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Dietary Intervention to Reverse Carotid Atherosclerosis

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Background—It is currently unknown whether dietary weight loss interventions can induce regression of carotid atherosclerosis.

Methods and Results—In a 2-year Dietary Intervention Randomized Controlled Trial—Carotid (DIRECT-Carotid) study, participants were randomized to low-fat, Mediterranean, or low-carbohydrate diets and were followed for changes in carotid artery intima-media thickness, measured with standard B-mode ultrasound, and carotid vessel wall volume (VWV), measured with carotid 3D ultrasound. Of 140 complete images of participants (aged 51 years; body mass index, 30 kg/m²; 88% men), higher baseline carotid VWV was associated with increased intima-media thickness, age, male sex, baseline weight, blood pressure, and insulin levels ($P < 0.05$ for all). After 2 years of dietary intervention, we observed a significant 5% regression in mean carotid VWV (-58.1 mm³; 95% confidence interval, -81.0 to -35.1 mm³; $P < 0.001$), with no differences in the low-fat, Mediterranean, or low-carbohydrate groups (-60.69 mm³, -37.69 mm³, -84.33 mm³, respectively; $P = 0.28$). Mean change in intima-media thickness was -1.1% ($P = 0.18$). A reduction in the ratio of apolipoprotein B₁₀₀ to apolipoprotein A1 was observed in the low-carbohydrate compared with the low-fat group ($P = 0.001$). Participants who exhibited carotid VWV regression (mean decrease, -128.0 mm³; 95% confidence interval, -148.1 to -107.9 mm³) compared with participants who exhibited progression (mean increase, $+89.6$ mm³; 95% confidence interval, $+66.6$ to $+112.6$ mm³) had achieved greater weight loss (-5.3 versus -3.2 kg; $P = 0.03$), greater decreases in systolic blood pressure (-6.8 versus -1.1 mm Hg; $P = 0.009$) and total homocysteine (-0.06 versus $+1.44$ μ mol/L; $P = 0.04$), and a higher increase of apolipoprotein A1 ($+0.05$ versus -0.00 g/L; $P = 0.06$). In multivariate regression models, only the decrease in systolic blood pressure remained a significant independent modifiable predictor of subsequent greater regression in both carotid VWV ($\beta = 0.23$; $P = 0.01$) and intima-media thickness ($\beta = 0.28$; $P = 0.008$) levels.

Conclusions—Two-year weight loss diets can induce a significant regression of measurable carotid VWV. The effect is similar in low-fat, Mediterranean, or low-carbohydrate strategies and appears to be mediated mainly by the weight loss-induced decline in blood pressure.

Clinical Trial Registration—<http://www.clinicaltrials.gov>. Unique Identifier: NCT00160108. (*Circulation*. 2010;121:1200-1208.)

Key Words: atherosclerosis ■ blood pressure ■ diet ■ imaging

Several recent studies suggest that lifestyle interventions can halt the progression of atherosclerosis,¹⁻³ whereas others show no effect.⁴ However, it is currently unknown whether dietary interventions can induce regression of carotid atherosclerosis, which could be detectable by B-mode and 3-dimensional ultrasound (3DUS).

Clinical Perspective on p 1208

Intima-media thickness (IMT) is a commonly used and direct assessment of early atherosclerosis and is generally considered a reliable surrogate end point of vascular outcomes.⁵ The average annual increase of IMT in untreated

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individuals is $0.015 \text{ mm}^3/\text{y}$,^{6,7} and thus requires large sample sizes and long duration of follow-up to show effects of therapy. In contrast, carotid plaque area has been shown to increase by $\approx 5 \text{ mm}^2/\text{y}$ and carotid plaque volume by $\approx 50 \text{ mm}^3/\text{y}$.⁸ Measurements of carotid arterial wall volume that include arterial plaque such as provided by 3DUS vessel wall volume (VWV) may serve as complementary measurements to IMT,⁹ exhibiting the requisite sensitivity, precision, and specificity that would permit detection of the effects of dietary intervention on changes in carotid atherosclerosis.¹⁰

In a 2-year Dietary Intervention Randomized Controlled Trial (DIRECT),¹¹ we found that Mediterranean and low-carbohydrate diets may be effective alternatives to low-fat diets for weight loss, with more favorable effects on lipids with the low-carbohydrate diet and on glycemic control with the Mediterranean diet. In the present DIRECT-Carotid study, we sought to assess whether these diets had measurable effects on established (IMT) and emerging (3DUS VWV) ultrasound measurements of carotid atherosclerosis and whether such effects could be predicted by alterations in lipoproteins and other less routinely measured cardiovascular biomarkers.

Methods

Study Population

The trial, previously described in detail,¹¹ was conducted between July 2005 and June 2007 in a research center workplace in Dimona, Israel. Eligible participants were aged 40 to 65 years with body mass index (BMI) $\geq 27 \text{ kg}/\text{m}^2$; individuals with type 2 diabetes mellitus or coronary heart disease were eligible regardless of age or BMI. Pregnant or lactating women and participants with a serum creatinine $\geq 2 \text{ mg}/\text{dL}$ ($\geq 176 \text{ }\mu\text{mol}/\text{L}$), liver dysfunction (≥ 2 -fold higher than the upper limit of normal in alanine aminotransferase or aspartate aminotransferase), intestinal problems that would prevent adherence to any of the test diets, or active cancer were excluded.

All participants in our study were allocated to the 3 diets in a random manner. To maintain balance within each diet group, we randomized by strata of sex, age (below or above the median), BMI (below or above the median), history of coronary heart disease (yes/no), type 2 diabetes mellitus (yes/no), and current use of statins (none/ <1 year/ ≥ 1 year). We stratified by use of statins to enable us to assess possible modification by statins for any changes in carotid atherosclerosis.

Among the 322 DIRECT participants, retention was 95% after 1 year and 85% after 2 years. For technical and budgetary reasons, we limited this substudy to the 175 participants with 3DUS and IMT evaluations within the period of baseline measurements. We further excluded 35 sets for which the observers noted inadequate image quality in 1 of the carotid sides or time points, yielding a total of 140 completed sets (4 images: 2 time points, right and left carotid sides) to evaluate change in carotid VWV after 2 years of intervention. Characteristics of the 140 participants with high-quality VWV imaging were similar to other DIRECT participants with respect to age, sex, and the final outcomes: change of weight and systolic blood pressure.

Participants received no financial compensation or gifts for participating. The study was approved and monitored by the Human Subjects Committee of Soroka Medical Center and Ben-Gurion University. Each participant provided written informed consent.

Interventions

The interventions were reported in detail previously.¹¹ Food diaries obtained from a subset of participants during the weight loss phase¹² showed distinct differences between low-carbohydrate and low-fat diet in fat intake (41% versus 26% in the low-fat diet), carbohydrate intake

(28% versus 48% in the low-fat diet), and dietary cholesterol intake (358 versus 174 mg/d in the low-fat diet). During the 2-year intervention, validated¹³ food frequency questionnaires revealed that the Mediterranean diet group consumed the highest dietary fiber and monounsaturated/saturated fat ratio ($P < 0.05$). The low-carbohydrate diet group consumed the least carbohydrates and the most fat, protein, and cholesterol and had a higher percentage of positive urinary ketone determinations ($P < 0.05$). Caloric deficit was similar among groups.¹¹ After analyzing the recipes using the Israeli nutritional database, we color-coded the labels of all food dishes daily served in the workplace cafeteria for each diet type to promote adherence.

Carotid Image Acquisition Parameters

High-resolution B-mode images were acquired with an ultrasound system optimized for carotid imaging (ATL HDI 5000, Philips, Bothel, Wash) with a 50-mm L12 5-MHz transducer with a central frequency of 7 MHz. Ultrasound imaging parameters such as gain, time depth compensation, and focal points were optimized for each patient by the sonographer, taking into consideration the neck size, carotid anatomy, and tissue depth penetration required. Scans of 3DUS were acquired by freehand scanning along the neck for a distance of $\approx 5 \text{ cm}$. Probe orientation and position were tracked with a magnetic tracking system, and 2-dimensional images were reconstructed into a 3D volume with the use of the 3D Echotech system (GE Medical Systems, Milwaukee, Wis). All scans were performed at baseline and at the end of the 2-year intervention period at the Soroka Medical Center by 3 qualified ultrasound technicians who had undergone previous training on this technique at the Robarts Research Institute in London, Ontario, Canada. Allocation of participants to each technician at the baseline examination was arbitrary and unrelated to the specific intervention group. The technicians were blinded with respect to the participant's dietary treatment. At the 2-year follow-up study, 70% percent of participants were successfully referred to the same technician who performed the examination at baseline.

3DUS VWV Measurement

Analysis of the IMT and 3D VWV was validated¹⁴ and performed at the Imaging Research Laboratories at Robarts Research Institute (London, Ontario, Canada). Allocation of images to the observers performing the measurements was randomized with each of 3 observers receiving approximately equal numbers of patients from each treatment group; the observers were also blinded to subject identity, time point, and treatment allocation. Images from both time points were analyzed simultaneously as a pair on separate 19-inch monitors together to facilitate the location of the carotid bifurcation. The lumen-intima and media-adventitia boundaries of the carotid artery were manually segmented in the transverse plane, although the image volume could be manipulated in any plane to verify the segmentation, as described previously.¹⁵ With the use of a WACOM Intuos pen and tablet (Wacom Technology Corporation, Vancouver, Wash), the common carotid artery was segmented 10 mm proximally from the bifurcation in 1-mm increments. Similarly, measurement of the internal carotid artery was performed distally into the internal carotid artery to a maximum of 5 mm. The area enclosed by each segmented boundary was multiplied by the interslice distance, and all areas were summed to obtain the final volume of the lumen and vessel wall. VWV was calculated as the difference between the volume enclosed by the arterial wall and the volume of the lumen, summed across both sides. It is important to note that lumen was delineated on the basis of echolucency with all viewing planes in the 3D volume to help to delineate the blood-intima boundary, minimizing the potential to include hypochoic and echolucent plaque within the manually segmented lumen volume. In a previous 3-month trial⁸ among 38 patients with carotid stenosis $>60\%$ (21 placebo and 17 atorvastatin), the mean (SD) change in plaque volume was $16.81 (\pm 74.10) \text{ mm}^3$ for placebo and $-90.25 (\pm 85.12) \text{ mm}^3$ for atorvastatin ($P < 0.0001$). More recent data¹⁵ showed that 3DUS VWV increased by $70 \pm 140 \text{ mm}^3$ in the placebo group and decreased by $30 \pm 110 \text{ mm}^3$ in the atorvastatin group ($P < 0.05$).

IMT Measurement

IMT was measured from lateral image planes extracted from the 3DUS images. One side was randomly selected for analysis from each subject, and images for IMT measurement from both time points were generated from video by the same observer to maximize observer consistency. Three observers were blinded to treatment and time point. In the 3DUS image volume, the carotid bulb was marked in the cross-sectional view, and a lateral plane parallel to the axis of the common carotid artery was isolated. The contrast and brightness were adjusted to maximize the visibility of the intima-media layer and the contrast with the lumen. Horizontal and vertical calibration lines with a length of 30 mm were drawn, and the image was saved as a 24-bit bitmap. Bitmaps were imported into Prowin 24.0 (Medical Technologies International Inc, Palm Desert, Calif) for analysis. After calibration, measurements were generated from a 10-mm segment of the far wall of the common carotid artery, 5 mm from the marked carotid bulb. A different segment was chosen if plaque was identified in that location (defined as focal thickening >1 mm) or if that segment was not included in the 3D image volume. Approximately 10 to 15 points were placed along the media-adventitia and the intima-lumen boundary with a mouse-driven cursor. For the measurement of IMT, the distance between the curves defined by the points was generated. Measurements were repeated 3 times per image, and the mean was calculated. During the image processing and measurement stages, images were removed from the analysis if there was no clear intima-media layer present for measurement.

Blood and Clinical Measurements

Blood biomarkers were analyzed in Leipzig University Laboratories, Leipzig, Germany. A blood sample was drawn by venipuncture at 8 AM, after a 12-hour fast, at baseline and at 6 and 24 months and stored at -80°C . Apolipoprotein A1 (apoA1) and apolipoprotein B₁₀₀ (apoB₁₀₀) were determined in serum by immunoturbidimetric assays (Tina-quant apoA1 version 2 and Tina-quant apoB₁₀₀ version 2, Roche, Germany) on an automated Cobas c 501 analyzer (Roche, Germany). The coefficients of variation were between 1.0% and 4.7% for apoA1 and between 1.1% and 3.1% for apoB₁₀₀. Lipoprotein(a) [Lp(a)] was determined in serum by a particle-enhanced immunoturbidimetric assay [Tina-quant Lp(a) (Latex), Roche, Germany] on an automated Cobas c 501 analyzer. The coefficients of variation were between 0.8% and 2.2%. Plasma total homocysteine was determined by high-pressure liquid chromatography according to the manufacturer's instructions (Chromsystems, Munich, Germany), with a coefficient of variation of 5.1% at 10.6 $\mu\text{mol/L}$ and 3.6% at 20.4 $\mu\text{mol/L}$. Measurements of additional biomarkers were reported previously.¹¹ Body weight was measured without shoes to the nearest 0.1 kg every month. Height was measured to the nearest millimeter with the use of a wall-mounted stadiometer at baseline for BMI determination. Blood pressure was determined every 3 months with the use of an automated system (Datascop acutor 4), after 5 minutes of rest by the subject. Clinic and laboratory staff members were blinded to treatment assignment.

Statistical Analysis

We calculated means of baseline characteristics across tertiles of the 140 participants ($n=46, 46, 48$ for the lower, median, and higher tertiles, respectively) of carotid VWV and evaluated the P for trend after adjusting for age. To evaluate the repeated blood measurements over time, we estimated generalized estimating equations to account for the nonindependence of the same bioindicator in the same individual over time. We compared the within-person change from baseline in each diet group using pairwise comparisons. We used group t tests to compare participants with a regression or null change of the carotid VWV ($\delta \leq 0$) with those with progression ($\delta > 0$). In a sensitivity analysis, we repeated the univariate comparison after excluding 14 participants with minor changes of between -2% and $+2\%$ in carotid VWV to avoid misclassification bias. We performed a multivariable regression model to predict change in carotid VWV and IMT as continuous variables. We included the covariates age,

sex, diet group, and baseline carotid VWV or IMT and the univariate significant predictors of changes in weight, systolic blood pressure, apoA1, and total homocysteine, taking into account the level of change in the first time point, whereas it was associated with significant subsequent changes in carotid VWV in univariate models. We used SPSS16 (SPSS Inc, Chicago, Ill) and STATA9 (StataCorp LP, College Station, Tex) for the statistical analysis.

Drs Shai, Spence, and Bolotin had full access to and take full responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and agree to the manuscript as written.

Results

Population Characteristics Across Baseline Carotid VWV

The mean age of the DIRECT-Carotid study population was 51 ± 6 years, with an 88% male cohort, mean body mass of 90 ± 13 kg, and BMI of 30.4 ± 3.2 kg/m^2 . At baseline, mean carotid VWV was 942 ± 255 mm^3 , and mean IMT was 0.817 ± 0.17 mm. Overall, 26% of subjects were using lipid-lowering therapy (20% statins), and 31% used antihypertensive therapy. During the study, there was little change in use of medications, with no significant differences among groups.¹¹ None of the characteristics exhibited significant differential distribution among dietary groups (data not shown). At baseline (Table 1), male sex, increased age, increased weight, and increased systolic and diastolic blood pressure were all significantly associated with higher baseline carotid VWV levels, and among biomarkers, only increased level of baseline fasting insulin was associated with increased VWV (age-adjusted P for trend across tertiles < 0.05 for all). Similar associations were observed in an analysis of participants stratified by use of statins (data not shown).

Two-Year Changes of Biomarkers, Carotid VWV, and IMT

Changes in biomarkers at 6 and 24 months among the 140 DIRECT-Carotid study participants are shown in Figure 1. ApoA1 (Figure 1A) increased significantly (0.09 ± 0.14 g/L; $P < 0.001$) within all diet groups, with a greatest increase after 6 months in the low-carbohydrate group ($P < 0.05$) compared with the low-fat group. Levels of apoB₁₀₀ (Figure 1B) increased slightly in the low-fat group. The change was significantly different from the decrease in the low-carbohydrate group after 6 months only ($P = 0.04$). Levels of Lp(a) (Figure 1C) decreased significantly within the Mediterranean group only (-0.08 ± 0.21 g/L; $P = 0.047$), with no significant differences between the diet groups. No material changes were observed in total homocysteine (Figure 1D). Overall, after 2 years, a reduction in the ratio of apoB₁₀₀ to apoA1 (Figure 1E) was observed in the low-carbohydrate group compared with the low-fat group (-0.08 ± 0.23 versus 0.00 ± 0.19 units; $P < 0.01$ at 6 months, $P < 0.08$ at 24 months, and an overall $P = 0.001$ for the interaction between diet group and time).

Carotid VWV was measured at baseline and after 2 years, as illustrated in Figure 2A, permitting calculation of the dynamics of carotid vessel wall and plaque atherosclerosis during the trial. After 2 years of dietary intervention, there was a significant ($P < 0.001$) overall 4.9% regression (-58.07 mm^3 ; 95% confidence interval [CI], -80.98 to -35.14 mm^3) in the carotid

Table 1. Baseline Characteristics of the DIRECT-Carotid Study Population Across Tertiles of Baseline Carotid VWV

Baseline Characteristics	Entire Group (n=140)	Tertiles of Baseline Carotid VWV		
		Lowest	Middle	Highest
Carotid VWV, mm ³	941.50 (255.33)	685.39 (96.35)	904.40 (48.71)	1222.49 (188.32)
IMT, mm	0.817 (0.17)	0.777 (0.16)	0.759 (0.14)	0.909 (0.17)†
Assignment to dietary intervention group, %				
Low-fat	35	33	39	33
Mediterranean	39	47	33	35
Low-carbohydrate	26	20	28	32
Age, y	51.43 (6.15)	49.10 (6.56)	51.41 (5.88)	53.52 (5.36)†
Men, %	88	74	91	98†
Weight, kg	90.21 (13.05)	86.07 (14.27)	90.14 (10.68)	94.62 (12.56)†
Blood pressure, mm Hg				
Systolic	130.55 (13.57)	126.45 (12.50)	127.89 (13.31)	136.54 (12.66)†
Diastolic	79.31 (9.24)	77.43 (9.10)	77.36 (8.54)	82.583 (9.19)*
Use of statins, %	20	20	13	27
Use of antihypertensive therapy, %	31	24	35	33
Current smokers, %	12.9	19.6	13.0	6.3
Blood biomarkers				
Fasting insulin, μU/mL	13.93 (8.20)	12.98 (7.19)	12.54 (6.07)	15.84 (10.16)*
HOMA-IR	3.02 (2.07)	2.94 (2.32)	2.64 (1.41)	3.42 (2.28)
LDL-C, mg/dL	118.53 (32.97)	115.91 (37.90)	123.70 (30.29)	117.10 (32.08)
HDL-c, mg/dL	39.39 (9.70)	40.14 (11.01)	39.41 (8.58)	38.02 (9.22)
LDL-c to HDL-c ratio	3.15 (1.07)	3.04 (1.23)	3.24 (0.94)	3.23 (1.07)
Fasting triglycerides, mg/dL	158.27 (59.25)	160.16 (73.49)	149.55 (46.44)	166.77 (56.84)
ApoB ₁₀₀ , g/L	0.83 (0.15)	0.820 (0.22)	0.84 (0.17)	0.84 (0.17)
ApoA1, g/L	1.38 (0.21)	1.37 (0.22)	1.38 (0.20)	1.35 (0.20)
ApoB ₁₀₀ to apoA1 ratio	0.54 (0.25)	0.53 (0.32)	0.55 (0.24)	0.55 (0.22)
Lp(a), g/L	0.29 (0.23)	0.32 (0.27)	0.28 (0.22)	0.26 (0.18)
Total homocysteine, μmol/L	14.84 (4.82)	15.45 (5.56)	14.31 (2.99)	14.46 (5.27)

Data are expressed as mean (SD) unless indicated otherwise. HOMA-IR indicates homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; and HDL-C, high-density lipoprotein cholesterol.

**P*<0.05, †*P*<0.01 for age-adjusted *P* for trend (except of age) across tertiles of baseline carotid VWV. n=46, 46, 48 for lowest, median, and highest tertile, respectively.

VWV across all 3 diet groups, with a change of -60.69 mm³ (95% CI, -97.34 to -24.02 mm³) in the low-fat, -37.69 mm³ (95% CI, -77.19 to 1.80 mm³) in the Mediterranean, and -84.33 mm³ (95% CI, -129.97 to -38.69 mm³) in the low-carbohydrate diet groups (*P*=0.28 between groups).

Carotid IMT changed by -1.1% from 0.816 mm at baseline to 0.808 mm after 2 years (*P*=0.18), with no significant difference between diet groups (*P*=0.91). There was a trend toward significant correlation between the 2-year changes in carotid IMT and VWV (*r*=0.173, *P*=0.056).

Predictors of Regression in Carotid VMV and IMT

When we compared participants who exhibited regression in the carotid VWV (n=95; mean decrease=-128.00 mm³; 95% CI, -148.07 to -107.90) with participants who exhibited progression (n=45; mean increase=89.57 mm³; 95% CI, 66.58 to 112.57), several significant differences emerged.

The regression group had a higher mean baseline carotid VWV (986.51±243.3 versus 846.48±256.7 mm³ in the progression group; *P*<0.001); more weight loss (-5.30±5.8 versus -3.20±5.0 kg; *P*=0.03; Figure 1A in the online-only Data Supplement); a greater decrease in blood pressure (systolic: -6.81±13.9 versus -1.11±10.7 mm Hg; *P*=0.009; Figure 1B in the online-only Data Supplement; diastolic: -1.81±8.7 versus +1.18±8.3 mm Hg; *P*=0.053); and greater decline in total homocysteine (-0.06±4.1 versus +1.44±3.3 μmol/L; *P*=0.04). The regression group showed a trend toward greater increase in apoA1 levels (+0.05±0.13 versus -0.00±0.14 g/L; *P*=0.058). Age, sex, baseline BMI, statin use, prevalence of coronary heart disease, type 2 diabetes mellitus, or 6-month changes in lipoprotein cholesterol levels were not significantly different when we compared participants who demonstrated regression or progression of carotid VWV after 2 years of the weight loss diet

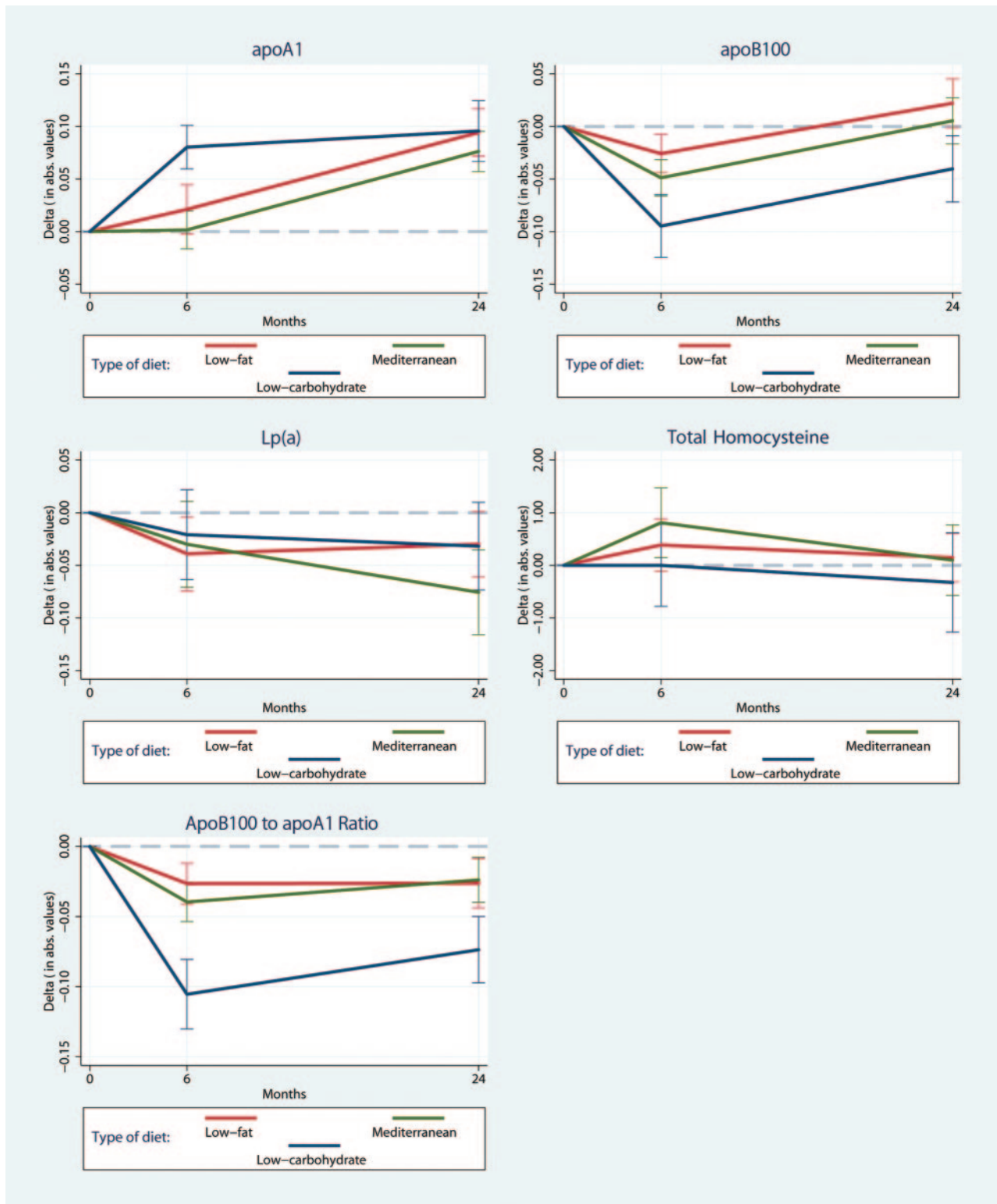


Figure 1. Changes in biomarkers according to diet groups during the maximum weight loss phase (1 to 6 months) and the weight loss maintenance phase (7 to 24 months) of the 2-year intervention.

intervention. We found similar patterns in a sensitivity analysis after excluding participants with no material 2-year change (within $\pm 2\%$) of VWV (n=14).

We conducted multivariate regression analyses (Table 2) adjusted for age, sex, diet group, and the univariate predictors

of changes in weight, systolic blood pressure, apoA1, and total homocysteine levels to predict changes in VWV and IMT. We found that only the decrease in systolic blood pressure remained a significant independent modifiable predictor of subsequent greater regression in both carotid VWV

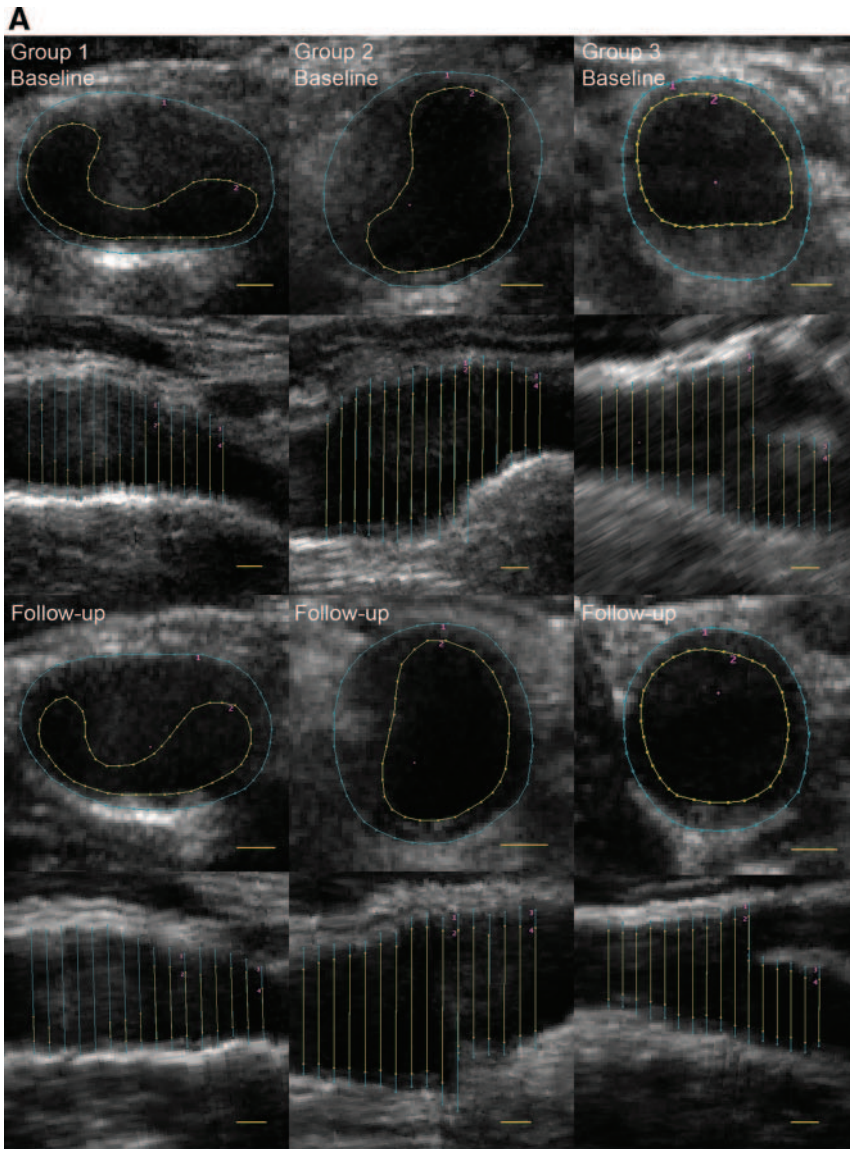


Figure 2. A, Examples of VWV measurements at baseline and follow-up. Images show segmentation of 1 slice of the common carotid artery at baseline and follow-up and the view of all segmentations from the lateral view. The average change for each group and artery was calculated, and patients were selected who had change in 1 artery close to the average for that side. Group 1 (low-fat diet), right side, change on that side is -24.3 mm, total change is -111.3 mm. Group 2 (Mediterranean diet), left side, change on that side is -21.5 mm, total change is -71.5 mm. Group 3 (low-carbohydrate diet), right side, change on that side is -61.4 mm, total change is -140 mm. In the images, lumen-intima boundary is yellow, and media-adventitia boundary is blue. Yellow scale bars indicate 2 mm. **B,** Two-year change in carotid VWV across quintiles of change in systolic blood pressure. Lines denote 1 SE.

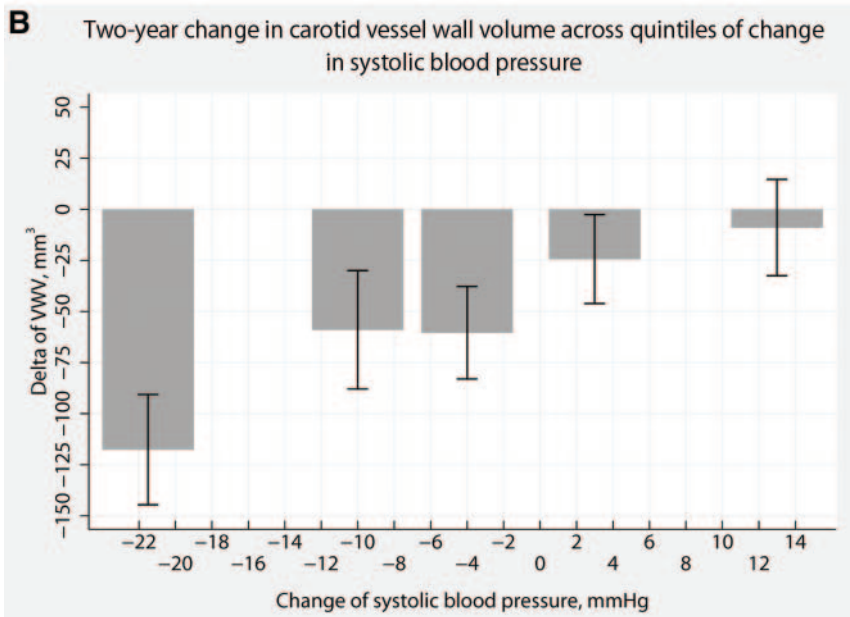


Table 2. Predictors of 2-Year Change in Carotid VWV and IMT: Multivariate Regression Model*

Variable in the Models	Change in Carotid VWV*; Multivariate Model 1		Change in Carotid IMT*; Multivariate Model 2	
	Standardized β	<i>P</i>	Standardized β	<i>P</i>
Baseline level, time 0 (carotid VMV, mm ³ in model 1; and carotid IMT, mm in model 2)	-0.319	0.001	-0.335	0.002
Change in systolic blood pressure, mm Hg	0.232	0.01	0.275	0.008
Change in weight, kg	0.010	0.92	-0.063	0.52
Change in total homocysteine, μ mol/L	0.028	0.75	-0.054	0.57
Change in apoA1, g/L	-0.072	0.43	-0.009	0.93
Age, y	0.064	0.48	-0.058	0.56
Male sex	0.019	0.83	-0.020	0.83
Mediterranean vs low-fat assigned diet	0.092	0.35	-0.020	0.85
Low-carbohydrate vs low-fat assigned diet	-0.046	0.64	0.074	0.50

*Dependent variables: 2-year changes (δ) of VWV in mm³, as a continuous variable in model 1, and 2-year changes (δ) of IMT in mm, as a continuous variable in model 2. Covariates of changes in parameters in the model were included at the first time point of significant indication of difference from univariate analysis. Change in systolic blood pressure was taken at month 9; change in weight loss was taken at 12 months; and changes in apoA1 and total homocysteine were taken at 6 months.

($\beta=0.23$; $P=0.01$) and IMT ($\beta=0.28$; $P=0.008$) after 2 years of weight loss intervention (Figure 2B).

Discussion

The main findings in this study are as follows: (1) Diet-mediated weight loss over a 2-year period induced a significant regression of carotid VWV; (2) carotid IMT and 3DUS VWV changes are mainly predicted by diet-induced changes in blood pressure over 2 years compared with the changes in lipoprotein levels; and (3) all diets provided a similar benefit, suggesting that the low-carbohydrate diet is at least as effective as the others and may be considered an alternative to low-fat and Mediterranean diets. However, our power to detect moderate differences in the effect of the 3 diets was limited.

Some limitations of our study warrant consideration. Although the evaluation of carotid IMT is a well-validated surrogate for carotid atherosclerosis and clinical outcomes,¹⁶ we measured changes in 3DUS VWV, which is considered a 3D IMT plus plaque measurement of VWV, rather than clinical end points. It is possible that the changes in VWV we detected may represent blood pressure-induced changes in the medial smooth muscle thickness rather than changes in atherosclerotic plaque. In addition, because very few women were enrolled, sex-specific effects may not have been probed adequately. The unique nature of the workplace that enabled a highly monitored dietary intervention over 2 years might limit the generalizability of our weight loss findings to

free-living populations. Nevertheless, we believe that similar strategies to maintain adherence could be applied elsewhere. VWV regression in the low-carbohydrate diet group (-84.33 mm³; 95% CI, -129.97 to -38.69 mm³) was somewhat higher than in the reference low-fat diet group (-60.69 mm³; 95% CI, -97.34 to -24.02 mm³); the difference was far from statistically significant, reflecting our inadequate statistical power to detect moderate differences among the 3 diet groups. Strengths of the study include a 1-phase design in which intervention began simultaneously for all participants, the relatively long duration of the study, and the high adherence rate.

Several previous studies have shown that lifestyle interventions can halt the progression of carotid IMT,¹⁻³ but others have shown no such effect.⁴ In a quantitative coronary angiography study among 35 patients with moderate to severe coronary heart disease, a 5-year intensive lifestyle program (10% fat whole foods vegetarian diet, aerobic exercise, stress management training, smoking cessation, group psychosocial support) significantly decreased coronary stenosis compared with a usual-care control group.¹⁷ Although coronary angiography does not quantify the extent of atherosclerosis within the vessel wall, this study demonstrated the potential for lifestyle-specific changes on coronary atherosclerosis. From this study, however, it was not clear which of the intensive lifestyle change components contributed most to the measured effects. An IMT study suggested that reduction of BMI, smoking cessation, and reduction of dietary cholesterol would reduce the annual rate of carotid wall IMT progression by 0.13 mm/y.¹⁸ In our intervention trial of 3 popular weight loss diets, moderate weight loss per se, whether from a low-fat, Mediterranean, or low-carbohydrate diet, was the main modifiable predictor of carotid VWV regression among moderately obese individuals, and this effect appears to be mediated primarily by reductions in blood pressure.

Our results suggest that among the lipid-related risk indicators, improvement of apoA1 had the greatest association with carotid VWV regression, although in the univariate analysis only. Although support for positive effects of aggressively increasing high-density lipoprotein cholesterol levels is debated,¹⁹ several studies suggest that a variety of apoA1/high-density lipoprotein cholesterol-raising therapies have the potential to stabilize or regress atherosclerosis.²⁰⁻²³ Levels of Lp(a) and total homocysteine barely changed on the various diets. Total homocysteine, an intermediary product of methionine metabolism that is not correlated with lipids²⁴ but is associated with variation in carotid plaque area,²⁵ had an association with carotid VWV regression, although in the univariate analysis only.

The effect of moderate weight loss on carotid regression appears to be mediated mainly by the reduction in systolic blood pressure. As in a previous drug study,²⁶ we found that systolic but not diastolic blood pressure or serum cholesterol was associated with alterations in carotid IMT. Because a significant dose-response relationship was found between the status of hypertension and the severity of carotid atherosclerosis, as measured by thicker IMT, hypertension is suggested to have a major role in the pathogenesis of atherosclerosis.²⁷

We quantified 3DUS carotid artery VWV to evaluate changes in atherosclerosis burden because it is noninvasive, has high sensitivity for detecting changes in VWV plus plaque, and is correlated with coronary disease.^{16,28–33} Our results suggest that in a predominantly male population, moderate weight loss, induced by a low-fat, Mediterranean, or low-carbohydrate diet, can cause significant decreases in carotid atherosclerosis measured by 3DUS VWV, mainly by decreased blood pressure, within 2 years. This effect is more pronounced among mildly obese persons who lose >5.5 kg body weight and whose systolic blood pressure decreases by >7 mm Hg. Increase in apoA1 and decrease in plasma total homocysteine levels are further associated with subsequent success in reversing carotid atherosclerosis.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The main findings in this study are as follows: (1) Diet-mediated weight loss over a 2-year period can induce a significant regression of carotid vessel wall volume. (2) Low-fat, low-carbohydrate, and Mediterranean diets provide similar degrees of carotid vessel wall volume regression. Thus, a low-carbohydrate diet is an alternative to low-fat and Mediterranean diets in reversing carotid atherosclerosis. (3) Over 2 years, changes in carotid intima-media thickness and 3-dimensional ultrasound are more clearly predicted by diet-induced changes in blood pressure than by changes in lipoprotein levels. For the practicing clinician, this study demonstrates that carotid atherosclerosis is reversible by long-term adherence to dietary strategies to induce weight loss. This effect is more pronounced among mildly obese persons who lose >5.5 kg body weight within 12 months and whose systolic blood pressure decreases by >7 mm Hg within 9 months. Increase in apolipoprotein A1 and decrease in plasma total homocysteine levels are also associated with subsequent success in reversing carotid atherosclerosis.