Effects of diet and serotonergic agonist on hepatic apolipoprotein B-100 secretion and endothelial function in obese men

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Summary

We studied the effects of a hypocaloric diet with or without a serotonergic agonist (dexfenfluramine, DF) on the hepatic secretion of very-low-density-lipoprotein (VLDL) apoB and endothelial function of the forearm microcirculation in 20 viscerally obese men. The kinetics of VLDL apoB were studied using an infusion of 1-[13C]-leucine. Isotopic enrichment of apo B was measured using gas-chromatography mass spectrometry, and a multicompartmental model was used to estimate kinetic functions. Forearm vaso-dilatation was measured following an ischaemic stimulus using strain-gauge plethysmography, and visceral adipose tissue mass using magnetic resonance imaging. Compared with leaner subjects, the obese men had significantly higher hepatic apoB secretion ($p < 0.05$) and lower forearm flow debt repayment ($p < 0.001$). Both treatments produced similar decreases ($p < 0.05$) in body weight, waist circumference, visceral adipose tissue and fasting plasma insulin. With diet alone, there was a significant decrease ($p < 0.05$) in the plasma concentration and pool size hepatic secretion rate of VLDL apoB, as well as a significant increase ($p < 0.05$) in post-ischaemic flow debt repayment. With diet plus DF, there were parallel responses in these variables, but only decreased forearm vascular resistance ($p < 0.05$) was statistically significant. Combining both data sets, there was a highly significant reduction in hepatic apoB secretion rate ($20.9 \pm 2.0 \text{ vs. } 14.7 \pm 1.6 \text{ mg/kg fat-free mass/day, } p = 0.005$), as well as an increase in both maximal forearm blood flow ($16.8 \pm 7.5 \text{ vs. } 22.2 \pm 8.5 \text{ ml/100 ml/min, } p = 0.006$) and flow debt repayment ($3.5 \pm 2.1 \text{ vs. } 5.4 \pm 2.8 \text{ ml/100 ml, } p = 0.01$), and a decrease in vascular resistance ($6.7 \pm 3.7 \text{ vs. } 5.1 \pm 4.4 \text{ mmHg/ml/100 ml/min, } p = 0.007$).

Obese men have increased hepatic secretion of apoB and endothelial dysfunction of the forearm microcirculation, and decreasing their visceral adipose tissue mass by diet (with or without a serotonergic agonist) improves these abnormalities. This may provide a mechanistic basis for the reduction in cardiovascular risk in obese patients who lose weight.

Introduction

Visceral obesity increases the risk of cardiovascular disease, possibly owing to insulin resistance, hypertension and dyslipidaemia, but the precise mechanisms remain undefined. We have previously shown that hepatic output of apolipoprotein B-100 (apoB), a determinant of the plasma concentrations of atherogenic lipoproteins, is elevated in visceral obesity. Endothelial dysfunction, a precursor of atherosclerosis, may also co-exist with visceral adiposity. No previous studies have investigated whether these abnormalities are reversible by reducing visceral fat. ApoB is constitutively expressed in human liver, and its metabolism is regulated post-translationally by the availability of lipid substrates. Abdominal adipocytes have a high lipolytic capacity that increases the flux of free fatty acids to the liver.
This enhances the supply of neutral lipids necessary for hepatic assembly and secretion of VLDL apoB, that may be quantified in vivo using endogenous labelling of apoB with a stable isotope. Increased hepatic synthesis of neutral lipids is associated with oversecretion of apoB measured by this technique. Insulin resistance may also contribute directly to abnormal apoB metabolism.

Endothelial dysfunction is an early feature of vascular damage due to abnormal release of vasoactive agents, in particular nitric oxide (NO), that control arterial tone, thrombogenesis and atherosclerosis. Endothelial dysfunction is seen in visceral obesity and may be due to elevated plasma levels of fatty acids, small dense LDL or apoB, or to insulin resistance. A simple in vivo test of endothelial function is the plethysmographic measurement of post-ischaemic flow-mediated dilatation of peripheral resistance vessels.

Dexfenfluramine (Df), the D-enantiomer of fenfluramine, is effective pharmacotherapy for weight loss in obese subjects. It decreases appetite centrally through inhibition of serotonin uptake in presynaptic neurons, and directly stimulates fatty acid oxidation. Df decreases visceral fat and improves insulin resistance, elevated plasma free fatty acid levels and dyslipidaemia. Because of serotonergic actions that stimulate the endothelial release of NO, Df may also improve endothelial function in coronary arterial beds. The independent effects of Df, or other serotonergic agonists, in the setting of dietary energy restriction on the increased hepatic output of apoB and endothelial dysfunction in visceral obesity, have not been previously investigated.

Methods

Patients and study design

Patients (all male) with visceral obesity (BMI ≥ 30 kg/m², waist-to-hip ratio ≥ 0.99, waist circumference ≥ 100 cm) and dyslipidaemia (fasting plasma triglyceride ≥ 1.5 mmol/l and HDL cholesterol ≤ 1.2 mmol/l) were studied. None had diabetes mellitus, genetic hyperlipidaemia, hepatic disease, proteinuria, or hypothyroidism, or had been treated with Df or other appetite suppressants. They were not taking medication known to affect plasma lipids, and none were smokers or gave a family history of hyperlipidaemia or coronary disease. The plasma transport rate of VLDL apoB and endothelial function of the forearm microcirculation in the obese subjects was compared with a group of age-matched lean men. The study was approved by the Ethics Committee at Royal Perth Hospital.

The study design was a randomized double-blind study comparing Df and placebo in obese subjects on a 1600 kcal reduced-fat diet (25% of energy from fat). After a 4-week run-in on an isocaloric diet, patients were randomized to Df or placebo for 3 months. During treatment, patients received one capsule (15 mg Df or placebo) with the evening meal for 1 week at the beginning of the treatment period and then one capsule (15 mg Df or placebo) with breakfast, and one capsule with the evening meal for the next 11 weeks. Tablet compliance was checked by tablet count. Patients were reviewed monthly. At the end of 3 months, baseline measurements were repeated.

Clinical protocol

Clinical tests

Weight and height were measured without shoes and in light clothing. Body mass index (BMI) (kg/m²) was calculated. Waist circumference (cm) was measured at the point midway between the costal margin and iliac crest in the mid-axillary line. Hip circumference (cm) was measured at the widest point around the greater trochanter. A bioelectrical impedance method using a Holtain Body Composition Analyser (Holtain Ltd) was used to estimate fat mass (FM) and fat free mass (FFM); this technique correlates well with underwater weighing and in our hands has an imprecision of < 5%. To assess glucose tolerance, a 75 g oral load of dextrose in 200 ml water was taken in the morning after a 14-h fast. Blood samples were collected through a venous cannula from an antecubital vein at 0 and 120 min for plasma glucose and insulin assays. Insulin resistance was assessed from the fasting plasma insulin and glucose concentrations using the homeostasis model.

Nutritional methods

For 4 weeks prior to randomization, patients consumed a weight maintenance diet. During this period, body weight did not vary by more than 3%. Subjects completed a 7-day food intake record at weeks 0, 4, 8 and 12. These were subsequently analysed by a dietitian using DIET4 Nutrient Calculation Software (Xyris Software) based on the Australian Food Composition Database (NUTTAB 95, Australian Government Nutrient Database). The prescribed weight loss diet provided 1600 kcal/day, with approximately 25% from fat. Patients were reviewed monthly to assess compliance with diet. They were instructed not to change physical activity during the study. Energy expenditure was estimated by a standard 7-day recall questionnaire.

Magnetic resonance imaging

At weeks 0 and 12, a magnetic resonance imaging (MRI) scan and glucose tolerance test was carried out. A 1.0 T MR scanner (Picker International) was
used to obtain 10 transverse axial images (field of view 40–48 cm, 10 mm thick) at various intervertebral levels from T10 to the pubis. Subjects lay supine in the magnet field, with arms placed straight above the head and to reduce errors caused by respiratory movements, the scans were obtained after quiet exhalation. The data for subcutaneous and visceral adipose tissue area from these intervertebral levels were calculated using software developed within the MRI department. For the purposes of this study, MRI measurements at the 4th lumbar vertebra (L4) were employed to define visceral fat.³

Stable isotope test of apoB kinetics

All patients fasted for at least 14 h and were allowed water only. Venous blood was collected for measurement of plasma concentrations of total cholesterol, triglycerides, HDL cholesterol, free fatty acids and assessment of apolipoprotein E genotype. A Teflon cannula was placed into a superficial vein of the left antecubital fossa, and 1-[¹³C]-leucine (99.5% enrichment) (Tracer Technologies) was administered by a primed (1 mg/kg), constant (1 mg/kg/h) intravenous infusion (10 h duration). Venous blood was collected via a cannula from the contralateral arm to measure the isotopic enrichment of apoB, plasma leucine and VLDL apoB concentration.

Plasma volume (litres) was measured using a standard isotopic dilution technique. Patients were injected with ¹²⁵I albumin (0.00185 mBq/kg body weight) and four blood samples were taken at 10-min intervals. The ratio of ¹²⁵I albumin administered to the activity of ¹²⁵I per litre was plotted against time, and plasma volume at time 0 was extrapolated.

Post-ischaemic vasodilatation of the forearm microcirculation

This was measured before and after weight reduction, and within 1 week of the stable isotope studies. Subjects were studied after 10 min rest in a supine position in a quiet room with ambient temperature controlled at 24°C, and after a 14-h fast. Heart rate and blood pressure were measured automatically using a Dinamap, Vital Signs Monitor 1846 SX. Measurement of forearm blood flow in the left arm was carried out using venous occlusion plethysmography with mercury-in-silastic strain gauges placed 5 cm below the antecubital crease (Hokansson, EC4 Plethysmograph connected Maclab/4e, AD Instrument). To isolate the forearm blood flow, the wrist cuffs were inflated to 200 mmHg during measurement periods. The upper arm cuff was inflated to 40 mmHg to impede venous outflow but allow arterial inflow. The cuffs were set to inflate for 10 s and deflate for 7 s. A sphygmomanometer cuff was placed over the left upper arm and inflated to 40 mmHg above systolic blood pressure for 4 min. After releasing the cuff, blood flow was measured at 1-min intervals until flow returned to baseline. Forearm blood flow was calculated by selection of the steepest gradient of each blood flow curve. The initial peak after ischaemia was defined as the maximal hyperaemic response (ml/100 ml of forearm/min). Flow debt repayment was defined as the area under the blood flow curve (ml/100 ml of forearm), equivalent to the excess blood flow during hyperaemia; the area under the curve was calculated using non-linear regression with the Prism statistical package. Minimum forearm vascular resistance was calculated as mean arterial blood pressure divided by maximal blood flow. Forearm length (l) and circumference (c) ... for measurement of plasma concentrations of total cholesterol, triglycerides, HDL cholesterol, free fatty acids and a circular cylinder. Within-observer coefficient of variation for forearm blood flow responses was < 3%.

Biochemical methods

Isolation and measurement of isotopic enrichment of VLDL apoB

A 3 ml aliquot of plasma was overlaid with 1.6 ml sodium chloride solution (d = 1.006 kg/l), and the samples were ultracentrifuged for 16 h at 147 000 g (Centrikon T-1190, Kontron Instruments). The isopropanol method was used to precipitate the apoB. In our hands, this technique is highly specific for apoB-100, and is positively associated with VLDL and plasma volume at time 0 was extrapolated. The precipitate was delipidated with ether ethanol (1:5 v/v), dried and hydrolysed. The amino acids were isolated by cation-exchange chromatography and eluted using 2 ml of 3M ammonia. The samples were derivatized using N-methyl-N-[(butylidemethylsilyl)-trifluoroacetamide in acetonitrile, and reconstituted in decane for gas chromatography-mass spectrometry (GCMS) analysis (Hewlett Packard 5890). Selected ion monitoring of derivitized samples at a mass-to-charge ratio (m/z) of 303 and 302, and using electron-impact ionization, was used to determine isotopic enrichment. Leucine enrichment was calculated using the formula:²³

\[
E(t) = \frac{R_t}{R_t + 1} - \frac{R_0}{R_0 + 1}
\]

where \( R_t \) is the \(^{13}C/^{12}C \) ratio at time \( t \) and \( R_0 \) is \(^{13}C/^{12}C \) ratio at baseline prior to the infusion of 1-[¹³C]-leucine. The coefficient of variation (CV) of this method was < 8.0% for isotopic enrichment of apoB with leucine. This was assessed by taking...
replicate samples (×4) at three time points in five of the studies.

**Quantification of VLDL apoB and other analytes**

From the onset of 1-[^13]C]-leucine infusion, plasma samples were combined to yield three pooled VLDL samples per study. VLDL apoB concentration in each pooled sample was measured using a modification of the Lowry method; interassay CV <4.5%. Conventional enzymic methods were used to measure plasma lipid concentrations. Apolipoprotein E genotyping was carried out using a standard polymerase chain reaction method. Plasma insulin was measured by an automated immunoassay (Tosoh) (CV <7.0%). Plasma glucose concentration was determined by an enzymic hexokinase reaction method using a Technicon Axon analyser (Bayer Diagnostics) (CV <3.1%). An enzymatic colorimetric assay was used to determine the concentration of free fatty acids (CV <3.0%).

**Calculation of VLDL apoB turnover rate**

\[ Z(t) = \frac{E(t)}{E(l) - E(t)} \]

where \( E(t) \) is the isotopic enrichment of VLDL apoB at time \( t \), and \( E(l) \) is the isotopic enrichment of the infusate. A multi-compartmental model was used to analyse the data. SAAM-II (SAAM Institute, University of Washington, Seattle) was used to fit the model to the data and the fractional secretion rate (FSR) of VLDL apoB (pools/day), equivalent to the fractional catabolic rate (FCR) at steady state concentration, was calculated. The model consisted of three compartments.

Compartment 1 is a plasma leucine compartment in which ^[13]C enrichment of plasma leucine was used as the forcing function for the precursor pool in the liver; compartment 2 is an adjustable intrahepatic delay compartment for the assembly and secretion of apoB; and compartment 3 is a plasma compartment for VLDL apoB secreted by the liver. This model is based on the following assumptions: (i) patients were in a metabolic steady state throughout the study; (ii) plasma leucine was the source of the leucine that was incorporated into apoB; and (iii) all apoB entered the plasma via VLDL particles. The absolute secretion rate of VLDL apoB was determined by multiplying FSR and pool size. Pool size was determined as the product of plasma volume and the mean plasma VLDL apoB concentration.

**Statistical analysis**

Data were log-transformed to normalize skewed distributions. The paired t-test was used to compare within-group changes. Between group differences were compared by the unpaired t-test and Wilcoxon signed rank test. Associations between variables were examined by linear regression analyses.

**Results**

The volunteers were middle-aged (45 ± 2.8 yrs), and of similar age in both groups. Their baseline and post-treatment data were compared with control groups of age, sex-matched, lean men who were non-smokers (mean age 46 ± 2.4 years, BMI 25 ± 1.4 kg/m², cholesterol 5.2 ± 0.6 mmol/l, triglyceride 1.1 ± 0.5 mmol/l). Table 1 shows the anthropometric characteristics before and after weight reduction with the hypocaloric diet and the hypocaloric diet plus Df. In both groups, there was a significant decrease \((p<0.001)\) in body weight, BMI, waist circumference, waist-to-hip ratio, fat mass and visceral adipose tissue area at L4. Of the subjects on placebo, six were apolipoprotein E3/E3 homozygotes and four were E4/E3 heterozygotes; one refused genetic tests. Of the subjects on Df, one was E4/E4 homozygote, six were E3/E3 homozygotes and two were E4/E3 heterozygotes. The frequency distributions of apolipoprotein E genotypes were not significantly different between the groups.

With diet alone, there was a significant reduction in total energy intake \((2600±256\ kcal vs. 1846±149\ kcal, p=0.002)\) and percentage of energy from fat \((37.3±1.6\% vs. 29.8±2.3\%, p=0.004)\), and a significant increase in percentage energy from carbohydrate \((42.5±2.0\% vs. 47.7±2.1\%, p=0.021)\). With diet plus Df, there was also a significant reduction in total energy intake \((2969±314\ kcal vs. 1937±121\ kcal, p=0.008)\) and percentage of energy derived from fat \((33.9±1.8\% vs. 28.0±1.4\%, p=0.05)\), and a significant increase in the percentage of energy from protein \((15.9±1.1\% vs. 19.8±1.1\%, p=0.004)\); dietary intakes were not significantly different between groups. There were no significant group differences in the level of physical activity before and after weight reduction.

Table 2 shows the biochemical characteristics of the patients studied. The plasma cholesterol and triglyceride concentrations were significantly higher than in the lean group \((p=0.019\) and \(p=0.006\), respectively). At week 0, the data did not differ significantly between the two groups. With diet alone, there was a significant decrease in the plasma concentrations of cholesterol, triglyceride,
and insulin; this was associated with an improvement in insulin resistance and an increase in HDL cholesterol. With Df, there was also a significant decrease in plasma cholesterol, insulin and free fatty acids. The lipid composition of VLDL did not alter significantly with weight reduction in either group, but the cholesterol:apoB ratio of VLDL increased with Df.

The tracer-to-tracee curves for plasma leucine and VLDL apoB in the two groups were similar before and after weight reduction. Representative curves for the dietary intervention group alone are shown in Figure 1. In all patients, the tracer-to-tracee ratios of plasma leucine reached steady state within 30 min, 14.7 ± 1.6 mg/kg fat-free mass/day, p = 0.005) of VLDL apoB. There were no significant correlations between the changes in hepatic secretion rate of VLDL apoB and any of the anthropometric, nutritional or biochemical variables measured. Compared with a retrospective group of 11 obese men studied twice while receiving a weight maintenance diet for 3 months, hepatic secretion of apoB fell significantly more with both interventions (change −6.2 ± 1.9 vs. 6.4 ± 4.9 mg/FFM/day, p = 0.034). Post-intervention hepatic apoB secretion was also not significantly different from the lean controls.

Table 1  Anthropometric data before and after diet or diet plus dexfenfluramine

<table>
<thead>
<tr>
<th></th>
<th>Diet (n = 11)</th>
<th>Diet plus dexfenfluramine (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>104.9 (3.3)</td>
<td>99.8 (3.5)*</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>33.7 (1.2)</td>
<td>32.1 (1.4)*</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110.2 (1.5)</td>
<td>103.1 (2.1)*</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>1.02 (0.01)</td>
<td>0.99 (0.01)*</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>37.6 (2.1)</td>
<td>31.0 (3.0)*</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>67.3 (2.3)</td>
<td>68.8 (2.6)</td>
</tr>
<tr>
<td>VAT area at L4 (cm²)</td>
<td>205.6 (15.9)</td>
<td>164.1 (15.2)*</td>
</tr>
</tbody>
</table>

Data are means (SEM). VAT, visceral adipose tissue. *p < 0.05 vs. 0 wk.

Table 2  Biochemical data before and after diet or diet plus dexfenfluramine

<table>
<thead>
<tr>
<th></th>
<th>Diet (n = 11)</th>
<th>Diet plus dexfenfluramine (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.52 (0.25)</td>
<td>5.15 (0.28)*</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>2.74 (0.41)</td>
<td>1.88 (0.25)*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.95 (0.05)</td>
<td>1.01 (0.05)*</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/l)</td>
<td>0.62 (0.11)</td>
<td>0.47 (0.08)</td>
</tr>
<tr>
<td>VLDL triglyceride (mmol/l)</td>
<td>1.47 (0.28)</td>
<td>1.08 (0.19)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.47 (0.25)</td>
<td>5.37 (0.17)</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>13.46 (1.82)</td>
<td>9.12 (1.63)*</td>
</tr>
<tr>
<td>Insulin resistance (mmol²/l²)</td>
<td>3.38 (0.58)</td>
<td>2.19 (0.40)*</td>
</tr>
<tr>
<td>Post-load glucose (mmol/l)</td>
<td>7.95 (0.68)</td>
<td>6.67 (0.48)</td>
</tr>
<tr>
<td>Free fatty acids (mmol/l)</td>
<td>1.09 (0.12)</td>
<td>1.09 (0.07)</td>
</tr>
</tbody>
</table>

* p < 0.05 vs. 0 wk; ** p = 0.06 vs. 0 wk. Data are means (SEM).
Figure 1. Rates of $^{13}$C enrichment of plasma leucine ($\bullet$) and VLDL apoB ($\bigcirc$) in the subjects before (a) and after (b) the 1600 kcal reduced-fat diet and placebo during infusion of 1-$^{13}$C leucine (mean ± SEM).

Table 3 Metabolic parameters and VLDL apoB kinetics before and after diet or diet plus dexfenfluramine

<table>
<thead>
<tr>
<th></th>
<th>Diet ($n=11$)</th>
<th>12 weeks</th>
<th>Dexfenfluramine ($n=9$)</th>
<th>0</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL apoB (mg/l)</td>
<td>82.6 (11.8)</td>
<td>57.5 (8.3)*</td>
<td>84.7 (15.4)</td>
<td>71.2 (7.0)</td>
<td></td>
</tr>
<tr>
<td>Plasma volume (l)</td>
<td>3.4 (0.2)</td>
<td>3.4 (0.2)</td>
<td>3.7 (0.1)</td>
<td>3.7 (0.2)</td>
<td></td>
</tr>
<tr>
<td>Pool size (mg)</td>
<td>281.6 (39.2)</td>
<td>197.2 (27.2)*</td>
<td>311.5 (57.9)</td>
<td>260.6 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Fractional catabolic rate (pools/day)</td>
<td>5.6 (0.8)</td>
<td>6.5 (0.9)</td>
<td>5.1 (0.9)</td>
<td>3.7 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Hepatic secretion rate (mg/day)</td>
<td>1431.9 (195.3)</td>
<td>1122.4 (159.4)*</td>
<td>1316.2 (175.2)</td>
<td>844.2 (147.9)**</td>
<td></td>
</tr>
<tr>
<td>Hepatic secretion rate (mg/kg FFM/day)</td>
<td>21.3 (2.8)</td>
<td>16.1 (2.0)*</td>
<td>20.5 (3.2)</td>
<td>12.9 (2.6)**</td>
<td></td>
</tr>
</tbody>
</table>

Data are means (SEM). FFM, fat-free mass. * $p<0.05$ vs. 0 wk; ** $p<0.06$ vs. 0 wk.

Table 4 shows the haemodynamic and forearm blood flow data before and after weight reduction with diet and diet plus Df. The volunteers were not hypertensive. Blood pressure, heart rate, forearm volume and basal blood flow were similar in both treatment groups and did not alter significantly with weight loss. Basal blood flow in the obese patients was not significantly different from that in a group of 19 lean men. Maximal forearm flow and flow debt repayment in the obese patients at baseline was significantly impaired compared with the lean controls ($16.8 \pm 1.7 \text{ vs. } 23.2 \pm 1.7, p=0.012$; $3.5 \pm 0.4 \text{ vs. } 17.1 \pm 1.4, p<0.001$, respectively). Post-ischaemic maximal forearm blood flow was similar in both the obese groups and increased by approximately 25% with weight reduction, but within-group
Table 4  Haemodynamic and forearm blood flow data before and after diet or diet plus dexfenfluramine

<table>
<thead>
<tr>
<th></th>
<th>Diet (n=11)</th>
<th>Diet (n=11)</th>
<th>Dexfenfluramine (n=9)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>12 weeks</td>
<td>0</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>125 (4.1)</td>
<td>122 (3.4)</td>
<td>126 (4.3)</td>
<td>130 (4.1)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71 (2.9)</td>
<td>69 (1.8)</td>
<td>72 (3.2)</td>
<td>73 (3.5)</td>
</tr>
<tr>
<td>Mean arterial BP (mmHg)</td>
<td>89 (3.0)</td>
<td>87 (2.0)</td>
<td>90 (3.5)</td>
<td>92 (3.5)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>64 (1.8)</td>
<td>61 (1.4)</td>
<td>63 (3.2)</td>
<td>62 (3.7)</td>
</tr>
<tr>
<td>Forearm volume (ml)</td>
<td>2086 (71.8)</td>
<td>2060 (59.8)</td>
<td>2094 (82.8)</td>
<td>2092 (79)</td>
</tr>
<tr>
<td>Basal blood flow (ml/100 ml/min)</td>
<td>3.0 (0.43)</td>
<td>2.3 (0.22)</td>
<td>2.3 (0.2)</td>
<td>2.6 (0.29)</td>
</tr>
<tr>
<td>Maximal blood flow (ml/100 ml/min)</td>
<td>17.2 (2.73)</td>
<td>21.8 (3.15)</td>
<td>16.2 (1.89)</td>
<td>22.8 (1.98)</td>
</tr>
<tr>
<td>Blood Flow debt (ml/100 ml)</td>
<td>2.8 (0.51)</td>
<td>5.0 (0.87)*</td>
<td>4.3 (0.76)</td>
<td>5.9 (0.92)</td>
</tr>
<tr>
<td>Vascular resistance (mmHg/ml/100 ml/min)</td>
<td>7.0 (1.39)</td>
<td>5.8 (1.77)</td>
<td>6.2 (0.77)</td>
<td>4.3 (0.42)*</td>
</tr>
</tbody>
</table>

Data are means (SEM). *p<0.05 vs. 0 wk.

changes just failed to reach conventional statistical significance. Flow debt repayment increased in both groups, the difference being significant only with diet-induced weight loss. There were parallel changes in these variables with Df, but only the decrease in forearm vascular resistance was significant. When data were pooled, weight loss was associated with a significant increase in both maximal forearm blood flow (p = 0.006) and flow debt repayment (p = 0.01) and with a decrease in forearm vascular resistance (p = 0.007) (Figure 2). However, the flow debt repayment following weight loss remained significantly lower than the lean group (p <0.01). The changes in basal and maximal blood flow, flow debt repayment and vascular resistance were not significantly correlated with the changes in fat mass, plasma lipids, lipoproteins or insulin concentrations, or hepatic secretion of apoB.

**Discussion**

The principal finding was that weight reduction of the order of 5% following a diet with or without Df improved both hepatic secretion of VLDL apoB and peripheral microcirculatory function in men with visceral obesity. To formally test the hypothesis that Df achieves greater improvement in these endpoints than a diet alone would have required a larger sample size. The results of this pilot study are nevertheless valuable, since they are consistent with the hypothesis that in visceral obesity weight reduction improves hepatic output of atherogenic lipoproteins and vascular dysfunction as a consequence of decreases in insulin resistance and lipid substrate supply to the liver.

Using a stable isotope technique, we have previously shown that the hepatic secretion of VLDL apoB is increased in viscerally obese subjects, unselected for having dyslipidaemia, and that this is reversible with a degree of weight reduction comparable to that achieved in the present study. Improvement in hepatic apoB secretion was probably a consequence of reduction in both lipid substrate supply to the liver and insulin resistance, resulting from loss of visceral adipose tissue mass. This may explain the hypolipidaemic effect of weight reduction. Df has previously been shown to increase fatty acid oxidation, which may account for the significantly lower plasma concentrations of free fatty acids demonstrated here. This did not, however, translate into a statistically significant decrease in hepatic apoB secretion. The 13% lower hepatic secretion of apoB with Df reflects a true population effect of Df in the setting of a hypocaloric diet, one would need to randomize 120 patients to test the null hypothesis with 80% power at the 5% level of significance. This may not be a clinically relevant study, however, given that both the interventions tested decreased hepatic apoB secretion into the reference range for lean subjects.

This is the first attempt at using post-ischaemic dilatation of the forearm as a measure of endothelial function in an intervention trial in viscerally obese subjects. Several mediators may contribute to postischaemic forearm hyperaemia, including local metabolites, adenosine, prostaglandins and NO. NO may be particularly more important in determining flow debt repayment than maximal blood flow. Forearm vasotonic responses may potentially reflect changes in the coronary circulation. The partial restoration in vasodilatory function noted with weight loss could be attributable partly to increased bioavailability of NO, as a consequence of improvements in dyslipidaemia, insulin resistance or fatty acid levels, or to reduction in adrenergic activity. The greater decrease in plasma free fatty acid levels with Df

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could account for the incremental improvement in forearm vascular resistance, given that free fatty acids impair NO synthase activity.\cite{28} We cannot, however, exclude the effect of Df on vascular function being due to a direct serotonergic effect on endothelial function.\cite{29} That apoB secretion was restored into a ‘normal range’, but flow debt was not in the present study suggests a differential effect of weight loss on these endpoints, and requires further examination.

Df was withdrawn from the market in 1997, owing to concerns of increased risk of pulmonary hypertension and valvular heart lesions associated with long-term use of the drug.\cite{29, 30} Follow-up of the nine patients participating in the present study with pulmonary function tests and echocardiography has not confirmed abnormalities, however. Risk of valvular heart disease with short-term use of Df has also recently been shown to be very low.\cite{31} Hence, the potential cardiac complications associated with short-term use of Df in obese patients may be outweighed by improvements in endothelial function and cardiovascular risk factors,\cite{32} including plasma apoB levels.

In conclusion, this pilot study suggests that weight reduction with a hypocaloric diet alone or a hypocaloric diet plus Df potentially improves both the kinetics of VLDL apoB and endothelial function in visceral obesity. Our findings should be viewed as hypothesis-generating in respect of the greater beneficial effects of similar anorectic agents on these endpoints than diet alone. Whether there are specific metabolic and vascular benefits with newer, serotonergic agents (e.g. sibutramine) for treating obesity requires investigation with, as indicated above, a larger sample size than used in the present study. More weight loss may also be required to normalize endothelial dysfunction than dyslipoproteinaemia in obesity.

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