmol/mol)	56.86 ± 7.11	51.14 ± 6.67*	57.93 ± 7.19	49.87 ± 3.96**
Fasting glucose (mmol/l)	7.52 ± 1.32	7.40 ± 1.18	7.70 ± 1.38	6.63 ± 1.16**
Insulin levels (mU/l)[a]	19.86 ± 16.38	15.72 ± 11.26	17.85 ± 9.44	17.02 ± 7.01
NEFA (μmol · l)	124.58 ± 47.73	153.78 ± 83.04	139.32 ± 54.28	130.92 ± 85.06
ADIPO-IR (mM · mU/l)	2.31 ± 2.27	2.27 ± 2.06	2.14 ± 0.99	2.14 ± 1.58
hs-CRP (mg/l)	1.53 ± 1.41	1.23 ± 1.23	1.48 ± 0.85	1.40 ± 1.16
Body fat characteristics				
Fat mass (%)	38.37 ± 8.00	34.77 ± 10.03*	37.39 ± 5.55	35.39 ± 6.77*
SCAT area (cm^2)[b]	308.62 ± 158.97	244.41 ± 169.74	235.94 ± 79.98	223.76 ± 91.71
VAT area (cm^2)[b]	225.30 ± 38.77	162.17 ± 86.40	203.37 ± 104.23	177.64 ± 103.73*

Inflammation markers

The impact of Liraglutide on inflammation was assessed through analysis of a marker common to the majority of human monocytes/macrophages: cluster of differentiation 14 (*CD14*) [21], the expression of which reflects macrophage infiltration; inflammatory cytokines produced by both adipocytes and resident macrophages: interleukine-6 (*IL6*), *MCP-1* and *TNFA* [20]. Diet-induced weight loss had a tendency to decrease *CD14* expression that did not change with Liraglutide. Surprisingly, AT RNA (Figure 1D) and protein expression (before: 0.37 ± 0.2 AU, after: 0.55 ± 0.3 AU, $P > 0.05$, $n = 6$) and circulating levels of MCP-1 (Figure 1E) increased with Liraglutide. Similarly, *TNFA* expression was significantly increased in response to Liraglutide (Figure 1F). Although changes in corresponding plasma concentrations were not significant, Liraglutide treatment and diet-induced weight loss seemed to differentially influence TNFA plasma concentrations (Liraglutide group's change: 0.15 ± 0.09 pg/ml; diet group's change: −0.30 ± 0.18 pg/ml; $P = 0.04$). *IL6* expression was not affected in any group.

ECM and regulators

To evaluate Liraglutide's effects on AT ECM remodelling, we analysed the expression of genes coding for the main ECM components [collagens, elastin (ELN) and fibronectin (FN1)], the respective ratios of which define AT's

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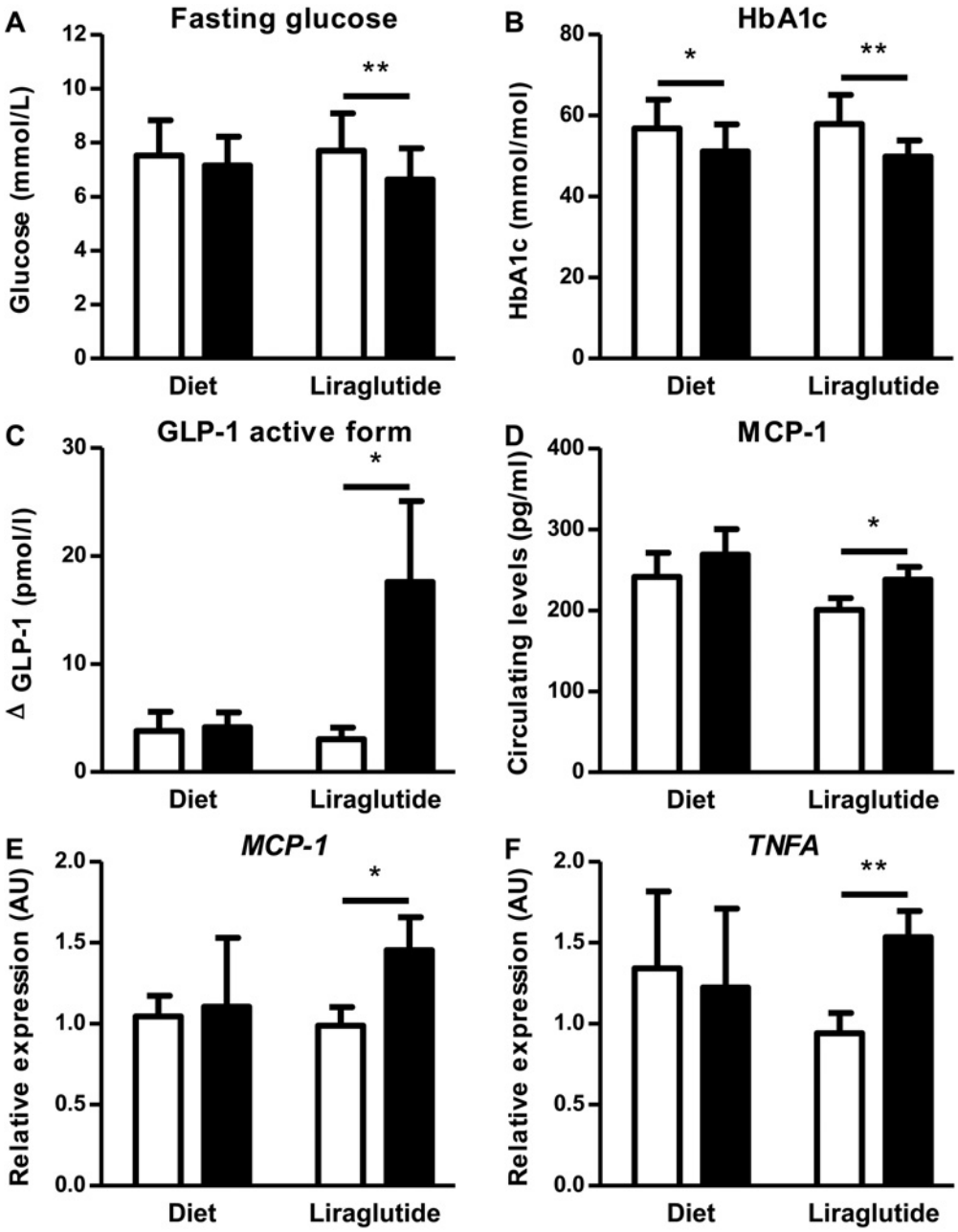

Figure 1. Liraglutide's effect on glucose metabolism and inflammation

Liraglutide improved glycaemic control, its treatment was associated with elevated active GLP-1 levels and it increased inflammatory cytokines in the circulation as well as respective expression of circulating genes in adipose tissue. Fasting glucose (**A**) and HbA1c (**B**) levels were determined before and after 4 months of either diet-induced weight loss or Liraglutide treatments. Glucose-stimulated GLP-1 secretion (**C**) was expressed as difference of GLP-1 active form plasma concentrations before and 20 min after a glucose uptake (referred to as ΔGLP-1). MCP-1 circulating levels (**D**) were measured by ELISA. *MCP-1* (**E**) and *TNFA* (**F**) expression in SCAT were assessed at baseline and after diet- or Liraglutide-induced weight loss. Data were analysed using a Wilcoxon matched-pairs signed rank test; $^{*}P < 0.05$, $^{**}P < 0.01$; white bars = baseline; black bars = after 4 months.

mechanical properties [22]. This included enzymes involved in collagens and ELN fibres cross-linking (lysyl oxidase, LOX and lysyl oxidase-like 2, LOXL2), degradation (matrix metalloproteinase 9, MMP9) and the pro-fibrotic factor TGFB1. TGFB1 is linked with fibrosis development in many organs and stimulates remodelling and production of fibrotic ECM components (including collagens and FN1). We showed that after Liraglutide treatment *TGFB1*

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Table 2 Gene expression in subcutaneous adipose tissue at baseline and 4 months after diet-induced weight loss or Liraglutide treatment

Data obtained from paired samples (Liraglutide group, $n = 13$; diet group, $n = 6$) were presented as AU and expressed as mean ± S.E.M.; $^{*}P < 0.05$, $^{**}P < 0.01$. Wilcoxon matched-pairs signed-rank test comparing baseline measurements with those taken after 4 months within group; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$. Wilcoxon matched-pairs signed-rank test comparing baseline measurements with those taken after 4 months within group excluding two subjects who have not lost weight with Liraglutide; $^{\dagger\dagger}P < 0.01$. Mann–Whitney test comparing changes after intervention between groups.

	Diet group			Liraglutide group		
	Baseline	**4 months**	**Mean change**	**Baseline**	**4 months**	**Mean change**
Adipokines						
ADIPOQ	0.92 ± 0.12	1.28 ± 0.22	0.36 ± 0.27	1.12 ± 0.10	1.13 ± 0.13	0.00 ± 0.10
LEP	1.10 ± 0.22	1.02 ± 0.16	− 0.08 ± 0.26	1.30 ± 0.11	1.17 ± 0.18	− 0.12 ± 0.12
Inflammation markers						
CD14	1.07 ± 0.18	0.69 ± 0.10	− 0.38 ± 0.17	1.28 ± 0.20	1.45 ± 0.31	0.16 ± 0.33
IL6	0.90 ± 0.12	0.99 ± 0.21	0.09 ± 0.22	1.18 ± 0.24	1.14 ± 0.18	− 0.04 ± 0.17
MCP-1	1.05 ± 0.13	1.11 ± 0.42	0.06 ± 0.40	0.99 ± 0.11	1.45 ± 0.20	0.47 ± 0.16$^{*,\$}$
TNFA	1.34 ± 0.47	1.22 ± 0.49	− 0.12 ± 0.53	0.94 ± 0.13	1.53 ± 0.16	0.59 ± 0.13$^{**,\$\$}$
ECM and its regulators						
COL1A1	1.15 ± 0.25	0.92 ± 0.12	− 0.21 ± 0.22	0.84 ± 0.09	1.49 ± 0.26	0.65 ± 0.28$^{*,\$\$}$
COL3A1	1.13 ± 0.15	1.30 ± 0.44	0.17 ± 0.39	0.92 ± 0.08	1.22 ± 0.16	0.30 ± 0.16$^{\$\$}$
COL4A1	0.93 ± 0.13	1.23 ± 0.23	0.30 ± 0.21	1.07 ± 0.09	1.19 ± 0.16	0.12 ± 0.11
ELN	1.08 ± 0.28	1.11 ± 0.20	0.04 ± 0.11	1.14 ± 0.17	1.07 ± 0.15	− 0.07 ± 0.15
FN1	1.66 ± 0.44	2.30 ± 0.89	0.64 ± 0.94	0.73 ± 0.06	1.11 ± 0.13	0.38 ± 0.14$^{*,\$\$}$
LOX	1.09 ± 0.11	1.51 ± 0.35	0.42 ± 0.29*	1.06 ± 0.08	0.91 ± 0.06	− 0.14 ± 0.05*,††
LOXL2	1.14 ± 0.18	1.10 ± 0.07	− 0.04 ± 0.16	1.24 ± 0.15	1.23 ± 0.13	− 0.01 ± 0.10
MMP9	2.10 ± 0.49	2.29 ± 1.44	0.19 ± 1.01	0.80 ± 0.21	1.34 ± 0.27	0.54 ± 0.32$^{\$\$}$
TGFB1	1.22 ± 0.30	1.18 ± 0.34	− 0.05 ± 0.29	0.73 ± 0.09	1.00 ± 0.13	0.27 ± 0.10$^{**,\$\$}$
Others						
CD31	1.15 ± 0.14	1.27 ± 0.20	0.12 ± 0.14	1.00 ± 0.07	1.21 ± 0.16	0.22 ± 0.18
HIF1A	1.32 ± 0.22	1.50 ± 0.54	0.19 ± 0.58	0.88 ± 0.06	1.10 ± 0.09	0.22 ± 0.10$^{*,\$}$
LPL	0.81 ± 0.09	0.82 ± 0.11	0.01 ± 0.07	1.14 ± 0.09	1.03 ± 0.14	− 0.11 ± 0.13
PPARG	0.91 ± 0.12	1.02 ± 0.14	0.11 ± 0.11	1.14 ± 0.11	1.15 ± 0.14	0.01 ± 0.15

expression increased unlike with diet-induced weight loss (Figure 2A). *COL1A1*, one of the most abundant collagens in human AT [17] was increased only by Liraglutide treatment (Table 2 and Figure 2B), whereas *COL3A1* or *COL4A1* expression were not affected. We also found an increase in *FN1* expression in both groups, which only reached significance in the Liraglutide group (Figure 2C). Liraglutide was associated with a decrease in *LOX* expression, whereas it increased with diet-induced weight loss (Table 2 and Figure 2D). The comparison of changes (Δ after 4 months – baseline) showed significant differences between groups. We observed a non-significant trend to increased *MMP9* expression at follow up in both arms ($P = 0.08$); however, there was no change in *ELN* or *LOXL2*.

Other relevant proteins of AT function

Hypoxia inducible factor 1 α (HIF1A) is a key transcription factor in the hypoxia stress response, with increased expression in response to adipocyte hyperplasia-induced hypoxia [23]. We observed an increased *HIF1A* expression after Liraglutide treatment independent of weight change (Table 2 and Figure 2E) and no change in the expression of the endothelial-related factor cluster of differentiation 31 (*CD31*), the lipoprotein lipase (*LPL*) a lipid uptake regulator or peroxisome proliferator-activated receptor γ (*PPARG*), the key regulator of adipogenesis (Table 2) in any of the treatment arms.

Conclusions

This is the first RCT assessing the effect of the GLP-1 analogue, Liraglutide, on obesity and diabetes-associated AT dysfunction *in vivo*. Most information about the effect of GLP-1 and its analogues on AT physiology are derived

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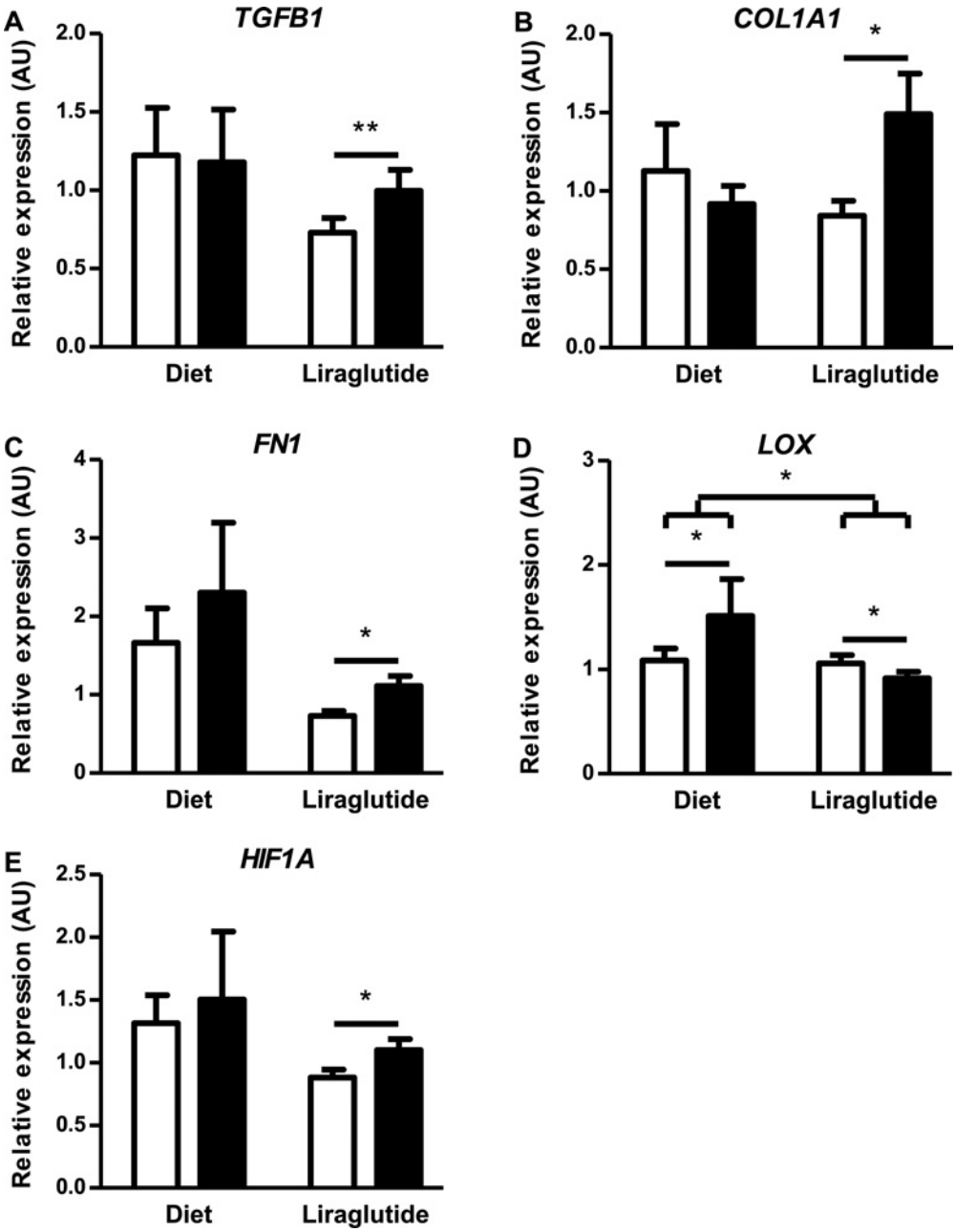

Figure 2. Liraglutide's effects on ECM remodelling and HIF1A

Liraglutide-induced weight loss promoted ECM remodelling and stimulated *HIF1A* expression. Changes in *TGFB1* (**A**), *COL1A1* (**B**), *FN1* (**C**), *LOX* (**D**) and *HIF1A* (**E**) expression measured in SCAT by TLDA before (white bars) and after intervention (black bars) of either dietary advice-based weight loss or Liraglutide. Statistical significance between baseline and after treatment measurements were determined using a Wilcoxon matched-pairs signed-rank test whereas changes between groups were evaluated with a Mann–Whitney test; $^{*}P < 0.05$, $^{**}P < 0.01$.

mainly from *in vitro* studies [6] or deduced from *in vivo* studies that make only indirect conclusions by monitoring circulating adipokines/inflammatory cytokines rather than examining AT *per se* [24,25]. The only previous report is a pilot *in vivo* study of Liraglutide's effect on SCAT in three participants and does therefore not permit any conclusions [26]. For the purpose of repeat biopsies in a clinical trial and an attempt at controlling for weight loss, the study of visceral tissue and its biopsy is not feasible. Moreover, subcutaneous tissue is of more interest, as its

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expandability is linked with metabolic health and is a key determinant of fatty acid overspill into ectopic depots [27]. Here, we have found that, despite its leading to weight loss, Liraglutide treatment for 4 months does not ameliorate and may even worsen inflammation; that it appears to influence ECM remodelling and does not improve adipogenesis as also suggested by our recent *in vitro* data with Exendin-4 [5]. Here, we present results suggesting that Liraglutide does not reproduce the beneficial changes typical of weight loss, i.e. improvement in AT-inflammation. This should be considered in particular when dealing with patients who do not respond to GLP-1 treatment with weight loss.

Treating obese/overweight patients with Type 2 diabetes with Liraglutide, we observed, as expected, some weight loss in most participants, a consecutive decrease in BMI and fat mass percentage, an improvement in glucose homoeostasis (greater than by calorie reduction-induced weight loss) and a decrease in systolic BP similar to previous reports [25,28]. Even though not all subjects lost weight with Liraglutide, which made it a challenge to correct for weight loss with the dietary arm, we noted a decrease in abdominal visceral adipose area as measured by MRI scan, similar to Suzuki et al. [29], though no change in the SCAT volume.

In response to Liraglutide, we found no change in either leptin or adiponectin AT expression and showed an increase in circulating adiponectin, in those who lost weight. Human studies mainly comment on circulating levels of adipokines after GLP-1 analogue treatment of varied duration and dosage. Studies report varied outcomes if any change [3,25] in circulating adipokines and typically do not take account of the associated effect of weight change. Studies on effects of AT differences similarly fail to account for change in expression of adipokines with GLP-1 [26] or show no change in rodent AT despite weight loss [30]. Weight loss is commonly associated with a decrease in leptin and an increase in adiponectin expression and secretion [20], though in our study the weight loss in the diet arm may have been too modest (i.e. lower than 10%) and thus with less impact on leptinaemia and adiponectinaemia [31].

Pro-inflammatory proteins (expression and secretion) in SCAT, as studied here, decrease with considerable weight loss [12,14]. However, after Liraglutide treatment, we found an up-regulation of *TNFA* and *MCP-1* expression in AT and an elevation in circulating MCP-1 levels without change in *CD14* or circulating hs-CRP levels, in contrast with a tendency to lower *CD14* in our diet group and a significant difference in *TNFA* between groups. On the other hand in rodent studies, Exenatide does not change inflammatory marker expression in AT from high-fat fed rats [32], whereas recombinant adenovirus producing GLP-1 decreases pro-inflammatory cytokine expression and reduces macrophage infiltration in AT of ob/ob mice that lack functional leptin [33] and may thus not be the best model for inflammation studies as leptin influences inflammation [34]. So far as we are aware, no previous human clinical trial evaluated AT's expression of inflammatory factors after GLP-1 analogue treatment and few took account of the concomitant weight loss that independently affects AT. Most studies on the effects of GLP-1 on circulating cytokines did not observe any significant modification [25,35] whereas studies that reported a decrease in MCP-1 and IL-6 plasma concentrations attributed the decrease not to AT changes, but to GLP-1 analogues' anti-inflammatory properties on blood mononuclear cells [36,37]. Further studies are necessary to establish changes in the numbers of macrophages infiltrating AT and their phenotypes, which will help to clarify GLP-1 analogues' inflammatory properties. The increase in TGFB1 that has a pivotal role in fibrosis development may also be a result of increased macrophage infiltration, which also express *TGFB* [38], though we did not find a change in *CD14* to support this.

So far as we are aware, there are no previous reports about the impact of GLP-1 analogues on AT fibrosis. Weight loss in obese subjects is characterized by a change in genes coding for the main matrix fibres and enzymes involved in their maturation and cross-linking [39] in a pattern that is linked with an improvement of AT fibrosis [15,39]. Importantly, increased SCAT fibrosis was suggested to hamper fat mass loss in bariatric patients [15] and as elaborated above, fibrosis is linked with ectopic fat deposition and metabolic complications. We observed after Liraglutide treatment, that *TGFB1*, *COL1A1* and *FN1* were up-regulated suggesting formation of a pro-fibrotic environment. However, in the context of a down-regulation of *LOX* and increase in *MMP9* expression, a metalloproteinase involved in collagen fibres' degradation [8], the impact of Liraglutide on AT stiffness is less clear as it would suggest reduced fibre cross-linking and potentially a higher ratio of soluble fibres that do not partake in tissue stiffness. A recent study reports that bariatric surgery-induced weight loss is associated with considerable ECM remodelling with increased accumulation of unbound collagens [39]. As most studies rely on picrosirius red staining in the assessment of fibrosis, it is not clear whether the collagen increase results in increased insoluble fibres that define fibrosis, or soluble fibres due to an increase in degradation and lack of cross-linking of collagen [39]. This requires studies assessing AT stiffness in larger cohorts and study of changes in ectopic fat distribution linked with impairment in AT's storage capacity. We can thus only confirm an effect of GLP-1 on AT's ECM remodelling that is not in line with weight changes and note that GLP-1 analogues have been reported to affect ECM in other organs (liver and heart) where they constrain fibrosis development [40,41].

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Liraglutide's effect on adipogenesis and AT's lipid metabolism that are associated with obesity and diabetes has so far not been studied in humans *in vivo*. As their key regulators, *PPARG* (adipogenesis) and *LPL* (lipid metabolism) expression remained unchanged after Liraglutide treatment, an AT effect by Liraglutide independent of weight change appears unlikely. This is in agreement with our *in vitro* study on effects of Exendin 4 on subcutaneous and omental tissues [5]. Results from recent *in vitro* studies on adipogenesis appear conflicting between differentiation of human SCAT stem cells [6] and murine 3T3-L1 adipocytes in which increased differentiation was observed [42–44]. Adipogenesis is important for healthy tissue expansion; findings from genetic mouse models suggest that acute inflammation may augment adipogenesis [45]. Inflammation in this circumstance would be regarded as beneficial as it would allow for increased capacity for AT expansion. Observing inflammation with no indication of increased adipogenesis as in our study, however, does not explain the need of inflammation in this context. Of note is that inflammatory cytokines, such as *TNFA*, which were up-regulated in the GLP-1 treatment arm, increase lipolysis and block adipocyte differentiation [46].

A weakness of the present study is that we are unable to make any comments on deeper SCAT or visceral/omental AT that contribute to circulating levels of inflammatory adipokines. The changes in circulating levels observed may in part be due to the influence of other fat depots. A limitation of the present study is the small sample size. Our patient numbers are limited particularly for the diet arm, so type II errors cannot be excluded and not all participants agreed to an MRI scan. Future research is required to establish the effect of long-term treatments with GLP-1 analogues that would permit the monitoring of long-term changes in ECM remodelling and inflammation and ensure that not only transitional processes of AT homoeostasis were detected. Particularly interesting would also be to study the effect of GLP-1 discontinuation on AT as the observed AT dysfunction may be further aggravated as result of subsequent weight regain. Should GLP-1 treatment impair AT expansion and storage capacity, this may not be relevant till subjects are exposed to an energy surplus and the capacity for AT expandability becomes more important.

In summary, despite the marked improvement in systolic BP and glucose metabolism that was greater with Liraglutide than with diet-induced weight loss, 4 months of GLP-1 analogue therapy results in an increase in AT chemokine and inflammatory markers and with no improvement in systemic inflammation. The observed increase in pro-fibrotic proteins and collagens suggests that AT's ECM remodelling takes place with GLP-1 treatment which, despite concomitant weight loss, appears different to changes due to weight loss by calorie reduction. Although the potential induction of increased AT stiffness by GLP-1 analogues that may hinder AT expansion requires further study, we can confidently conclude that Liraglutide does not improve obesity-associated AT inflammation.

Clinical Perspectives

- GLP-1 analogues are molecules commonly used to improve glycaemic control of patients with Type 2 diabetes and used for obesity care. In addition to weight loss, GLP-1 analogue treatment could also directly influence AT function that is compromised in obesity and that expresses GLP-1 receptors.
- In comparison with dietary restriction based weight loss supported by counselling, treatment with Liraglutide (a GLP-1 analogue) increased inflammation and enhanced a pro-fibrotic environment in AT. Despite a stronger improvement of glycaemic control, Liraglutide was not effective in amelioration of obesity-associated AT dysfunction.
- As inflammation and fibrosis limit AT's storage ability of surplus and contribute to systemic subclinical inflammation, the effect of GLP-1 on AT may not be relevant for patients in calorie deficit and losing weight but may become an issue for those exposed to an energy surplus, e.g. after Liraglutide discontinuation in whom the AT dysfunction may be further exacerbated.

Author Contribution

Laura McCulloch, Rebecca Ward, Shivam Joshi and Emilie Pastel performed experiments and contributed to discussion. Kim Gooding and Angela Shore contributed to study design, recruitment, discussion and writing of the manuscript. Emilie Pastel and Katarina Kos analysed the data and wrote the manuscript. Katarina Kos designed the study.

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Abbreviations

ADIPO-IR, index of adipose tissue insulin resistance; ADIPOQ, adiponectin; AT, adipose tissue; AU, arbitrary units; BMI, body mass index; BP, blood pressure; CD14, cluster of differentiation 14; CD31, cluster of differentiation 31; COL1A1, collagen type 1 alpha 1; COL3A1, collagen type 1 alpha 1; COL4A1, collagen type 1 alpha 1; ECM, extracellular matrix; ELN, elastin; FN1, fibronectin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; HbA1c, glycated haemoglobin; HIF1A, hypoxia inducible factor 1α; hs-CRP, high-sensitivity C-reactive protein; IL6, interleukine-6; LEP, leptin; LOX, lysyl oxidase; LOXL2, lysyl oxidase-like 2; LPL, lipoprotein lipase; MCP-1, macrophage chemoattractant protein-1; NEFA, non-esterified fatty acid; ns, non significant; OGTT, oral glucose tolerance test; PPARG, peroxisome proliferator-activated receptor γ; PPIA, peptidylprolyl isomerase A; RCT, randomized controlled trial; SCAT, subcutaneous AT; TGFB, transforming growth factor-β; TLDA, TaqMan low-density array; TNFA, tumour necrosis factor-α; UBC, ubiquitin C; VAT, visceral AT.

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