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INTERVENTION AND PREVENTION

Diet-Induced Weight Loss in Overweight or Obese Women and Changes in High-Density Lipoprotein Levels and Function

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Diet-induced weight loss in women may be associated with decreases not only in plasma levels of low-density lipoprotein cholesterol (LDL-C), but also in high-density lipoprotein cholesterol (HDL-C). Whether a decrease in HDL-C is associated with altered HDL function is unknown. One hundred overweight or obese women (age 46 ± 11 years, 60 black; 12 diabetic) were enrolled in the 6-month program of reduced fat and total energy diet and low-intensity exercise. Serum cholesterol efflux capacity was measured in ^{3}H-cholesterol-labeled BHK cells expressing ABCA1, ABCG1, or SR-B1 transporters and incubated with 1% apolipoprotein B (apoB)-depleted serum. Antioxidant properties of HDL were estimated by paraoxonase-1 (PON1) activity and oxygen radical absorbance capacity (ORAC). Endothelial nitric oxide synthase (eNOS) activation was measured by conversion of L-arginine to L-citrulline in endothelial cells incubated with HDL from 49 subjects. Participants achieved an average weight loss of 2.2 ± 3.9 kg ($P < 0.001$), associated with reductions in both LDL-C (–6 ± 21 mg/dl, $P = 0.004$) and HDL-C (–3 ± 9 mg/dl, $P = 0.016$). Cholesterol efflux capacity by the ABCA1 transporter decreased by 10% ($P = 0.006$); efflux capacities by the ABCG1 and SR-B1 transporters were not significantly altered. ORAC decreased by 15% ($P = 0.018$); neither PON1 activity nor eNOS activation was significantly altered by reduction in HDL-C. Findings were similar for diabetic and nondiabetic subjects. Diet-induced weight loss in overweight or obese women is associated with a decrease in HDL-C levels, but overall HDL function is relatively spared, suggesting that decrease in HDL-C in this setting is not deleterious to cardiovascular risk.

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INTRODUCTION

A strong inverse association between high-density lipoprotein cholesterol (HDL-C) levels and the risk of developing atherosclerotic vascular disease has been frequently reported (1). Significantly, there is mounting evidence supporting the importance of HDL function over the measurement of HDL-C levels (2). In this regard, HDL has been shown to have a variety of atheroprotective properties, including reverse cholesterol transport, nitric oxide stimulation, and protection from lipoprotein oxidation (3–5). Recently, cholesterol efflux capacity from macrophages was found to have a strong inverse association with carotid intimamedia thickness and the likelihood of angiographic coronary artery disease independent of the HDL-C levels, reinforcing the notion that HDL function is more informative to atherosclerotic risk than measuring HDL-C concentration (6).

Increased physical activity has been shown to be associated with a reduction in the risk of cardiovascular disease, likely a result, at least in part, of an improvement in the lipoprotein profile including a rise in HDL-C levels (7–11). HDL-C increases, however, appear to require aerobic exercise of substantial intensity, duration, and frequency (10,11). In contrast, diet with weight loss is often associated with actual reductions in HDL-C (12,13). In this regard, we previously reported a decline in HDL-C levels in participants at a worksite wellness program at our institution (14). We therefore considered whether diet-induced changes in HDL-C levels are associated with unfavorable alterations in functional properties of HDL.

METHODS AND PROCEDURES

Study design

This study was conducted at the Clinical Center of the National Institutes of Health with 100 overweight (BMI ≥25 kg/m^2) female employees enrolled in Keep the Beat, a worksite wellness program initiated by the National Heart, Lung, and Blood Institute. The program included meeting with a registered dietician, web-based dietary information and access to exercise equipment on campus. Subjects in our 6-month protocol had

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no history of atherosclerotic vascular disease and underwent cardiovascular physical examination, with no abnormalities detected. All participants provided written consent to participate in the protocol which was approved by the institutional review board of the National Heart, Lung, and Blood Institute (NCT00666172). Fasting venous blood samples were collected, and plasma and serum aliquots were prepared and frozen (−80 °C) for subsequent assays. Standard lipid profiles were measured using enzymatic assay from EDTA plasma aliquots (Wako Chemical USA, Richmond, VA). Apolipoprotein A-I (apoA-I) was measured by turbidimetric immunoassay (Kamiya Biomedical Company, Seattle, WA) in serum depleted of apolipoprotein B (apoB) by polyethylene glycol precipitation. For women reporting menses, testing was done during the follicular phase (day 1–10) of the menstrual cycle. No medications were started, stopped, or changed in dose during participation in the study. Any subject taking medication had to be on a stable dose for at least 2 months before entry in the protocol. All functional testing was performed on paired baseline and 6-month samples.

Adiposity was assessed by total fat mass and truncal fat (percent of total fat), as measured by dual-energy X-ray absorptiometry (DXA Software Gncore 11.10; GE Lunar Medical Systems, Madison, WI). Exercise fitness was measured by exercise duration and by peak oxygen consumption (VO_2 peak) during graded treadmill exercise (Bruce Protocol) using a Sensor Medics Vmax Spectra 229c metabolic cart (Carefusion, San Diego, CA). Insulin resistance was estimated by the homeostatic model assessment, which is based on fasting glucose (mg/dl) and fasting insulin (mU/l) levels (15).

Nutritional assessment and weight loss recommendations

For 90 subjects, a 24-h dietary recall was recorded for three days at baseline and at 6 months. This information was analyzed by the Nutrition Data System for Research (16) to determine the participants' daily nutritional composition. Participants were provided nutritional information based on the guidelines set forth by both the Department of Health and Human Services and the US Department of Agriculture as outlined in the MyPlate plan (17,18) and advised to reduce their daily caloric intake by 500 kcal. They were also instructed to increase their daily energy expenditure by an additional 5,000 steps/day over baseline activity, as measured by a pedometer issued to all participants.

Cholesterol efflux assay

A modified procedure (19,20) was used to measure the capacity of a serum specimen to accept cholesterol effluxed by either the ABCA1, ABCG1, or SR-B1 transporters in a stably transfected BHK cell line expressing one of the three human transporters. BHK cells transfected with a hygromycin-resistant control plasmid were used as the control cell line. Cholesterol efflux was conducted at 37 °C in cells labeled with ^{3}H-cholesterol for 24 h, washed, and incubated for 10 h with subject's serum, previously depleted of apoB lipoproteins by precipitation with polyethylene glycol (MW 8000; Sigma-Aldrich, St Louis, MO). The equivalent of 1% serum was used in each efflux reaction. The percent of efflux specific to each of the three transporters was calculated by subtracting the radioactive counts in the blank medium (α-minimal essential medium with 0.1% bovine serum albumin) from the radioactive counts in the presence of apoB-depleted serum and dividing by the sum of the radioactive counts in the medium plus the cell fraction. Each sample was run in triplicate with a mean coefficient of variation of 4.4%. Efflux values for each sample were normalized by dividing the efflux capacity of pooled sera from healthy subjects that was run with each assay. As a secondary analysis, efflux values were adjusted to apoA-I content in serum to account for changes in the apoA-I level between baseline and 6-month samples from the same subject.

ORAC assay

The method used was adapted from Cao and Prior (21,22). Solutions of 1.60 μmol/l fluorescein-disodium (Sigma-Aldrich, St Louis, MO) and 0.32 mol/l 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH) (Wako Chemical USA) were prepared and protected from light. A standard curve with the following concentrations (μmol/l) of Trolox was used and run in duplicate: 100, 50, 25, 12.5, and 6.25, blank. Ten microliter of HDL isolated by density gradient ultracentrifugation of serum depleted of apoB lipoproteins by dextran sulfate in solutions of KBr (1.2 g/ml) and dialyzed against phosphate-buffered saline (PBS) containing 0.02% EDTA was diluted in 40 μl of 75 mmol/l PBS and added to the plate. The Synergy 4 (BioTek, Winooski, VT) was programmed to add 50 μl fluorescein-disodium to each well, incubate for 5 min, add 25 μl of AAPH to each well, and measure the fluorescence every minute for 120 min. Results are reported as antioxidant activity in Trolox equivalents, and were normalized to the sample cholesterol concentration to account for different concentrations of HDL isolated in each sample following density gradient ultracentrifugation. Each sample was run in triplicate with a mean coefficient of variation of 5.8%. As a secondary analysis, oxygen radical absorbance capacity (ORAC) values were adjusted to apoA-I content in serum to account for changes in the apoA-1 level between baseline and 6-month samples from the same subject.

PON1 assay

Paraoxonase-1 (PON1) activity was determined using spectrophotometric measurement of rate of cleavage of phenylacetate (Sigma-Aldrich) to produce phenol in serum by monitoring the increase in absorbance at 270 nm at 25 °C. The resulting phenol concentration was calculated by the molar extinction coefficient, $\varepsilon = 1{,}310\ M^{-1}$ (pH 8.0). Each sample was run in triplicate with a mean coefficient of variation of 1.8%. Activity of paraoxonase is expressed as kU/l of serum.

eNOS activity

Nitric oxide synthase (NOS) activation was determined in bEND.3 immortalized endothelial cells by measuring [^{3}H]L-arginine conversion to [^{3}H]L-citrulline (23,24) during incubation with HDL isolated by polyethylene glycol precipitation from each sample to remove apoB-containing particles. On the day of the assay, serum-free medium was changed, incubated for 2 h, and then placed in 2 ml of NOS PBS (NOS PBS, pH7.4, 4.2 mmol/l KCl, 1.3 mmol/l $MgSO_4$, 2.5 mmol/l $CaCl_2$, 7.5 mmol/l glucose, 10 mmol/l Hepes, 120 mmol/l NaCl, 1.2 mmol/l Na_2HPO_4, 0.37 mmol/l KH_2PO_4) at 37 °C for 15 min. The NOS PBS was then removed from the wells and replaced with 500 μl NOS PBS containing 0.1 μCi/ml L-[^{14}C] arginine (PerkinElmer Life and Analytical Sciences, Boston, MA) with the amount of subjects' serum equaling 100 μg apoA-I. The cells were incubated for 60 min and the reaction was terminated by the addition of 750 μl of ice-cold stop solution 2 (15 mmol/l EGTA, 200 mmol/l HEPES buffer, pH 5.5). The cells were frozen and thawed twice, collected into a 1.5 ml tube, then spun down for 2 min; 0.5 ml of the supernatant was applied to Dowex AG50WX-8 (Tris form; Sigma) columns and eluted with 1 ml of stop solution 1 (40 mmol/l HEPES buffer, pH 5.5, 2 mmol/l EDTA and 2 mmol/l EGTA). The eluate, containing L-[^{14}C]citrulline, was collected and quantified by liquid scintillation counting. NOS inhibition by 1 mmol/l Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) was used to establish NOS-independent background activity. Untreated cells were assayed to obtain basal activity. To confirm that the NOS activity is from cellular NOS activation and not arginase-1 activity of the plasma, the NOS PBS with L-[^{14}C]arginine was incubated with the same concentration of plasma, and counting from the column eluate showed no difference compared to the background. Results are reported as fold-increase over basal activity. The mean coefficient of variation for samples run induplicate is 10.4%.

Statistical analysis

The data were expressed as mean ± SD. Statistical analysis was carried out using Instat3 statistical software (GraphPad Software, San Diego, CA) or SAS (SAS Institute, Cary, NC). Comparisons between baseline and 6-month values were made by two-tailed paired *t*-test. Associations between dependent and independent variables were quantified by

simple linear regression and the Pearson correlation coefficient. Significance was accepted at the 0.05 level of probability. As secondary analyses, changes in lipid levels and HDL functional properties were compared between diabetic and nondiabetic women.

RESULTS

One hundred women, average age 46 years (range 22–67 years); 60 black, 35 white, 3 Asian, 2 Hispanic were enrolled in the study. Thirty-five subjects were overweight (BMI 25–29.9 kg/m^2) and 65 were obese (BMI ≥30 kg/m^2). The group included 6 cigarette smokers, 12 adult-onset diabetics on glucose-lowering medications, 22 on treatment for hypertension, 13 on HMG CoA-reductase inhibitors (statins) for cholesterol reduction (including 6 diabetic subjects) and 9 on thyroid-replacement medications. Fifty-five women were premenopausal, of whom 12 were using hormonal contraception. The remainder was perimenopausal (4), postmenopausal (32), or had undergone hysterectomy (9), of whom two were on hormonal replacement therapy. All subjects taking medications were on stable doses for at least 2 months before entry in the protocol, with no change in dose during the 6-month study.

Dietary composition

The dietary composition for the 90 participants who completed food records at baseline and after completion of the program is reported in Table 1. At baseline, there were no significant correlations between any lipid values and any of the measured energy sources (all $r < 0.195$, $P > 0.069$). At 6 months, participants reported consuming fewer calories and less fat, carbohydrates and protein than at baseline. Participants consumed significantly fewer calories from fat and more calories from protein, with insignificant changes in the contribution of carbohydrates to caloric intake. The reduction in fat was associated with reduced intake of saturated fatty acids, mono-unsaturated fatty acids, and polyunsaturated fatty acids.

Table 1 Nutritional composition of 90 participants who completed dietary recalls

	Baseline	Six month	*P* value
Energy (kcal)	2,017 ± 641	1,619 ± 536	**<0.001**
Total fat (g)	85 ± 36	68 ± 40	**<0.001**
Total carbohydrates (g)	235 ± 83	193 ± 64	**<0.001**
Total protein (g)	80 ± 23	71 ± 23	**<0.001**
Calories from fat (%)	36.4 ± 6.9	34.0 ± 6.9	**0.005**
Calories from carbohydrates (%)	45.6 ± 8.2	46.4 ± 9.1	0.433
Calories from protein (%)	16.7 ± 4.4	17.8 ± 4.1	**0.013**
Calories from SFA (%)	11.5 ± 2.7	10.8 ± 2.6	**0.019**
Calories from MUFA (%)	13.1 ± 3.0	12.3 ± 2.7	**0.039**
Calories from PUFA (%)	8.9 ± 2.8	7.8 ± 2.5	**0.008**

Values are mean ± SD. Bold values identify comparisons that achieved statistical significance. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

Clinical characteristics

Following program completion, there were significant decreases in weight (–2.2 ± 3.9 kg), fat mass (–1.6 ± 3.4 kg), and percent truncal fat (–1.3 ± 2.9%) (Table 2). There was significant improvement in exercise performance as measured by duration of treadmill exercise (+45 ± 72 s) and VO_2 peak (+1.6 ± 3.2 ml O_2/kg/min). A significant reduction in insulin resistance, as measured by homeostatic model assessment, was also observed (–0.7 ± 1.0), due to reductions in both fasting glucose (–4 ± 8 mg/dl) and fasting insulin (–2.6 ± 3.9 mcU/ml) levels. All lipoprotein-associated cholesterol levels in plasma were significantly reduced: total cholesterol (–11 ± 24 mg/dl), low-density lipoprotein cholesterol (LDL-C) (–6 ± 21 mg/dl), HDL-C (–3 ± 9 mg/dl). There was a statistically nonsignificant change in apoA-I levels (+3 ± 28 mg/dl).

Cholesterol efflux capacity

At baseline, the mean efflux capacity from BHK cells expressing the ABCA1 transporter was 10.8% (range 1.7–27.9%), from cells expressing the ABCG1 transporter: 9.5% (range 1.1–27.4%), and from cells expressing the SR-B1 transporter: 6.4% (range 0.1–17.7%). After adjustment to the efflux capacity of an HDL pool run with each test, the relative activity of the ABCA1 transporter was 1.3 ± 0.8, ABCG1: 0.9 ± 0.5, and SR-B1: 1.2 ± 1.1. There were no statistically significant correlations between efflux capacity from any of the transporters and levels of serum HDL-C (all $r < 0.130$, $P > 0.203$). ABCA1 transporter activity was marginally positively correlated with apoA-I levels ($r = 0.173$, $P = 0.088$); no significant correlations

Table 2 Clinical characteristics for 100 participants

	Baseline	Six month	*P* value
Adiposity			
Weight (kg)	89.6 ± 18.6	87.5 ± 19.1	**<0.001**
Fat mass (kg)	39.3 ± 11.3	37.7 ± 11.9	**<0.001**
% Truncal fat (%)	46.7 ± 6.1	45.3 ± 6.3	**<0.001**
Fitness			
Duration (s)	413 ± 109	458 ± 128	**<0.001**
VO_2 peak (ml O_2/kg/min)	23.6 ± 5.3	25.2 ± 6.0	**<0.001**
Lipids			
Total cholesterol (mg/dl)	185 ± 33	176 ± 34	**<0.001**
LDL-C (mg/dl)	110 ± 24	104 ± 26	**0.004**
HDL-C (mg/dl)	55 ± 14	53 ± 13	**0.016**
Triglycerides (mg/dl)	94 ± 47	84 ± 40	**0.006**
ApoA-I (mg/dl)	127 ± 34	131 ± 38	0.206
Insulin sensitivity			
Fasting glucose (mg/dl)	93 ± 10	89 ± 10	**<0.001**
Fasting insulin (μU/ml)	8.8 ± 6.8	6.4 ± 6.5	**<0.001**
HOMA	2.1 ± 1.7	1.5 ± 1.6	**<0.001**

Values are mean ± SD. Bold values identify comparisons that achieved statistical significance. apoA-I, apolipoprotein A-I; duration, time spent exercising on the Bruce protocol; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostatic model assessment; LDL-C, low-density lipoprotein cholesterol; VO_2, peak oxygen consumption;

were noted between apoA-I and either ABGG1 or SR-B1 transporter activity (both $r < 0.091$, $P > 0.374$).

Cholesterol efflux capacity by the ABCA1 transporter decreased significantly by 10% at 6 months ($P = 0.006$), while efflux capacities by the ABCG1 and SR-B1 transporters were nonsignificantly changed (both <2%, $P > 0.740$) (**Figure 1**). Change in efflux capacity by the three transporters did not correlate with changes in either serum HDL-C or apoA-I levels (all $r < 0.147$, $P > 0.150$). After adjustment to sample apoA-I levels in order to account for changes in the apoA-I level between baseline and 6-month samples from the same subject, the decrease in ABCA1 efflux at 6 months (–7%) lost statistical significance ($P = 0.119$), and changes in function of the ABCG1 and SR-B1 transporters remained small (both < 2%) and statistically nonsignificant ($P > 0.311$). Differences in changes in efflux capacity for the three transporters were statistically nonsignificant between diabetic and nondiabetic subjects (all $P > 0.374$).

Antioxidant properties of HDL

At baseline, ORAC averaged 290 Trolox equivalents (range 135–532 Trolox equivalents) and correlated with both serum HDL-C ($r = 0.2077$, $P = 0.045$) and serum apoA-I ($r = 0.258$, $P = 0.014$) levels. When adjusted to the cholesterol content of the sample following density gradient ultracentrifugation to account for differences in HDL concentration, baseline ORAC was inversely correlated with serum HDL-C ($r = -0.343$, $P < 0.001$) and serum apoA-I ($r = -0.251$, $P = 0.014$) levels. ORAC adjusted to sample cholesterol content significantly decreased at 6 months (–15%; **Figure 2**). This change correlated significantly and inversely with changes in serum HDL-C levels ($r = -0.368$, $P < 0.001$), but not with changes in serum apoA-I levels ($r = -0.167$, $P = 0.105$). When further adjusted to the apoA-I content of the sample to account for changes in apoA-I levels between baseline and 6-month samples from the same subject, a reduction of 10% was observed ($P = 0.002$), but without correlation with either HDL-C or apoA-I at baseline or 6 months or changes in values during this interval (all $r < 0.091$, $P > 0.390$). Differences in the change in ORAC were statistically nonsignificant between diabetic and nondiabetic subjects ($P > 0.221$).

At baseline, serum PON1 activity averaged 139 kU/l (range 54–206 kU/l) and correlated with both serum HDL-C ($r = 0.482$, $P < 0.001$) and serum apoA-I ($r = 0.342$, $P < 0.001$) levels. There was no correlation between PON1 activity and ORAC, whether unadjusted ($r = 0.174$, $P = 0.102$) or normalized to sample cholesterol content following density gradient ultracentrifugation ($r = -0.102$, $P = 0.340$). At 6 months, the serum PON1 activity was nonsignificantly altered (+1%; **Figure 2**). Change in PON1 activity tended to be greater in diabetic subjects compared with nondiabetic subjects (9.6 ± 17.1 vs. 0.7 ± 16.9 kU/l, $P = 0.092$).

Endothelial nitric oxide stimulation by HDL

The assay for endothelial nitric oxide synthase (eNOS) was based on the apoA-I content of the analyzed specimen. At baseline, eNOS activation by subject samples averaged 4.0-fold over basal activity (range 2.4–10.5) and was nonsignificantly decreased by 5% at 6 months (**Figure 3**). There were no significant correlations between eNOS activity and serum HDL-C or serum apoA-I at baseline or with the changes in these values between baseline and 6 months (all $r < 0.098$, $P > 0.517$). Likewise, there were no significant correlations between eNOS activity at baseline and either ORAC or PON1, or in changes in these values at 6 months (all $r < 0.081$, $P > 0.595$). Differences in the change in eNOS activity were nonsignificant between diabetic and nondiabetic subjects ($P = 0.443$).

DISCUSSION

To our knowledge this study is the first to report effects of diet with reduced fat and total energy intake, based on guidelines set forth by the US Department of Agriculture (17,18), coupled with low-intensity exercise on HDL function. We found that weight loss achieved by this regimen was associated with significant reductions in HDL-C levels, representing an ~6% decline from baseline values averaging 55 mg/dl. This decline in HDL-C levels, however, was associated with only relatively small changes in HDL-mediated reverse cholesterol transport as determined in BHK cells transfected with ABCA1, ABCG1 or SR-B1 transporters, with baseline and 6-month samples paired in an effort to minimize assay variance. We chose two assays to investigate effects of weight loss and HDL-C reduction on antioxidant properties of HDL; the ORAC assay which has been previously used to measure the "total" antioxidant capacity of samples (22) and activity of PON1, primarily associated with HDL particles, which degrades oxidized lipids, especially cholesterol esters and phospholipids (4). ORAC significantly decreased at 6 months; PON1 activity was not significantly altered. Adjustment of HDL functional tests to HDL cholesterol concentration or apoA-I levels had little effect on the

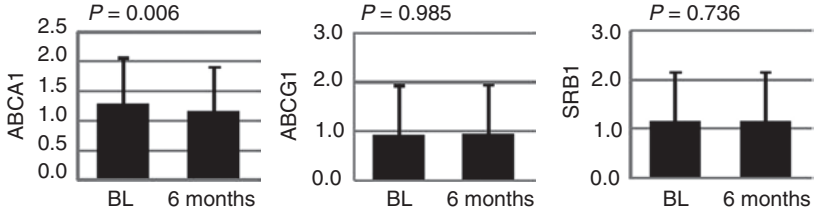

Figure 1 Comparison of baseline and 6-month cholesterol efflux in apolipoprotein-B-depleted serum samples by ABCA1, ABCG1, and SR-B1 transporters expressed in stably transfected BHK cell lines. All values are adjusted to the efflux capacity of pooled serum from healthy subjects. Data are expressed as mean values and SEM.

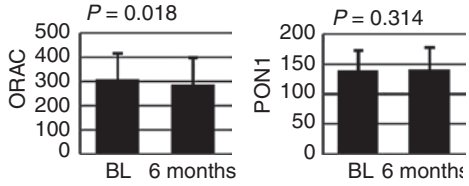

Figure 2 Comparison of baseline and 6-month antioxidant properties of high-density lipoprotein (HDL). Oxygen radical absorbance capacity assay (ORAC) was used in samples following HDL isolation by density gradient ultracentrifugation and normalized to the cholesterol content of the sample. Results are reported as antioxidant activity in Trolox equivalents. Paraoxonose-1 activity (PON1) was determined using spectrophotometric measurement of rate of cleavage of phenyl acetate to produce phenol in serum samples, and is expressed as kU/l of the sample. Data are expressed as mean values and SEM.

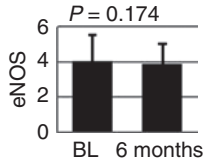

Figure 3 Comparison of baseline 6-month endothelial nitric oxide synthase (eNOS) activity in endothelial cells by measuring [^{3}H]L-arginine conversion to [^{3}H]L-citrulline. All testing was performed on a serum sample aliquot containing 100 µg apolipoprotein A-I: Results are reported as fold-increase over basal activity. Data are expressed as mean values and SEM.

magnitude of change or level of statistical significance with the diet and exercise intervention. eNOS activity, as measured by conversion of L-arginine to L-citrulline in cultured endothelial cells with HDL normalized to apoA-I content, increased 4.0-fold over background activity, but was nonsignificantly altered at 6 months.

Diets restricted in cholesterol and fat administered in controlled environments have been reported to lower lipoprotein cholesterol levels, including HDL-C, for three decades (25). Tracer studies indicate that fat-restricted reduction in HDL-C is associated with increased apoA-I catabolism and decreased apoA-I appearance rate from liver into plasma (12). Diets used to reduce HDL-C in these studies, however, are difficult to achieve in the population setting. Wood *et al.* (10) reported that fat-reduced diet (30% daily energy intake) reduced HDL-C by 10% after 1 year in 31 overweight premenopausal women. Sacks *et al.* (26) reported that overweight men and women randomized to a low fat (20% daily energy intake), average protein diet had a small decline in HDL-C at 6 months (–0.4 mg/dl), in addition to declines in total cholesterol and LDL-C values, which increased by 2 years with increase in fat intake and weight above baseline measurements. Comparison of dietary effects on lipids by sex was not reported. In the Women's Health Initiative Randomized Controlled Modification Trial, dietary intervention that aimed to reduce total fat intake to 20% of daily energy intake in postmenopausal women resulted in significantly lower HDL-C levels (including HDL_2 and HDL_3 subfractions) at year 1 compared to the control group, but no significant difference in HDL particle size by NMR spectroscopy in a subset of participants (27). Weight was minimally changed in both groups as macronutrient modification, and not reduction in total energy intake, was the aim of this study.

Our cohort of women was predominately obese, with two-thirds African-American representation, and the aim of participation in the worksite wellness program was to achieve weight loss. Although exercise has been reported to ameliorate diet-induced reduction in HDL (7), low-intensity exercise (primarily walking and use of aerobic exercise equipment at the worksite) by our study participants that was sufficient to significantly improve exercise performance did not prevent a reduction in HDL-C, which significantly correlated with the reduction in total cholesterol. No significant univariate correlations were found between the change in HDL-C and changes in macronutrient intake, body weight, fat mass, exercise duration or VO_2 peak, suggesting a multifactorial explanation for HDL-C reduction in our study.

Because of the recent interest in HDL function and association with cardiovascular disease, we considered whether diet-induced changes in HDL-C, which might be considered undesirable if reduced, are associated with changes in functional properties of this lipoprotein. On the one hand, lower levels of HDL-C might indicate fewer particles to mediate its multiple functions, although apoA-I, an imperfect metric of the number of HDL particles, did not decline in our subjects following participation in the diet and exercise program. On the other, if decreased HDL-C is an indication of more nascent, less cholesterol-laden lipoproteins, reverse cholesterol transport, at least by the ABCA1 transporter, might be facilitated. In this regard, reverse cholesterol transport is the transfer of cholesterol from peripheral cell sources (especially macrophages) to the liver for catabolism or excretion as bile (3). Phospholipid-associated nascent apoA-I secreted from the liver and intestines binds to the ABCA1 transporter on macrophages or other cholesterol-rich cells, generating HDL containing limited amounts of free cholesterol. Esterification of cholesterol within these particles produces spherical HDL, which can then incorporate additional cholesterol by the ABCG1 transporter on macrophages and the scavenger receptor type B1 (SR-B1) in the liver and other cells. Khera *et al.* (6) measured cholesterol efflux from macrophages containing radiolabeled cholesterol, incubated with apoB-depleted serum from healthy subjects and a larger cohort of patients who underwent coronary angiography. Efflux measured after 4 h correlated with HDL-C and apoA-I levels, with carotid intimal-medial thickness in healthy subjects and the presence of angiographic coronary artery disease in patients. We chose BHK cells instead of macrophages to permit individual assessment of the major cholesterol transporters by separate transfection with ABCA1, ABCG1 and SR-B1, and then incubated cells for 10 h with apoB-depleted serum. Baseline and 6-month samples were paired in an effort to minimize assay variance. Cholesterol efflux by the ABCA1 transporter—normalized to the efflux capacity of pooled sera from healthy subjects run with each assay—decreased by 10%; this change was no longer statistically significant following adjustment for apoA-I content in samples. Efflux capacity

through the ABCG1 and SR-B1 transporters was not significantly altered during the 6-month diet and exercise program. Overall, these results indicate that despite the modest decrease in HDL-C levels observed after the diet and exercise intervention, the cholesterol efflux capacity of HDL was relatively preserved.

A limitation of this study is that medications were not stopped, although for protocol eligibility subjects (including the 13 women on statin medications) had to be on stable doses for at least 2 months with no change in dose during the 6-month study. Furthermore, the assay we employed to determine cholesterol efflux has not been demonstrated to be associated with cardiovascular disease. Of interest, however, the mean values and range of values for cholesterol efflux reported by Khera *et al.* (6) using macrophages that naturally express the transporters transfected in our BHK cells, were similar to our assay values. A strength of our study is inclusion of a large proportion of African-American women, in whom obesity is rising in prevalence. Furthermore, our study represents what is in practice often achievable in a workplace environment, especially for employees with largely sedentary job activities.

We conclude that diet-induced weight loss in overweight women is associated with a significant decrease in HDL-C levels, but overall HDL function is relatively spared, suggesting that decrease in HDL-C in this setting may not be associated with increased cardiovascular risk.

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DISCLOSURE

The authors declared no conflict of interest.
See the online ICMJE Conflict of Interest Forms for this article.

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