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Effect of caloric restriction with and without exercise training on oxidative stress and endothelial function in obese subjects with type 2 diabetes

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Aim: Effects of dietary weight loss on endothelial function, particularly when combined with exercise training, is largely unknown in type 2 diabetes. We sought to determine whether aerobic exercise training provided any additional improvements in endothelial function, oxidative stress or other established markers of cardiovascular risk when combined with an energy-restricted diet in patients with type 2 diabetes.

Methods: In a parallel study design, 29 sedentary, overweight and obese patients with type 2 diabetes (age 52.4 ± 1.4 years and BMI 34.2 ± 0.9 kg/m^2) were randomized to a 12-week moderate energy-restricted diet (~5000 kJ/day and ~30% energy deficit) with or without aerobic exercise training [diet only (D), n = 16 and diet plus exercise (DE), n = 13]. Body weight, cardiovascular risk markers, malondialdehyde (MDA, oxidative stress marker), 24-h urinary nitrate/nitrite and flow-mediated dilatation (FMD) of the brachial artery were measured pre- and postintervention.

Results: Both interventions reduced body weight (D 8.9%, DE 8.5%, time effect $p < 0.001$). Significant reductions in body fat, waist circumference, blood pressure, glycated haemoglobin, glucose, insulin resistance, lipids and MDA and increases in urinary nitrite/nitrate were observed in both groups (time effect $p \leq 0.05$); however, these changes were not different between treatments. At baseline, FMD was similar in both groups (D 2.5 ± 0.9%, DE 4.2 ± 1.2%; $p = 0.25$) and did not change after the interventions ($p = 0.59$).

Conclusions: These results suggest that lifestyle interventions incorporating diet with or without exercise improve glycaemic control, reduce oxidative stress and improve other cardiovascular risk factors but do not improve FMD in obese subjects with type 2 diabetes.

Keywords: cardiovascular risk factors, diabetes, energy restriction, exercise training, flow-mediated dilatation
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Introduction

The obesity epidemic has led to an increasing prevalence of type 2 diabetes [1]. Type 2 diabetes is associated with a number of macrovascular and microvascular complications and a threefold to sixfold increase in the risk of cardiovascular disease–related mortality [2]. Endothelial dysfunction (ED) is an early event in the aetiology of atherosclerosis [3]. ED has been reported in patients with type 2 diabetes and is considered to be a major determinant of diabetic vascular complications [4]. Endothelial function can be assessed non-invasively

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using brachial artery flow-mediated dilatation (FMD) [5]. During FMD, increased shear stress stimulates the production of endothelium-derived nitric oxide (NO) that causes vascular smooth muscle relaxation and arterial dilatation [6], a response that is impaired when the vascular endothelium is dysfunctional [7]. Brachial artery FMD correlates with coronary artery dilatory function and as such is an independent predictor of future cardiac events [8].

It is well established that lifestyle therapies that combine energy restriction and physical activity independently improve a number of cardiovascular disease risk factors in type 2 diabetes including insulin resistance, impaired glucose tolerance, dyslipidaemia and hypertension [9,10]. Accordingly, modifications in diet and physical activity are recognized as the cornerstone in the treatment and prevention of type 2 diabetes. However, studies examining the effect of these lifestyle interventions on endothelial function in overweight/obese patients with type 2 diabetes are few. Although controversy still exists, most [11–16], but not all studies [17], have shown no improvement in FMD with weight loss. Alternatively, a number of studies have consistently shown benefits of exercise training for FMD in a range of populations [18–22], including patients with type 2 diabetes [23–26]. Maiorana *et al.* [24] showed that 8 weeks of combined aerobic and resistance exercise training improved FMD in patients with type 2 diabetes but did not investigate the effects of concomitant dietary restriction. Similarly, Sakamoto *et al.* [25] had rats with type 2 diabetes undergo caloric restriction or exercise and found that while both energy restriction and exercise training improved plasma levels of glucose, insulin, cholesterol and triacylglycerol and reduced abdominal fat accumulation, only exercise training and not caloric restriction improved endothelial function. In the only study of its kind, Hamdy *et al.* [23] showed that 6 months of weight loss by caloric restriction combined with exercise training improved FMD in a number of obese patient groups, including type 2 diabetes, but this study lacked an appropriate control group and the design did not allow for determination of whether the addition of exercise training to weight loss was responsible for the improvement in FMD. To date, no human studies have allowed for the determination and comparison of the separate effects of caloric restriction and exercise training on FMD in patients with type 2 diabetes.

While relatively few studies have investigated the effects of lifestyle modification on endothelial function in type 2 diabetes, even fewer have evaluated the mechanisms by which any benefits are mediated. The hyperglycaemia that is apparent in type 2 diabetes is associated with elevated oxidative stress [27]. Increased oxidative stress can lead to quenching or inactivation of NO [28], resulting in diminished NO availability in type 2 diabetes [29], and this has been thought to play a role, at least in part, in the impairment of FMD [30]. Consequently, the administration of antioxidant agents has been shown to improve endothelial function in type 2 diabetes [30]. Both weight loss and exercise training have been associated with reduced oxidative stress [31,32] and improved NO availability in type 2 diabetes [33], but whether these changes contribute to any improvements in endothelial function is not known.

The aim of this study was to determine whether the addition of aerobic exercise training to a moderate calorie-restricted diet had any effect on FMD in overweight and obese patients with type 2 diabetes and whether any changes were associated with changes in oxidative stress and NO availability.

Methods

Subjects

A total of 37 overweight/obese sedentary men and women [body mass index (BMI) 27–44 kg/m^2; age between 33 and 62 years] with type 2 diabetes were recruited by public advertisement (figure 1). Subjects completed a health and physical activity screening questionnaire and were not eligible to participate if they were smokers, had known proteinuria, a malignancy, a history of liver, kidney, cardiovascular, respiratory or gastrointestinal disease, cardiac (heart) abnormalities, uncontrolled hypertension or were pregnant or lactating. Subjects were also excluded if they had taken beta blockers or glucocorticoids within the previous 3 months or dietary supplements (vitamins, minerals, carotenoids or traditional herbs) in the previous 2 months. Subjects who had a musculoskeletal injury, joint or peripheral vascular disease sufficient to impede exercise or had participated in regular physical exercise (greater than two 30-min sessions of moderate/vigorous exercise more than six metabolic equivalents per week) during the 6 months prior to study were also excluded from participation. The protocol and the potential risks and benefits of participating in the study were explained to each subject before they provided written informed consent. The study was approved by the Human Ethics Committee of the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the University of South Australia.

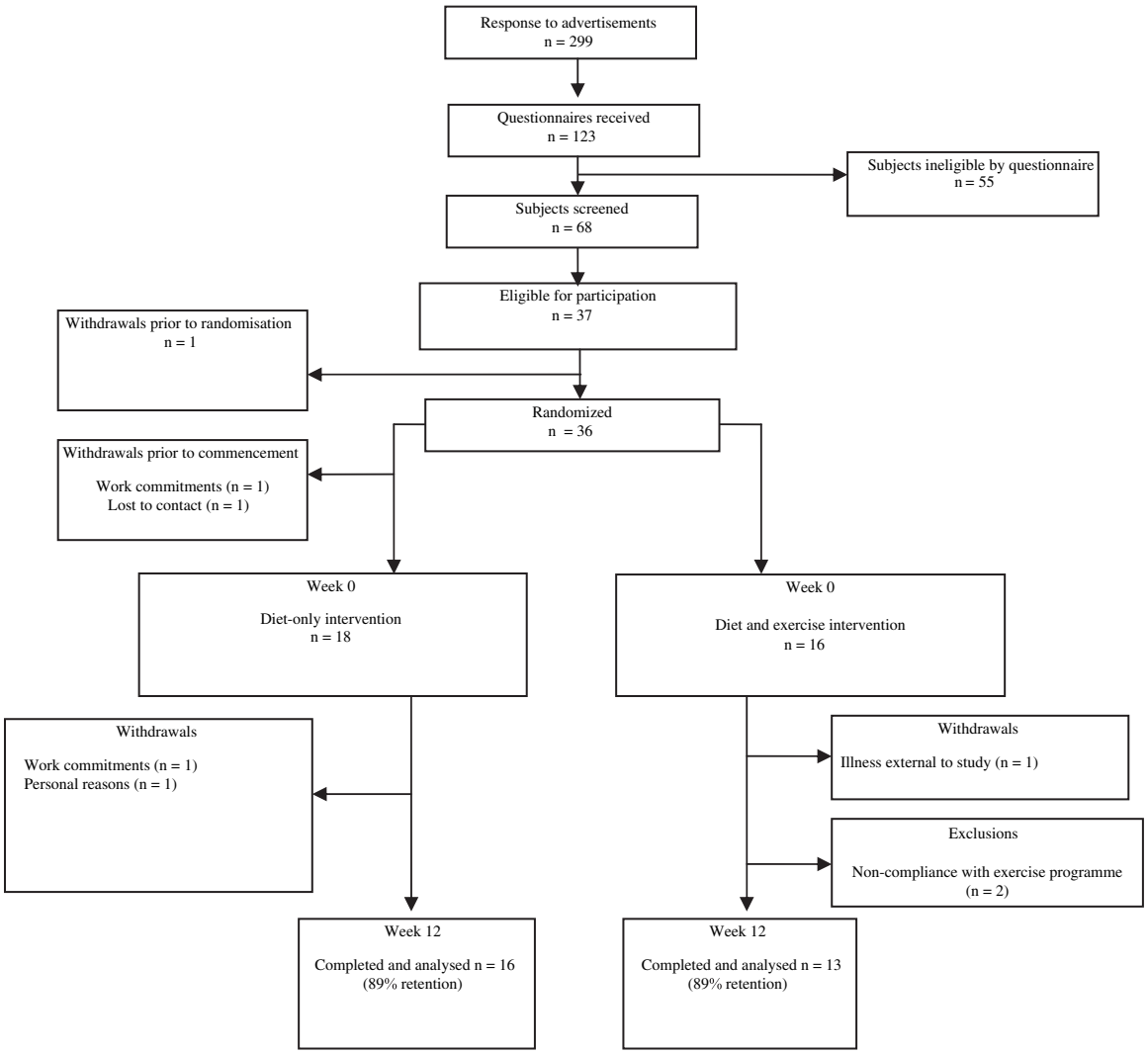

Fig. 1 Flow chart of subject participation.

Experimental Design

In a parallel study design, subjects were block matched for age, gender and BMI and then randomized to one of two 12-week lifestyle interventions: diet alone (D) or diet plus exercise (DE). Before the start (week 0) and at the end (week 12) of the intervention, subjects attended the CSIRO clinical research unit on two consecutive days after an overnight fast for testing. Subjects were asked to abstain from alcohol consumption and participation in vigorous physical activity during the 24 h prior to these visits. On the first visit, height, weight and blood pressure were measured before a blood sample was collected for measurement of plasma glucose, glycated haemoglobin (HbA1c), insulin and lipid concentrations (total cholesterol, HDL, LDL and triglycerides), total antioxidant status (TAS) and malondialdehyde (MDA) levels. Subjects then performed a maximal graded treadmill test to exhaustion. On the following day, subjects returned to the clinic and waist circumference and body composition (bioelectrical impedance) were measured prior to the assessment of endothelial function by brachial artery FMD. The next day, subjects commenced the appropriate intervention. In addition, during the 24 h prior to the first clinic visits at weeks 0 and 12, subjects collected a 24-h urine sample for assessment of urinary nitrate/nitrite. Subjects were requested not to modify their lifestyles during the 12-week intervention period other than to comply with the requirements of the study.

Anthropometric Measurements and Blood Pressure

Height was measured using a stadiometer (SECA, Hamburg, Germany), and body mass was measured using calibrated electronic digital scales (AMZ 14; Mercury, Tokyo, Japan). Fat mass and fat-free mass were measured in a supine position using single-frequency (50 kHz) bioelectrical impedance analysis according to the manufacturer's instructions (IMP5™ BIA; Impedimed, Brisbane, Australia).

Blood pressure was measured seated after a minimum 5-min rest using an automated sphygmomanometer (Dinamap™ 8100; Critikon, Tampa, FL, USA). Three blood pressure measurements were performed, each separated by 2 min. An average of the three readings was used as the measured value.

Maximal Oxygen Consumption and Heart Rate

Peak oxygen consumption (VO_{2peak}) was measured during a graded incremental exercise test to volitional fatigue on a motorized treadmill (Trackmaster TMX425CP; Full Vision, Newton, KS, USA) using the modified Bruce protocol [34]. Metabolic data (VO_2 consumption and CO_2 production) were measured during exercise using indirect calorimetry (TrueMax 2400; ParvoMedics, Sandy, UT, USA). Heart rate was recorded throughout the incremental test as 5-s averages using a personal heart rate monitor (Polar Beat; Polar Electro, Oulu, Finland). Peak oxygen consumption was deemed to have been reached when subjects achieved the primary criteria of a plateau in VO_2 with increasing workload or at least two of the following three secondary criteria: (i) a peak respiratory exchange ratio of >1.0; (ii) reaching $\geq 85\%$ age-predicted maximum heart rate (HR_{max}); or (iii) achieving a rating of perceived exertion (Borg Scale) ≥ 17. A continuous three-lead electrocardiogram recording was monitored throughout the test, and blood pressure was measured at the end of each exercise stage to ensure patient safety.

Flow-mediated Dilatation

Endothelial-dependent brachial artery FMD measurements were conducted under conditions previously described [5]. B-mode ultrasound with a 7.5-MHz linear array transducer (Acuson Aspen Duplex; Acuson, Mountain View, CA, USA) was used to image the brachial artery in the distal third of the upper arm. A sphygmomanometer cuff was placed around the forearm 2 cm distal to the olecranon process and inflated to 200 mmHg for 5 min to provide a stimulus for forearm ischaemia. Images were recorded 30 s before cuff deflation and every 30 s for 3 min after deflation. The FMD response expressed as a percentage change in the diameter of the artery was used as the measure of endothelium-dependent vasodilation. After a 10-min rest phase, endothelium-independent dilatation was assessed after the administration of 300 μg sublingual glyceryl trinitrate (GTN). Images were recorded 30 s before administration of GTN and every minute for 10 min following. All FMD assessments were performed by the same operator, and the intraobserver coefficient of variation for FMD in this operator's hands was 10.6% based on data for normal healthy individuals ($n = 10$) who were scanned on two separate occasions after an overnight fast prior to commencement of the study, which is similar to that reported in other laboratories [35,36].

Dietary Intervention

Both groups consumed a high-protein, energy-restricted diet (~5500 kJ/day) for a planned weight loss of 8–12 kg. The dietary plan consisted of two meal replacements (KicStart™, Pharmacy Health Solutions, New South Wales, Australia) added to 200 ml of skim milk (47% of total energy as protein, 41% as carbohydrate and 12% as fat) and one self-prepared high-protein meal. Overall, the macronutrient content of the three meals comprised 40% of total energy as protein, 20% as fat and 40% as carbohydrate. To facilitate compliance, the diet was structured to include specific daily quantities of foods in a checklist, which subjects completed daily. Dietary instruction, assistance with meal planning and monitoring of completion of checklists were conducted at baseline and every 2 weeks by a qualified dietician to assist with dietary adherence.

Exercise Intervention

In addition to the dietary programme, subjects in the DE group undertook a walking/jogging exercise programme comprising four to five exercise sessions per week, with the exercise intensity progressing from 25–30 min at 60–65% HR_{max} during the first week to 35–40 min at 65–70% HR_{max} by week 3, 50–55 min at 70–75% HR_{max} by week 7 and 55–60 min at 75–80% HR_{max} by week 12. The training heart rate was calculated based on the HR_{max} achieved in the treadmill test that was conducted at week 0, and personal heart rate monitors (Polar Beat; Polar Electro) were provided and worn for all sessions to assist subjects in exercising at the appropriate heart rate. Subjects were required to

participate in a minimum of one group exercise session each week that was monitored by a qualified exercise trainer, the remaining sessions were conducted at a place of the subject's own choosing either in groups or individually. Subjects were required to keep a training diary of the date, duration and heart rate (intensity) for each training session, and compliance with non-monitored sessions was discussed with the exercise trainers weekly. Only data from subjects who completed at least 75% of the exercise programme were included for analysis. Compliance with the exercise programme was based on total minutes from completed exercise sessions; an exercise session was deemed to be complete only if the session was 20 min or longer in duration and performed with an average heart rate of no less than 15 beats/min below the prescribed intensity. Apart from the exercise intervention in the DE group, all subjects were required to maintain their usual patterns of physical activity during the study period. Compliance with this request was discussed during fortnightly visits to the clinical research unit.

Biochemical Analysis

Fasting blood samples were collected from a forearm vein into tubes containing no additive for lipids, sodium fluoride/EDTA for glucose and insulin, lithium heparin for total antioxidant capacity and potassium/EDTA for HbA1c and MDA measurements. Biochemical assays were performed in a single assay at the completion of the study except for HbA1c, which was analysed after each test at a certified laboratory (IMVS, Adelaide, Australia) using high-performance liquid chromatography (between-assay Coefficient of variation (CV) = 3.6%). An automated analyser (Hitachi 902; Hitachi Science Systems, Ibaraki, Japan) was used to measure lipids and glucose using enzymatic kits (Roche Diagnostics, Indianapolis, IN, USA) (within-assay CV's: total cholesterol = 0.8%, HDL = 0.9% and triglycerides =1.5%). Plasma TAS was measured using a colorimetric kit (Randox Laboratories, Ardmore, UK) (within-assay CV = 0.5%). The Friedewald equation was used to calculate LDL concentrations [37]. Insulin was measured in duplicate using a commercial enzyme-linked immunoassay kit (Mercodia AB, Uppsala, Sweden) (within-assay CV = 3.4%). The computerized homeostatic model assessment (HOMA2-IR) was used as a surrogate measure of insulin resistance based on fasting glucose and insulin concentrations [38,39]. Plasma MDA was measured in triplicate using a modification of the high-performance liquid chromatography technique described by Fukunaga *et al.* [40] (CV = 6.4%, within-subject triplicate assay variability). The 24-h urine was analysed for nitrate/nitrite metabolite excretion (NOx) using a colorimetric assay (780001; Cayman Chemical, Ann Arbor, MI, USA) (within-assay CV = 2.7%).

Statistical Analysis

Statistical analyses were performed using SPSS for Windows (version 14.0, SPSS, Chicago, IL, USA). Unpaired *t*-tests were used to assess differences at baseline. Repeated measures analysis of variance were used to evaluate treatment effects with time (i.e. weeks 0 and 12) using time as the within-subjects factor and treatment as the between-subjects factor. Analysis of covariance was used to control for baseline differences where appropriate. Pearson's correlation coefficients were used to determine the relations of the changes between variables. Data are presented as means ± s.e.m. Effect sizes (Eta^2) and 95% confidence intervals are also given. Statistical significance was set at $p < 0.05$.

Results

Subjects and Medications

Of the 37 subjects, 29 completed the study (D = 10 males/6 females, DE = 6 males/7 females; tables 1 and 2) (figure 1). Six subjects withdrew (one prior to study commencement, three because of personal commitments, one because of illness unrelated to the study and one lost to follow-up), and two were excluded because of non-compliance with the exercise programme (failure to complete 75% of the minimum required time spent exercising at the prescribed intensity). A number of subjects were taking antihypertensive, hypoglycaemic and lipid-lowering agents. Of the 29 subjects who completed the study, 19 were taking oral hypoglycaemic medication (D = 12 and DE = 7), nine were taking hypertensive medication (D = 4 and DE = 5) and 7 (D = 5, DE = 2) were using lipid-lowering medication. Ten subjects (D = 4 and DE = 6) were not taking any medication for diabetes, and none of the subjects required insulin. Throughout the study, four subjects reduced their oral hypoglycaemic medication (D = 3 and DE = 1). Subjects on antihypertensive or lipid-lowering medication were asked to maintain the same dose throughout the study, and the dosages of these medications were not changed during the study except one subject in the DE group who had reduced their antihypertensive medication. No adverse events were reported for either of the treatment groups.

Table 1 Anthropometric variables, blood pressure, aerobic capacity and endothelial function at baseline (week 0) and in response to a 12-week intervention of weight loss or weight loss plus exercise

	Baseline				Change from baseline				
	D	DE	Difference (95% CI)	p value	D	DE	Difference (95% CI)	p value	Eta^2
Age (years)	53.0 ± 1.8	51.7 ± 2.4	−1 (−7 to 5)	0.66					
BMI (kg/m^2)	34.6 ± 1.2	33.6 ± 1.3	−1.0 (−4.6 to 2.7)	0.59	−3.1 ± 0.3*	−2.9 ± 0.3*	0.2 (−0.5 to 1.0)	0.55	0.014
Weight (kg)	105.5 ± 5.2	96.3 ± 3.2	−9 (−22 to 4)	0.16	−9.4 ± 0.8*	−8.2 ± 0.8*	1.1 (−1.3 to 3.5)	0.34	0.034
Waist circumference (cm)	116.3 ± 3.9	108.0 ± 2.5	−8.3 (−18.3 to 1.8)	0.10	−9.9 ± 0.9*	−10.7 ± 1.0*	−0.9 (−3.6 to 1.9)	0.52	0.015
Systolic blood pressure (mmHg)	136 ± 3	132 ± 5	−4 (−16 to 8)	0.48	−11 ± 3‡	−4 ± 3‡	7 (−1 to 14)	0.09	0.104
Diastolic blood pressure (mmHg)	78 ± 3	73 ± 3	−6 (−14 to 3)	0.19	−5 ± 2‡	−2 ± 2‡	3 (−2 to 9)	0.24	0.050
Fat mass (%)	35.79 ± 0.71	34.49 ± 1.01	−1.30 (−3.92 to 1.31)	0.32	−2.03 ± 0.53*	−2.11 ± 0.29*	−0.08 (−1.45 to 1.29)	0.91	<0.001
FMD (%)	2.45 ± 0.88	4.20 ± 1.24	1.75 (−1.32 to 4.81)	0.25	0.03 ± 0.26	−0.52 ± 1.06	−0.55 (−2.65 to 1.54)	0.59	0.011
GTN (%)	14.94 ± 1.74	17.06 ± 1.84	2.12 (−3.09 to 7.33)	0.41	1.03 ± 1.22	2.42 ± 1.50	1.39 (−2.56 to 5.33)	0.48	0.021
VO_{2peak} (ml/kg/min)	23.25 ± 0.96	26.08 ± 1.50	2.82 (−0.86 to 6.50)	0.13	1.74 ± 0.59*	4.16 ± 1.05*,§	2.42 (−0.01 to 4.84)	0.05	0.145

BMI, body mass index; CI, confidence interval; D, diet only; DE, diet plus exercise; Eta, effect size; FMD, flow-mediated dilatation; GTN, glyceryl trinitrate.
Data are means ± s.e.m.
*$p < 0.001$, ‡$p < 0.05$ significantly different from week 0, §$p < 0.05$ significant time × treatment interaction for change in DE compared to D.

Table 2 Blood lipids, glucose, HbA1c, insulin, total antioxidant status, malondialdehyde and 24-h urinary nitrate/nitrite excretion at baseline (week 0) and in response to a 12-week intervention of weight loss or weight loss plus exercise

	Baseline				Change from baseline				
	D	DE	Difference (95% CI)	p value	D	DE	Difference (95% CI)	p value	Eta^2
Glucose (mmol/l)	10.48 ± 0.62	10.54 ± 1.24	1.31 (−2.63 to 2.74)	0.97	−2.67 ± 0.71*	−2.49 ± 0.69*	0.18 (−1.87 to 2.23)	0.86	0.001
Insulin (mU/l)	17.74 ± 3.21	13.06 ± 2.27	−4.69 (−13.05 to 3.68)	0.26	−7.44 ± 2.15	−5.11 ± 2.10	1.31 (−4.53 to 7.14)	0.65	0.008
HBA1c (%)	8.17 ± 0.46	7.55 ± 0.44	−0.62 (−1.95 to 0.70)	0.34	−1.67 ± 0.44*	−1.17 ± 0.22*	0.50 (−0.59 to 1.59)	0.36	0.032
HOMA2-IR	2.36 ± 0.43	1.81 ± 0.27	−0.55 (−1.64 to 0.53)	0.31	−0.93 ± 0.20*	−1.07 ± 0.31*	0.14 (−0.66 to 0.95)	0.69	0.005
Cholesterol (mmol/l)									
Total	4.33 ± 0.21	4.74 ± 0.34	0.41 (−0.37 to 1.19)	0.29	−0.50 ± 0.21†	−0.47 ± 0.15†	0.03 (−0.53 to 0.58)	0.93	<0.001
HDL	1.07 ± 0.07	1.25 ± 0.08	0.18 (−0.04 to 0.40)	0.11	0.02 ± 0.04	0.03 ± 0.05	0.01 (−0.12 to 0.13)	0.94	<0.001
LDL	2.21 ± 0.21	2.35 ± 0.35	0.32 (−0.49 to 1.14)	0.42	−0.23 ± 0.19‡	−0.22 ± 0.10‡	0.02 (−0.44 to 0.48)	0.94	<0.001
Triglycerides (mmol/l)	2.34 ± 0.31	2.04 ± 0.32	−0.30 (−1.22 to 0.63)	0.51	−0.97 ± 0.24*	−0.72 ± 0.29*	0.25 (−0.52 to 1.02)	0.51	0.017
TAS (Trolox equivalents, mmol/l)	1.57 ± 0.02	1.58 ± 0.04	0.01 (−0.08 to 0.09)	0.87	0.03 ± 0.02	0.02 ± 0.02	−0.01 (−0.07 to 0.05)	0.72	0.006
Malondialdehyde (μmol/l)	0.81 ± 0.06	0.82 ± 0.12	0.13 (−0.25 to 0.28)	0.91	−0.22 ± 0.07*	−0.21 ± 0.06*	0.02 (−0.19 to 0.22)	0.87	0.001
24-h urinary nitrate/nitrite (mmol)	1.05 ± 0.11	1.29 ± 0.14	0.25 (−0.12 to 0.61)	0.18	0.91 ± 0.24†	0.90 ± 0.41†	−0.01 (−0.95 to 0.93)	0.98	<0.001

Eta, effect size; HbA1c, glycated haemoglobin; HOMA2-IR, homeostatic model assessment 2 of insulin resistance; TAS, total antioxidant status.
Data are means ± s.e.m.
*$p < 0.001$, †$p < 0.01$ and ‡$p < 0.05$ significantly different from week 0.

Body Weight and Composition

There was an overall mean weight loss of 8.8% ($p < 0.001$ for time), and the magnitude of weight loss was not different between treatment groups ($p = 0.34$) (table 1). Similarly, BMI, percentage body fat and waist circumference were reduced by week 12 ($p < 0.001$), with no difference between treatments ($p > 0.55$).

Exercise Adherence and Aerobic Capacity

At week 0, there was no difference between groups in aerobic fitness as measured by VO_{2peak} (table 1). DE had shown a significant increase in VO_{2peak} by week 12 compared with D during the study. Two subjects (D) did not satisfy the criteria for a maximal treadmill test and were removed from this section of the analysis. Compliance with the exercise programme (based on total exercise duration at no more than 15 beats/min below prescribed intensity for any given session) for subjects who successfully completed the study was 111% of the minimum-prescribed exercise duration.

Cardiovascular Risk Markers

Both D and DE significantly improved blood pressure (table 1), but the improvements were not different between treatments, with an overall net reduction of 7.8 ± 1.9 mmHg in systolic and 3.4 ± 1.4 mmHg in diastolic pressure.

Weight loss was associated with significant improvements in a number of metabolic risk parameters, with reductions in waist circumference, fasting glucose, insulin, triglycerides, total cholesterol, LDL and HOMA1-IR at week 12 in both groups (table 2). There was no difference between the treatments for these parameters. Reductions in waist circumference correlated with reductions in systolic blood pressure ($r = 0.38$ and $p < 0.05$), diastolic blood pressure ($r = 0.47$ and $p < 0.05$) and LDL cholesterol ($r = 0.45$ and $p < 0.05$) and increases in aerobic capacity ($r = -0.44$ and $p < 0.05$) and NO bioavailability ($r = -0.46$ and $p < 0.05$).

Total Antioxidant Capacity, Oxidative Stress and Urinary NOx

There was a small but non-significant increase in TAS with both treatments ($p < 0.08$; table 1). Both D and DE resulted in significant reductions in plasma MDA ($p < 0.001$) and increases in urinary nitrite/nitrate ($p < 0.01$), with no differential effect between treatments (table 2). Correlates of change in plasma MDA were change in plasma glucose ($r = 0.97$ and $p < 0.001$), change in HbA1c ($r = 0.72$ and $p < 0.001$), change in HOMA2-IR ($r = 0.50$ and $p = 0.006$) and change in systolic blood pressure ($r = 0.41$ and $p = 0.03$). Change in weight, BMI, diastolic blood pressure, blood lipids, insulin, TAS or 24-h urinary nitrate/nitrite did not correlate with change in plasma MDA nor was there any correlation between changes in TAS and changes in urinary nitrate/nitrite.

Flow-mediated Dilatation

There were no differences in resting arterial diameter between groups at week 0 or 12 ($p = 0.47$). At week 0, there was no difference in brachial artery FMD between the groups ($p = 0.25$), and no change was observed in FMD with either treatment ($p = 0.59$; table 1). Similarly, the endothelium-independent vasodilatory response to GTN was not different between the groups at week 0 (D 14.9 ± 1.7, DE 17.1 ± 1.8 and $p = 0.41$) and did not change significantly in either group after the intervention ($p = 0.48$).

Controlling for small non-significant differences in baseline characteristics did not influence the statistical results for any outcome measures.

Discussion

The main finding of this study was that weight loss with or without aerobic exercise training in overweight and obese patients with type 2 diabetes improved a number of cardiovascular disease risk markers, reduced oxidative stress and increased NO availability but had no effect on FMD.

Brachial artery FMD and GTN responses in our subjects were similar to those previously reported in patients with type 2 diabetes [24,41], which is significantly lower compared with normal populations [41], indicating impaired endothelial function. In the present study, we found no change in FMD despite considerable weight loss. Although we did not have a specific control group for weight loss, this finding agrees with other well-controlled studies in non-diabetic subjects [12], including data from our laboratory [13], which failed to show any improvement in FMD despite improvements in several cardiovascular risk factors following moderate weight loss (~6.5%) over a 3-month period. While some other studies have shown improvements in FMD with weight loss [17,42–44], they used either a very low–calorie diet that resulted in very rapid weight loss [17], sibutramine therapy [42] or surgically induced weight loss in severely obese patients [43,44]. Hence, the positive

effect on FMD may have resulted from some aspect of the treatment other than weight loss. The role of weight loss for improving FMD in overweight and obese populations with heightened metabolic risk including patients with type 2 diabetes requires further clarification. Moreover, the longer term effects of weight loss on endothelial function in type 2 diabetes also warrant further investigation.

In a rat model of type 2 diabetes, Sakamoto *et al.* [25] showed that 16 weeks of exercise training rather than caloric restriction improved endothelial function. A number of human studies have shown that exercise training can improve endothelial function when assessed by FMD [21,22]. While ultrasound assessment of brachial artery FMD is associated with biological and measurement variability, it has been shown to be sufficiently sensitive that it can detect relatively small changes with limited sample sizes [42]. In this study, we had sufficient power to detect a change in FMD of 4% ($p = 0.05$ and power $= 0.8$), which is less than the approximate Eta^2 that has been observed previously with exercise training in type 2 diabetes [23]. In the subjects in the DE group, VO_{2peak} increased by about 16%, which is comparable with the magnitude of improvement reported in other studies examining patients with type 2 diabetes after short-term (8–12 weeks) aerobic exercise training [45]. However, our results showed no influence of training on FMD. No change in peak oxygen consumption in the D group suggests that subjects in this group maintained their habitual activity levels. Miche *et al.* [46] also showed that exercise training provided no improvement in FMD for patients with type 2 diabetes and chronic heart failure in whom endothelial function was impaired. Similarly, Middlebrooke *et al.* [47] also found no improvement in endothelial function with 6 months of exercise training in type 2 diabetic patients; however, they did not use FMD to assess endothelial function and observed no difference in VO_{2peak} during the intervention compared with the sedentary control group. In contrast, Hamdy *et al.* [23] showed improved FMD following an energy-restricted diet (~7% weight loss) combined with a moderate intensity exercise programme (60–80% HR_{max}). However, this study lacked an appropriate control group, but perhaps more importantly, the initial FMD response reported by Hamdy *et al.* was not different from that of the non-diabetic controls assessed, suggesting that the particular patient group studied did not have significant ED. It is possible therefore that the improvements in FMD reported by Hamdy *et al.* may have occurred because FMD was relatively normal in the patients they studied, whereas FMD was significantly impaired in the patients in the current study and in other studies, which have also shown no improvement of FMD [46].

Maiorana *et al.* [24] showed that an 8-week physical exercise programme improved FMD in patients with type 2 diabetes. However, a training regime that combined both aerobic and resistance training was used, and whether training mode influences endothelial function is not known. It is well recognized that aerobic training increases blood flow under relatively moderate pressure for prolonged periods of time, while resistance training increases blood flow for short periods of time under much higher pressure [48], so resistance training may have provided a greater shear stress stimulus for the production of NO by the endothelium with a resultant improvement in FMD. While Maiorana *et al.* [24] were able to show an improvement in FMD with exercise, they also showed that the improvements were independent of changes in cardiovascular risk factors [24,49]. This is consistent with other studies that have found no correlation between changes in FMD and known metabolic risk markers following chronic exercise training [21,46], suggesting that these risk factors and FMD are regulated independently.

Obesity and type 2 diabetes are associated with elevated oxidative stress [50]. MDA is the end product of lipid peroxidation by reactive oxygen species and is frequently used as a biomarker of oxidative stress [51]. This is the first known study to show a significant reduction in MDA levels following weight loss induced by caloric restriction in type 2 diabetes, a finding that is consistent with previous studies in other obese populations [32,52]. However, whether this was a direct effect of energy restriction and weight loss *per se* or whether the reduction in MDA was the consequence of other factors changed by the dietary regime cannot be determined. There was a high correlation between the change in MDA and plasma glucose levels, which is supported by other investigations in type 2 diabetes [27,53]. Reductions in MDA were also associated with reductions in insulin resistance and blood pressure. Our results are in agreement with Roberts *et al.* [33] who found a 3-week combined diet and exercise programme resulting in ~4% weight loss, reduced oxidative stress and increased NO production in men with type 2 diabetes. It is well established that weight loss is associated with significant improvements in the control of cardiovascular disease risk factors and that reductions in plasma MDA levels have been related to weight loss [52]. Taken together, this suggests that the reduction in oxidative stress in the present study was most likely a result of the improved metabolic profile and glycaemic control associated with weight loss. In addition, in the

present study, the decrease in MDA levels was not accompanied by a change in total antioxidant capacity. This further suggests that the lowered oxidative stress was not principally because of an enhanced removal of reactive oxygen species but was more likely to reflect a reduced reduction of reactive oxygen species resulting from the downregulation of the polyol pathway, glucose auto-oxidation, advanced glycation end products, protein kinase C or NADPH oxidase activation [54]. Lipid peroxidation is believed to be involved in oxidative modifications of LDL that is associated with atherogenesis [55] and is also thought to be an important factor in DNA damage and cell death [32]. Therefore, although the improvement in oxidative stress in the present study did not result in an improvement in FMD, reductions in oxidative stress are important for improving other risk factors for cardiovascular disease [56], which highlights the clinical importance of reduced MDA levels associated with weight loss.

A 24-h urinary nitrate/nitrite is regarded as a reliable indicator of longer term changes in whole body NO production [57]. After the interventions, there was a significant increase in urinary nitrate/nitrite concentrations, which provides evidence of increased NO production. Although the source of the NO recovered in the urine could not be determined, it is likely that it was derived from the endothelium because endothelial NO is the most prevalent source of NO in any 24-h period [58]. There is evidence that insulin stimulates NO production [59] and that insulin resistance impairs endothelial NO production by reducing NO synthase activity [60]. Therefore, the reduction in insulin resistance with weight loss in the present study might have resulted in an increase in endothelial NO production. An increased availability of NO combined with reduced oxidative stress (i.e. reduced MDA) suggests that less NO could have been oxidized and inactivated, making more available for biological functions, but there was no change in FMD in the present study. This suggests that either the increased NO was derived from non-endothelium origins or some disconnect exists between increased NO availability with lifestyle modification and the dilatory response of the endothelium during FMD. The concept of a disconnect is supported by the findings of a recent study that indicated that in type 2 diabetes, FMD does not reflect changes in basal NO function [58]. Therefore, it seems that NO availability may not be the limiting factor for FMD in type 2 diabetes; instead, arterial dilatory function may be limited by structural changes within the arterial walls that limit responsiveness to NO. Our finding of an impaired dilatory response to the administration of GTN compared with the response reported in healthy subjects [61] provides evidence for such a structural limitation.

Although no improvement in FMD was observed, the lifestyle interventions used in this study were effective in achieving ~9% weight loss and substantial metabolic improvements by reducing body fat, blood pressure, waist circumference, glucose, total cholesterol, triglycerides, LDL cholesterol and insulin resistance and improving glycaemic control. These results are directly comparable with other studies that have reported reductions in cardiovascular disease risk factors in type 2 diabetes using a similar level of energy restriction over a similar period of time [62], highlighting the importance of weight loss in reducing cardiovascular disease risk in type 2 diabetes. In particular, the magnitude of reduction in HbA1c of ~1.5% observed in the present study has been associated with a ~31.5% reduction in diabetes-related mortality [63]. While exercise appeared to provide no additional benefit for the metabolic factors measured, which is in direct agreement with a recent systematic review of other short-term, randomized, controlled trials [64], it did improve physical endurance, which is in itself an independent risk factor for the prevention of disease and mortality [65].

In this study, we did not address the possible adverse renal consequences of the high-protein diet used in the weight loss programme. Previous short-term, randomized, controlled studies have shown no change or differences in renal function following the consumption of either a high-protein or a normal-protein diet either during weight loss or energy balance in patients with type 2 diabetes without overt renal dysfunction [66,67], but dietary protein may accelerate renal decline in individuals with renal insufficiency [68]. Because type 2 diabetes is often associated with compromised renal function, the use of energy-restricted higher protein diets warrants further investigation to understand the applicability of its use as a strategy for weight management in this population.

In conclusion, in overweight and obese patients with type 2 diabetes, exercise training provided no additional improvement in the metabolic profile, reduction in oxidative stress or increase in NO availability beyond that achieved through caloric restriction alone, and neither caloric restriction nor exercise training had any observable effect on FMD. Nevertheless, combining exercise with caloric restriction improved physical fitness, supporting the importance of this combined lifestyle modification for reducing metabolic and cardiovascular disease risk in patients with type 2 diabetes. Further studies are required to better characterize the role and effects of

lifestyle therapies for the management of ED in this patient group.

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