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Effects of insulin lispro and chronic vitamin C therapy on postprandial lipaemia, oxidative stress and endothelial function in patients with type 2 diabetes mellitus

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Abstract

Background Insulin therapy may influence cardiovascular disease (CVD) and lipid metabolism in type 2 diabetes (T2D). Exaggerated postprandial lipaemia (PPL) is a feature of diabetic dyslipidaemia affecting CVD via enhanced oxidative stress (OS) and endothelial dysfunction. We assessed endothelial function and OS during PPL following insulin and vitamin C. Twenty (17 M) T2D patients were studied (mean Hba1c 8·4%) at baseline, following 6 weeks of insulin lispro (0·2 Iu kg^{-1}) and vitamin C 1-g daily. Eight-h lipid and glucose profiles were measured following a fatty meal. Endothelial function (flow-mediated vasodilatation: FMD) and OS were measured at fasting, 4 h and 8 h.

Materials and methods Glucose, body mass index, and total and LDL cholesterol remained unchanged. FMD improved. Placebo group: fasting, 1·1 ± 1·2 to 4·2 ± 1·1% ($P < 0·001$); 4-h, 0·3 ± 1·2 to 3·1 ± 0·9% ($P < 0·01$); 8-h, 0·7 ± 1·1 to 3·76 ± 1·1% ($P < 0·001$). Vitamin C group: fasting, 0·9 ± 1·1 to 6·1 ± 1·3% ($P < 0·001$); 4-h, 0·7 ± 1·5 to 4·9 ± 2·1% ($P < 0·001$); 8-h, 0·8 ± 0·9 to 5·8 ± 0·6% ($P < 0·01$). Post-prandial lipaemia was attenuated: TG area-under-curve (mmol L^{-1} 8 h^{-1}), 52·6 ± 11 to 39·1 ± 12·5 (placebo group), $P < 0·02$; and 56·9 ± 8 to 40·1 ± 10·3 (vitamin C group), $P < 0·02$. Oxidative stress was reduced, with greater changes in the vitamin C group.

Conclusion Insulin may thus exert vascular benefits in T2D, by modifying fasting and postprandial lipid metabolism resulting in reduced OS and improved EF. Vitamin C therapy may augment the vascular benefits of insulin in T2D through additional effects on OS and EF.

Keywords Diabetes, endothelium, insulin, oxidative stress, post-prandial lipaemia.
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Introduction

Coronary heart disease (CHD) is a common complication of type 2 diabetes (T2D) [1]. Several risk factors for atherosclerosis cosegregate in T2D [2], including dyslipidaemia characterized by reduced HDL-cholesterol (HDL-C), hypertriglyceridaemia and abnormal postprandial lipaemia (PPL) [3]. Postprandial lipaemia represents a state in which triglyceride (TG) metabolism is compromised, and exaggerated PPL associates with atherosclerotic disease severity and progression in subjects with and without diabetes [3]. As many factors involved in postprandial lipid metabolism are insulin-sensitive [3], there is clear potential for abnormal PPL in T2D, including prolonged and exaggerated excursions in TG and TG-rich lipoproteins (TGRL) [3].

Endothelial dysfunction (ED) is a pivotal event in atherogenesis and is a consistent finding in T2D. Potential mechanisms are complex [3], with abnormal lipid metabolism increasingly recognized as an important factor [3]. Indeed TGRL associated with enhanced (OS) appears particularly important in the aetiology of ED associated with PPL [4].

Transient ED has been described in association with PPL in diabetic and nondiabetic subjects [5], correlating with

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enhanced OS and TG-rich VLDL [5]. Furthermore, modifying PPL and reducing TGRL levels improve endothelial function and reduces OS associated with PPL in T2D [6].

Insulin therapy via improved glycaemic control retards the progress of microvascular complications in T2D [7] and may reduce macrovascular risk [7]. The potential mechanisms accounting for such effects are complex, including effects on platelet and endothelial function and lipid effects, namely suppression of hepatic free fatty acid release, and attenuated synthesis of atherogenic lipoproteins [8–10].

We therefore investigated the hypothesis that the vascular benefits of insulin therapy may be the consequence of improved endothelial function and reduced OS related to modified fasting and postprandial lipid metabolism.

T2D is also associated with reduced endogenous vitamin C concentrations [11], which may be an independent cardiovascular risk factor [12]. Vitamin C therapy improves endothelial function in type 2 diabetes mellitus (T2DM) [13] and attenuates transient ED associated with both hyperglycaemia and PPL in healthy subjects [14,15]. Controversy however, persists regarding the long-term potential cardiovascular benefits of chronic vitamin C therapy. We therefore sought to assess whether chronic vitamin C therapy would augment the vascular and antioxidant potential of insulin therapy.

Methods

Subjects

Twenty type 2 diabetic subjects (mean age 53·2 years; age range 39–59 years; 17 men, mean Hba1c 8·4%) were studied (Table 1). All were nonsmokers, receiving standard oral hypoglycaemic agents. Six subjects in the vitamin C group and seven in the placebo group were taking metformin only. Three subjects in the vitamin C group and two in the placebo group were taking a combination of metformin and sulphonylurea. One subject in each group was taking sulphonylurea, either gliclazide or glibenclamide, therapy only. Patients with vascular disease taking antihypertensives, lipid-lowering agents, aspirin or supplemental vitamins were excluded. Females were premenopausal and studied in the follicular phase. All subjects had normal 24-h urinary creatinine clearance, no detectable microalbuminuria and were free of any complications. There were no demographic differences between the men and the women. Written consent was obtained from all subjects with local Ethic Committee approval.

Study design

Studies commenced following a 12-h overnight fast with omission of hypoglycaemic agents for 36 h before the study day. After 30 min of supine rest, endothelial function was assessed and venous blood was drawn for measurement of total LDL and HDL cholesterol, triglyceride, insulin, glucose and glycosylated haemoglobin (HbA1C), and 5 mL of venous blood was drawn to enable measurement of OS. Each subject received a fatty meal [5,6] containing 80 g of saturated fat. Lipid levels and glucose were measured every 2 h over the following 8 h. Endothelial function and OS were reassessed at 4 and 8 h. Subsequent to these baseline investigations, oral therapy was replaced in all subjects by premeal bolus insulin lispro (0·2 U kg^{-1}) combined with either placebo (n = 10) or vitamin C 1 g daily (n = 10). Subjects were closely supervised, with individual dose adjustments made to match prestudy glucose levels based on a 4-week period of home blood glucose monitoring before entry into the study. After 6 weeks of therapy, PPL and associated changes in OS and endothelial function were reassessed with omission of all treatment on the study day.

Table 1 Patient characteristics and biochemical profiles. Data expressed as mean ± SD

	vitamin C (Baseline) n = 10, 2 F	Placebo (Baseline) n = 10, 1 F	vitamin C (+ Insulin)	Placebo (+ Insulin)
Age (years)	52·7 ± 6·9	53·6 ± 7·9	52·7 ± 6·9	53·6 ± 7·9
BMI (kg m^{-1})	29·2 ± 4·8	28·6 ± 5·5	29·3 ± 4·9	28·7 ± 5·2
TC (mmol L^{-1})	5·4 ± 0·7	5·5 ± 0·6	5·4 ± 0·7	5·5 ± 0·6
LDL-C (mmol L^{-1})	3·3 ± 0·3	3·4 ± 0·5	3·3 ± 0·3	3·4 ± 0·5
HDL-C (mmol L^{-1})	0·92 ± 0·1	0·93 ± 0·1	1·21 ± 0·1*	1·19 ± 0·14*
TG (mmol L^{-1})	2·1 ± 0·3	2·2 ± 0·4	1·4 ± 0·2*	1·3 ± 0·4*
Systolic BP (mm hg^{-1})	151 ± 11	149 ± 13	148 ± 13	148 ± 14
Diastolic BP (mm hg^{-1})	85 ± 10	84 ± 9	84 ± 9	82 ± 7
Fasting insulin (mu L^{-1})	30·6 ± 10·8	28·3 ± 12·9	25·9 ± 12·8	23·7 ± 15·6
Fasting Glc (mmol L^{-1})	8·4 ± 0·7	9·1 ± 1·3	8·3 ± 1	8·5 ± 0·8
Hba1c (%)	8·5 ± 0·8	8·4 ± 0·7	8·2 ± 0·8	8·2 ± 0·9
AUC-Glc (mmol L^{-1} 8 h^{-1})	62·9 ± 11·6	64·8 ± 17·8	59·8 ± 14·7	60·6 ± 15·1
AUC-Glc (mmol L^{-1} 4 h^{-1})	34·1 ± 6·9	33·1 ± 9·1	34·9 ± 7·9	33·9 ± 8·4
AUC-Glc (mmol L^{-1} 2 h^{-1})	19·7 ± 7·7	18·8 ± 8·4	20·1 ± 8·9	18·2 ± 9·1

*$P < 0·05$ post insulin vs. baseline.

Endothelial function assessment

Changes in brachial artery diameter (flow mediated dilatation: FMD) were measured using an ultrasonic vessel wall-tracking system (resolution ± 3 μm) [5,6]. Baseline measurements of artery diameter and blood flow were taken after > 10 min of rest. Releasing a paediatric sphygmomanometer wrist cuff inflated to supra-systolic pressure for 5 min produced reactive hyperaemia. Blood flow was recorded for 15 s before and until 90 s after cuff release. Vessel diameter was measured 60 s after cuff release and repeated after 15 min to confirm recovery. Repeat measurements were taken 3 min after sublingual glyceryl trinitate (GTN, 400 μg; endothelium-independent response). Data are presented as percentage change in diameter.

Biochemical measurements

Total cholesterol and TG were quantified enzymatically, and HDL-C after precipitation of apolipoprotein B with phosphotungstate/magnesium. LDL-C was calculated following lipoprotein separation [5,6]. Glucose was measured by a hexokinase-based technique, HbA1c via enzyme immunoassay, and plasma insulin by a commercial radioimmunoassay (INS-RIA-100, medgenix diagnostics, Brussels, Belgium).

Lipoprotein separation

Lipoprotein separation and analysis was carried out as previously described and validated [20]. Chylomicrons were removed from plasma by centrifugation, and aliquots of chylomicron free plasma (CF-plasma) retained. For each gradient, 1·6 mL of CF-plasma was mixed with 0·4 mL of Liposep™ (Liverpool John Moores University, UK), and 4·25 mL of Hepes-buffered saline was mixed with 0·25 mL of Liposep™; 1·4 mL of the latter mixture was pippetted into optiseal tubes with 1·4 mL of the Liposep-plasma mixture being layered beneath, filled with Hepes pH 7·4 and centrifuged. Gradients were collected into 20 fractions from the bottom of the tube. Cholesterol and TG was assayed in duplicate for each fraction and in triplicate on the plasma and CF-plasma. The lipoprotein classes in each gradient fraction were identified by gel electrophoresis. VLDL, LDL and HDL cholesterol and TG content were determined from the distribution profiles of the lipids on the gradients. Chylomicron lipids were calculated from the differences between the plasma and the CF-plasma.

Measurement of oxidative stress

Measurement of venous free radicals

The formation of lipid radicals follows oxidative damage *in vivo*. We used a fully validated technique of *ex vivo* spin trapping to measure free radical levels [7]. Free radicals decay quickly, but may be stabilized when chemically trapped ('spin-traps'), forming radical adducts quantifiable by electron paramagnetic resonance (EPR) spectroscopy.

Venous blood was placed directly into a sealed glass tube containing a spin-trap μ-phenyl *N*-tert-butyl-nitrone (PBN). Following centrifugation, the PBN adduct was extracted with toluene, dried under nitrogen gas and reconstituted in degassed chloroform. Electron paramagnetic resonance spectra were recorded on a Varian E104 spectrometer, and EPR spectral parameters were obtained from data acquisition and processing using in-house software. Electron paramagnetic resonance spectral peak heights were taken as a correlation of spin-adduct concentration after confirmation of peak-to-peak line width conformity and double integration on samples. The separation between sets of peaks in the EPR spectrum (coupling constants) identified the radicals trapped as carbonyl and alkoxyl.

Thiobarbituric acid-reactive substances (TBARS)

Levels of TBARS (μmol L^{-1}) as a measure of lipid peroxidation were determined using a spectophotometric assay [6]. The results give values for malonaldehyde (MDA) and 4-hydroxyalkenals (4-HNE). The reproducibility of this assay in our study has a SEM < 5%.

Statistical analysis

Conventional methods were used for calculating mean, standard deviation and checks for normal distribution. Group differences in variables were determined by two-tailed *t*-test. Statistical significance for differences between groups was tested by two-way analysis of variance. As a measure of plasma glucose and the total amounts of lipid and lipoprotein present during PPL, areas-under-curve (AUC) were calculated for plasma concentrations without subtraction of baseline values. Simple logistic regression analysis was used to study relationships between variables with logarithmic transformation of skewed data. This study was powered to detect changes in endothelial function and OS of the same order of magnitude as seen in our previous studies of the effect of ciprofibrate therapy on PPL, OS and endothelial function and on baseline differences in PPL and endothelial function between control and T2D subjects [6,7].

Results

Blood pressure and BMI remained unchanged. In order to maintain patients in a euglycaemic state compared with baseline, mean (± SD) individual daily insulin doses of 16·9 ± 4·4 U day^{-1} were required in the vitamin C-treated subjects and 15·7 ± 6·3 U day^{-1} were required in the placebo-treated subjects.

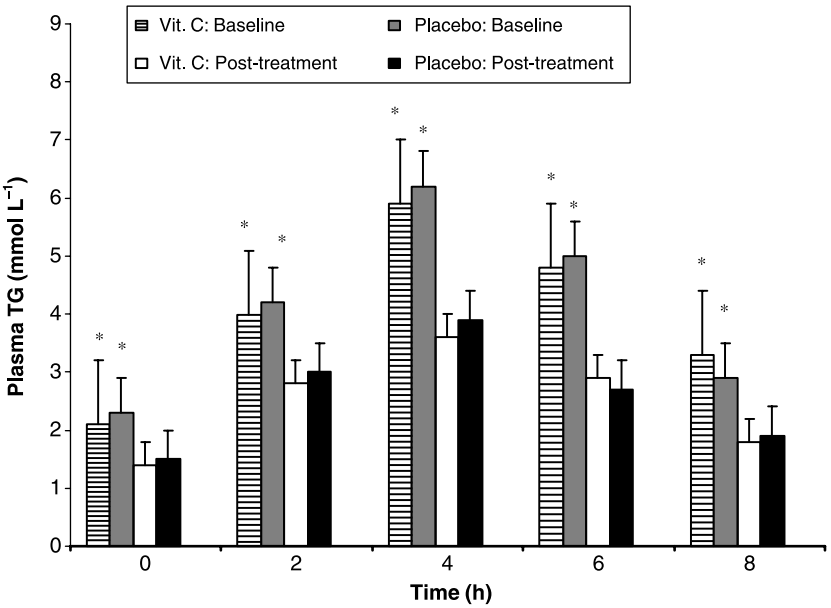

Figure 1 Post-prandial plasma triglyceride (TG) levels in the vitamin C or placebo group at baseline and after insulin therapy. $^{*}P < 0{\cdot}05$ for plasma TG levels following insulin therapy compared with baseline.

Table 2 Baseline and post-treatment qualitative lipoprotein profiles in the fasted state. Cholesterol and triglyceride content of the major lipoprotein subclasses are expressed as μmol mL^{-1} with data presented as mean ± SD

	vitamin C (Baseline)	Placebo (Baseline)	vitamin C (+ Insulin)	Placebo (+ Insulin)
VLDL-TG	3·79 ± 1·91	3·89 ± 2·28	2·83 ± 1·27*	2·91 ± 1·34*
LDL-TG	2·11 ± 0·98	2·05 ± 0·72	1·51 ± 0·42*	1·48 ± 0·37*
HDL-TG	1·12 ± 0·31	1·25 ± 0·44	0·79 ± 0·22*	0·81 ± 0·26*
VLDL-C	2·81 ± 1·13	2·77 ± 1·26	2·86 ± 1·19	2·79 ± 1·33
LDL-C	4·15 ± 1·84	4·13 ± 1·89	4·09 ± 2·10	4·11 ± 1·91
HDL-C	0·93 ± 0·11	0·97 ± 0·21	0·89 ± 0·2	0·95 ± 0·17

$^{*}P < 0{\cdot}05$ baseline vs. post-insulin.
TG, triglyceride content; C, cholesterol content.

Post-prandial lipaemia

Post-prandial TG AUC (mmol L^{-1} 8 h^{-1}) was reduced following insulin (Fig. 1) (AUC-TG – 56·9 ± 8 to 40·1 ± 10·3 [vitamin C group], 52·6 ± 11 to 39·1 ± 12·5 [placebo group]; $P < 0{\cdot}05$) associated with reduced lipoprotein TG content (Table 2). Following insulin, postprandial triglyceride excursions were reduced in both groups (AUC-TG [mmol L^{-1} 8 h^{-1}]; vitamin C group: 41·4 ± 11·9 and placebo group: 40·8 ± 13·9). Insulin therapy resulted in a significant depletion in TG content of all major lipoproteins during postprandial lipaemia (Table 3).

Plasma insulin levels rose during PPL, with small reductions following treatment: 66·7 ± 18·5 (4 h), 49·4 ± 13·9 (8 h) baseline vitamin C to 61·4 ± 23·6 (4 h), 42·8 ± 21·7 (8 h) post-treatment vitamin C; and 63·1. ± 17·8 (4 h), 43·6 ± 17·2 (4 h) baseline placebo to 59·8 ± 21·4 (4 h), 40·9 ± 24·2 (4 h) post-treatment placebo. There were no significant changes in insulin levels following insulin only.

Vascular data

Baseline FMD was similar in both groups (Fig. 2). Before entry into the study, a coefficient of variation of < 1% in FMD was observed in the study group following a repeated assessment of FMD over a 4-week period. Fasting FMD improved following insulin, with augmented changes in the vitamin C group, which were maintained postprandially (Fig. 2).

In the insulin- and placebo-treated group, the effects of vitamin C disappeared so that FMD was similar in both groups: 4·1 ± 0·6 (placebo fasting), 3·2 ± 1·1% (placebo 4 h), 3·71 ± 0·9% (placebo 8 h); and 4·2 ± 0·5 (vitamin C fasting), 3·3 ± 0·9%, (vitamin C 4 h), 3·8 ± 0·8% (vitamin C 8 h).

The GTN responses increased in both groups following insulin 9·8 ± 3·4–11·5 ± 3·1% ($P < 0{\cdot}01$) in the vitamin C group and 9·6 ± 4·2–11·8 ± 3·8% ($P < 0{\cdot}01$) in the placebo group. All other vascular parameters remained unchanged.

Table 3 Triglyceride content and cholesterol content (μmol $mL^{-1}8\ h^{-1}$) of major lipoproteins in both groups during postprandial lipaemia (expressed as 8-h area-under-curve measurements) pretreatment and after insulin

	vitamin C (Baseline)	Placebo (Baseline)	vitamin C (+ Insulin)	Placebo (+ Insulin)
CM-TG	32·5 ± 12·4	34·3 ± 15·8	22·6 ± 10·4*	23·7 ± 14·8*
VLDL-TG	23·8 ± 9·4	25·7 ± 9·8	18·5 ± 6·8*	19·7 ± 7·4*
LDL-TG	11·2 ± 5·7	12·6 ± 7·1	7·3 ± 4·2*	8·5 ± 5·2*
HDL-TG	4·5 ± 1·9	3·9 ± 2·7	2·4 ± 1·3*	1·9 ± 1·9*
CM-C	4·8 ± 2·1	5·2 ± 3·2	5·4 ± 3·1	5·6 ± 3·9
VLDL-C	5·87 ± 3·3	6·22 ± 4·1	6·1 ± 4·6	5·95 ± 3·9
LDL-C	7·5 ± 3·5	8·2 ± 2·9	7·9 ± 5·1	8·4 ± 3·6
HDL-C	2·5 ± 1·1	3·1 ± 1·4	2·9 ± 1·8	3·3 ± 1·5

*$P < 0·05$ baseline > post insulin when analyzed using the 2-sample Mann–Whitney test.
TG, triglyceride content; C, cholesterol content; CM, chylomicron.

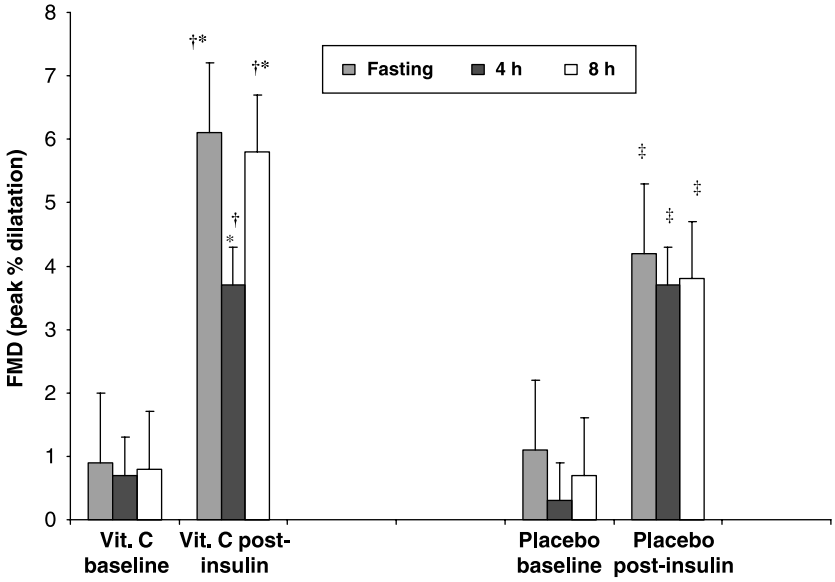

Figure 2 Fasting and post-prandial flow-mediated dilatation (FMD) of the brachial artery in the vitamin C- and placebo-treated groups at baseline and after insulin therapy. *$P < 0.05$ for the greater increase in FMD at 0, 4 and 8 h following combination insulin and vitamin C therapy compared with the increases produced by the insulin and placebo therapy. †$P < 0·05$ for the increase in FMD at 0, 4 and 8 h produced by the combined insulin and vitamin C therapy compared with baseline. ‡$P < 0·05$ for the increase in FMD at 0, 4 and 8 h produced by the combined insulin and placebo therapy compared with baseline.

Glyceryl trinitate induced dilatation, and resting and hyperaemic flow as well as resting arterial diameter were unaffected by postprandial lipaemia.

Oxidative stress:

Fasting and postprandial EPR and TBARS fell with insulin therapy, with augmented reductions in the vitamin C group (Figs 3 and 4). After insulin only, both EPR and TBARS measurements were similar in both groups, remaining reduced compared with baseline.

Correlation between endothelial function, PPL and oxidative stress

Pre-treatment fasting endothelial function correlated with HDL-C levels ($r = 0·49$, $P < 0·05$), while the postprandial deterioration in endothelial function correlated with TG enrichment of VLDL ($r = 0·43$, $P < 0·05$). Following insulin therapy the improvement in the fasting endothelial function in both groups correlated with the increase in HDL-C levels ($r = 0·46$, $P < 0·05$ [vitamin C group] and $r = 0·49$, $P < 0·05$ [placebo group]). The improvement in postprandial endothelial function following insulin correlated with the reduction in postprandial VLDL–TG content ($r = 0·51$, $P < 0·05$ [vitamin C group] and $r = 0·49$, $P < 0·05$ [placebo group]). The reduction in postprandial measures of oxidative stress in both groups following insulin correlated with the reduction in TG content of both HDL and LDL.

Discussion

Following 6 weeks of premeal insulin lispro, fasting and postprandial endothelial function (FMD) improved, with reduced OS. Endothelium-independent responses also increased while the FMD and OS changes were augmented by concomitant vitamin C. The low coefficient of variation

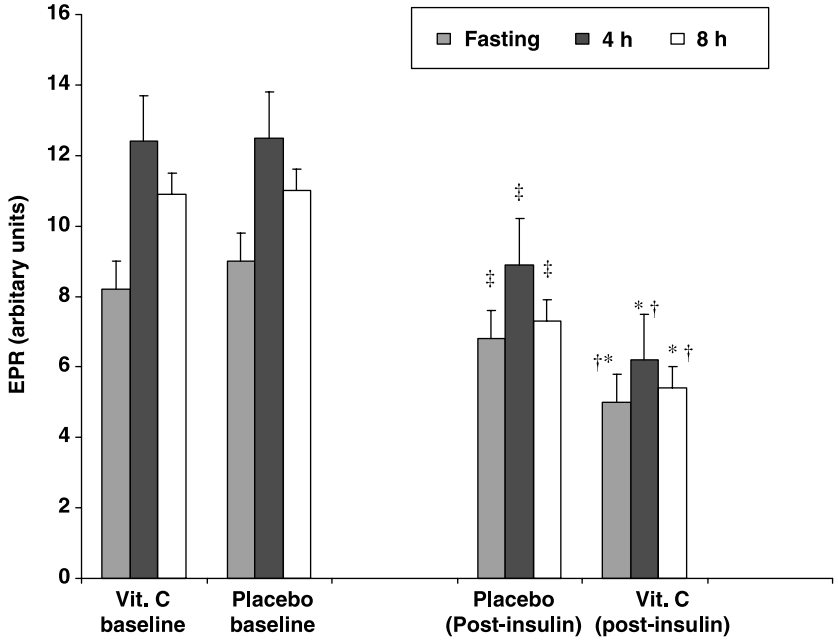

Figure 3 Fasting and post-prandial electron paramagnetic resonance (EPR) spectroscopy in the vitamin C- and placebo-treated groups at baseline and after insulin therapy. $^{*}P < 0{\cdot}05$ for the greater reduction in EPR at 0, 4 and 8 h following combination insulin and vitamin C therapy compared with the reduction produced by the combination insulin and placebo therapy. $^{\dagger}P < 0{\cdot}05$ for the reduction in EPR at 0, 4 and 8 h following the combined insulin and vitamin C therapy compared with baseline. $^{\ddagger}P < 0{\cdot}05$ for the reduction in EPR at 0, 4 and 8 h produced by the combined insulin and placebo therapy compared with baseline.

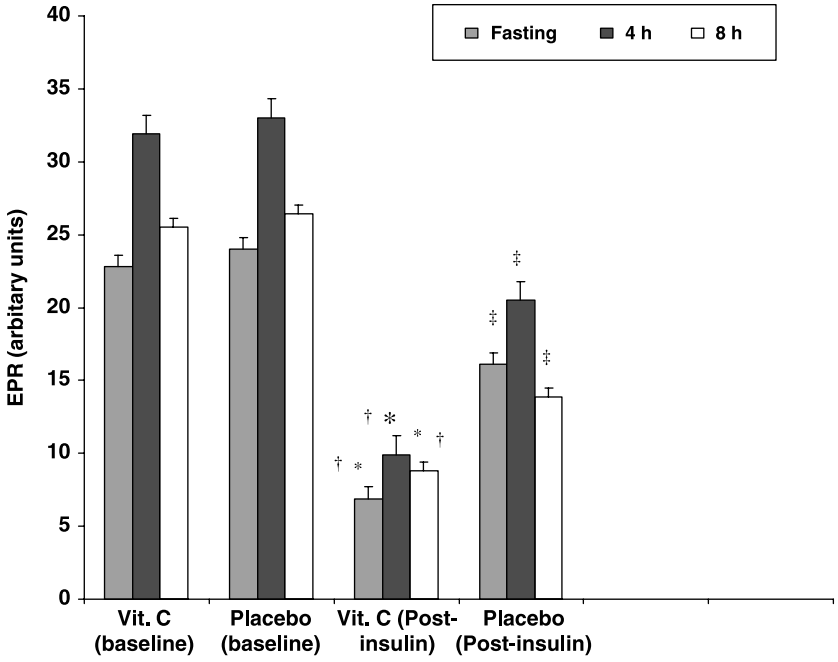

Figure 4 Fasting and post-prandial thiobarbituric acid-reactive substances (TBARS) in the vitamin C- and placebo-treated groups. $^{*}P < 0{\cdot}05$ for the greater reduction in TBARS at 0, 4 and 8 h following combination insulin and vitamin C therapy compared with the reduction produced by the combination insulin and placebo therapy. $^{\dagger}P = 0{\cdot}001$ for the reduction in TBARS at 0, 4 and 8 h following the combined insulin and vitamin C therapy compared with baseline. $^{\ddagger}P < 0{\cdot}01$ for the reduction in TBARS at 0, 4 and 8 h produced by the combined insulin and placebo therapy compared with baseline.

in repeated prestudy assessments of endothelial function implies that the changes observed during the study were the consequence of therapy rather than the result of a time-dependent phenomenon.

Insulin lispro is human insulin, with amino acids at positions 28 and 29 of the B chain reversed, resulting in rapid absorption and action kinetics [16]. Insulin lispro was therefore used in an attempt to supplement the impaired post-prandial early phase insulin release in T2DM [17].

Following insulin therapy, plasma TG fell, PPL was attenuated, and TGRL levels reduced with increased HDL-C. These effects were the consequence of chronic insulin therapy, as no insulin had been taken for at least 12 h before the study day.

Triglyceride-rich VLDL particles preferentially undergo endocytosis by macrophages forming foam cells [18], while lipolytic products of TG-rich VLDL are toxic to endothelial cells and macrophages [19]. Increased free fatty acid (FFA)

fluxes, particularly during postprandial lipaemia, further potentate the toxic effects of TG-rich VLDL on endothelial cells [4]. Increased levels of TG-rich VLDL may also promote ED by promoting increased synthesis of small dense LDLs [4]. Thus by reducing TG-rich VLDL concentrations and suppressing FFA release, insulin therapy may result in improved endothelial function. Triglyceride depletion of VLDL as a result of exogenous insulin has been described in T2DM [20] in relation to reduced hepatic synthesis as a result of reduced FFA levels, which are the major substrate for the production of TG-rich VLDL [4]. Although FFA levels were not directly measured in this study, FFA may directly induce endothelial dysfunction and the improvements in endothelial function following insulin may be primarily related to the suppression of FFA release by insulin. Furthermore, the augmented effects produced by vitamin C on endothelial function may reflect the recently described effects of vitamin C on attenuating FFA-induced endothelial dysfunction [21].

The observed associations between HDL-C concentrations and TG-rich LDL and VLDL levels with endothelial dysfunction in this study further supports the importance of HDL-C and TGRL, in particular TG-rich VLDL, in modulating vascular function in T2DM [4]. As the TG content of VLDL correlated most strongly with fasting and postprandial oxidative stress, this study also supports a role for TG-rich VLDL in the pathogenesis of enhanced oxidative stress in T2DM.

Insulin therapy increases HDL-C [10], which may also contribute to improved endothelial function. This increase in HDL-C may be a secondary consequence of attenuated TG metabolism, which may be partly result from the effects of insulin on TGRL synthesis [4]. The lipid effects of insulin therapy may also represent improved insulin sensitivity, supported by our observed reductions in endogenous insulin levels. Insulin therapy has indeed been shown to increase insulin sensitivity in T2DM [22]. Our observations were however, nonsignificant and provide no detailed assessment of insulin sensitivity.

Insulin also reduced LDL and HDL TG content. Excess hydrolysis of TG within TG-rich LDL particles results in increased production of small, dense particles with prooxidant properties. Thus reducing TG-rich LDL may influence OS and endothelial function through a reduced production of small dense LDL. Triglyceride-rich HDL particles demonstrate decreased endothelium protective and antioxidant properties [4]. Thus increased levels of TG-depleted HDL particles may further contribute to the enhanced antioxidant and endothelium protective potential as a result of increased HDL-C concentrations.

Reduced levels of TGRL following insulin may not only reflect reduced synthesis but also enhanced catabolism. The catabolism of TGRL is mediated by lipoprotein lipase (LPL), which may have reduced lipolytic activity in T2DM. Although acute administration of exogenous insulin may upregulate LPL enzymatic activity [23], there is no evidence that chronic administration of exogenous insulin has similar effects.

Insulin may also directly influence endothelial NO synthesis as a result of increased eNOS gene expression [24]. Such an effect may be augmented by any improvement in insulin sensitivity, as insulin sensitivity positively correlates with endothelial NO synthesis [25].

Insulin also increased endothelium-independent (vascular smooth muscle dependent) vasodilatation. This may be related to increased bio-availability of exogenous as well as endogenous NO, partly as a consequence of reduced OS. Vascular smooth muscle cells in the context of insulin resistance may exhibit nitrate resistance [26], thus any potential improvement in insulin sensitivity may be reflected in vascular smooth muscle cells as increased nitrate responsiveness.

T2DM is associated with enhanced OS [27], the aetiology of which is complex. Our study reaffirms the association between dyslipidaemia and OS in T2DM. Enhanced OS may directly induce endothelial dysfunction by decreasing the synthesis and release of NO and by inactivating NO in the subendothelial space [4]. Oxidative stress may also indirectly promote endothelial dysfunction by modifying the properties of lipoprotein particles, in particular LDL.

Vitamin C is a low-molecular weight antioxidant that scavenges free radicals. Treatment with vitamin C may supplement reduced endogenous levels in T2DM, thus reducing OS and so improving endothelial function. In our study all subjects treated with vitamin C demonstrated improved endothelial function and reduced oxidative stress, with no reported adverse events. These data thus support previous observations of beneficial effects of vitamin C on endothelial function in T2DM [15]. However, this study clearly demonstrates that chronic vitamin C therapy may attenuate endothelial dysfunction associated with PPL in T2DM. Moreover, this is the first study to demonstrate that the effects of vitamin C on endothelial function in T2DM may be augmented by concomitant insulin therapy.

Insulin therapy may improve endothelial function and reduce oxidative stress in T2D via improved glycaemic control [28, 29]. In this study there was however, no change in overall metabolic control as measured by Hba1c, while fasting and postprandial glucose levels (measured by AUC for 2-, 4- and 8-h post-prandial glucose levels) also remained unchanged. The effects of insulin therapy on endothelial function and oxidative stress in this study are likely to be have been the consequence of changes in lipid and FFA metabolism.

The additional improvement in endothelial function and OS produced by concomitant vitamin C therapy suggests that any potential benefits of insulin therapy on cardiovascular outcome in T2D may be enhanced by vitamin C supplementation. This hypothesis however, requires further evaluation in randomized controlled end-point studies.

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