Effects of a 2-y dietary weight-loss intervention on cholesterol metabolism in moderately obese men

Alexander B Leichtle, Christin Helmschrodt, Uta Ceglarek, Iris Shai, Yaakov Henkin, Dan Schwarzfuchs, Rachel Golan, Yftach Gepner, Meir J Stampfer, Matthias Blüher, Michael Stumvoll, Joachim Thiery, and Georg M Fiedler

ABSTRACT
Background: Long-term dietary weight loss results in complex metabolic changes. However, its effect on cholesterol metabolism in obese subjects is still unclear.

Objective: We assessed the effects of 2 y of weight loss achieved with various diet regimens on phytosterols (markers of intestinal cholesterol absorption), lanosterol (marker of de novo cholesterol synthesis), and changes in apolipoprotein concentrations.

Design: We conducted the 2-y Dietary Intervention Randomized Controlled Trial (DIRECT—a study of low-fat, Mediterranean, and low-carbohydrate diets). We assessed circulating phytosterol and lanosterol concentrations and their ratios to cholesterol and apolipoproteins A-I and B-100 in 90 DIRECT participants at 0, 6, and 24 mo.

Results: We observed a significant upregulation of the markers of cholesterol absorption (campesterol: +16.8%, \( P < 0.001 \)) and a downregulation of the markers of cholesterol synthesis (lanosterol: \(-16.5\%, P = 0.008\)) during the active weight-loss phase (first 6 mo), weight loss of 5%, 6%, and 10% in the 3 diet groups, respectively), followed by a rebound (campesterol: \(-6.2\%, P = 0.045\); lanosterol: +43.7%, \( P < 0.001 \)) during the next 18 mo (weight gain of 1%, 1%, and 2% in the 3 diet groups, respectively). HDL cholesterol continuously increased during the study (17.0%, \( P < 0.001 \)), whereas LDL cholesterol remained constant. At the end of the 24-mo follow-up period, campesterol (\( P < 0.001 \)) and lanosterol (\( P = 0.016 \)) amounts were significantly higher than baseline values. The concentration of apolipoprotein B-100 correlated with cholesterol metabolism (\( \hat{\rho} = 0.299 \) and \( P = 0.020 \) for lanosterol; \( \hat{\rho} = -0.105 \) and NS for campesterol), and the homeostasis model assessment of insulin resistance correlated with lanosterol (\( \hat{\rho} = 0.09 \), \( P = 0.001 \)).

Conclusions: Long-term weight loss is related to a characteristic response suggestive of altered cholesterol and apolipoprotein metabolism. Various diets have a similar effect on these effects. DIRECT is registered at clinicaltrials.gov as NCT00160108. Am J Clin Nutr 2011;94:1189–95.

INTRODUCTION
Whole-body cholesterol homeostasis is determined by a balanced regulation of intestinal cholesterol absorption and biliary cholesterol excretion as well as endogenous cholesterol de novo synthesis, which are linked by a reciprocal relation (1). Genetics, physiologic factors (eg, body weight), and external interventions (eg, plant sterol supplementation, ezetimibe, and statin therapy) differentially affect cholesterol metabolism, which results in changes in serum lipoprotein concentrations and the associated risk of CVD (2). The cholesterol homeostasis can be assessed by measuring circulating noncholesterol sterols, such as phytosterols (eg, campesterol) and cholesterol precursors (eg, lanosterol) and their ratios to cholesterol (relative concentrations) as reliable surrogate markers for the efficiency of dietary cholesterol absorption and endogenous de novo synthesis of cholesterol, respectively (2–4). The validity of these markers has also been documented in subjects with different degrees of insulin resistance (5, 6).

Insulin resistance states, such as obesity, metabolic syndrome, and type 2 diabetes, are commonly associated with characteristic changes in cholesterol metabolism, reflected in upregulated cholesterol synthesis and downregulated cholesterol absorption (1, 7). These changes may additionally modify the CVD risk and influence the effectiveness of cholesterol-lowering drugs, such as statins or ezetimibe (8–16). Short-term dietary weight loss can induce a partial remission of these changes in cholesterol metabolism (5, 6, 17, 18). However, it is not known to what extent long-term weight loss affects cholesterol metabolism and whether various diet regimens have a differential influence on these effects.

1 From the University Institute of Clinical Chemistry, Inselspital – Bern University Hospital, Bern, Switzerland (ABL and GMF); the Institute of Laboratory Medicine, Clinical Chemistry, and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany (CH, UC, and JT); The S. Daniel Abraham Center for Health and Nutrition, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, Israel (IS, RG, and YG); the Department of Cardiology, Soroka University Medical Center, Beer-Sheva, Israel (YH); the Nuclear Research Center Negev, Dimona, Israel (DS); the Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, and Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA (MJS); and the Department of Endocrinology, University of Leipzig, Leipzig, Germany (MB and MS).

2 ABL and CH contributed equally to this work.

3 Supported by grants from The Israeli Ministry of Health, Chief Scientist Office (project no. 300000-4850) and The Dr. Robert C. and Veronica Atkins Research Foundation.

4 Address reprint requests and correspondence to AB Leichtle, Center of Laboratory Medicine, University Institute of Clinical Chemistry, Inselspital – Bern University Hospital, Inselspital INO-F 502, CH-3010 Bern, Switzerland. E-mail: alexander.leichtle@insel.ch.

5 Abbreviations used: apo, apolipoprotein; CVD, cardiovascular disease; DIRECT, Dietary Intervention Randomized Controlled Trial; HOMA-IR, homeostasis model assessment of insulin resistance.

Received May 2, 2011. Accepted for publication August 16, 2011.
First published online September 21, 2011; doi: 10.3945/ajcn.111.018119.
DIRECT (www.clinicaltrials.gov; NCT00160108) analyzed the effectiveness and safety of 3 different nutritional protocols (low-fat, restricted-calorie diet; Mediterranean, restricted-calorie diet; and low-carbohydrate, nonrestricted-calorie diet) in moderately obese subjects over a 2-y period (19, 20). We evaluated cholesterol metabolism in 90 representative participants of this trial by quantitating plasma concentrations of phytosterols (as markers of cholesterol absorption), lanosterol (as a marker of cholesterol synthesis), and serum apo A-I and apo B-100 concentrations with respect to diet group assignment and insulin resistance status throughout the 2-y study period.

SUBJECTS AND METHODS

Study population

DIRECT was previously reported in detail (19). Eligible participants were aged 40–65 y, had a BMI (in kg/m\(^2\)) ≥27, and/or had type 2 diabetes (according to American Diabetes Association criteria; 21) or coronary heart disease. Exclusion criteria included pregnancy or lactation, a serum creatinine concentration ≥2 mg/dL (176.8 μmol/L), liver dysfunction (defined as an increase ≥2-fold the upper limit of normal in alanine aminotransferase and aspartate aminotransferase), intestinal problems that would prevent consumption of any of the test diets, active cancer, or participation in another diet trial. Starting in December 2004, participants were randomly assigned to a low-fat, Mediterranean, or low-carbohydrate diet within the strata of sex, age (below or above the median), BMI (below or above the median), history of coronary heart disease (yes or no), type 2 diabetes (yes or no), and current use of statins (none, duration <1 y, or duration ≥1 y) by Monte-Carlo simulations for randomization.

We focused on a representative subgroup of the overall DIRECT participants according to the following criteria, which we applied stepwise: to exclude sex-associated variability, we included 90 moderately obese men (aged 40–62 y). To avoid any drug-associated bias, we included only DIRECT participants who did not take any cholesterol-lowering drug during the study period. Furthermore, samples from each of the 3 time points had to be available for each participant. The characteristics of our subgroup (age, prevalence of coronary heart disease, baseline carotid vessel wall volume, weight, 2-y changes in BMI, and total energy intake) were similar to those of the overall DIRECT participants. The 90 participants were allocated to the 3 different diet groups as follows: 28 to the low-fat diet, 28 to the Mediterranean diet, and 34 to the low-carbohydrate diet.

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

Clinical and laboratory characteristics

Body weight was measured in subjects without shoes to the nearest 0.1 kg every month. Height was assessed to the nearest millimeter at baseline for the determination of BMI with a wall-mounted stadiometer. Blood samples were drawn by venipuncture at 0800 after a 12-h fast, at baseline (0 mo), and at 6 and 24 mo and were stored at −80°C until analyzed, except that fasting plasma glucose was measured in fresh samples. HDL cholesterol and LDL cholesterol were directly analyzed by a homogeneous enzymatic colorimetric test (HDL cholesterol plus third generation, LDL cholesterol plus second generation; provided by Roche). Triglycerides were measured by using an enzymatic colorimetric test (triglycerides; provided by Roche). Concentrations of apo A-I and apo B-100 were measured in serum by using immunoturbidimetric assays (Tina-quant apo A-I version 2 and Tina-quant apo B-100 version 2; Roche) on an automated Cobas e 501 analyzer (Roche). Fasting plasma insulin concentrations were measured by using an enzyme immunometric assay (ImmunoLite Automated Analyzer; Diagnostic Products). HOMA-IR was calculated according to the following equation (22): insulin (μU/mL × fasting glucose (mmol/L))/22.5. Details of the methods were described previously (23).

Sterol analysis

Noncholesterol sterols (eg, lanosterol or campesterol) and cholesterol were analyzed by atmospheric pressure photoionization liquid chromatography–tandem mass spectrometry with an interassay CV between 3.9% and 9.9% (24). For the quantification of free and esterified sterols, 10 μL of calibrators, controls, or plasma samples were mixed with 490 μL internal standard solution. After centrifugation (10 min at 11,400 g), the supernatant fluid was transferred into a glass vial. Supernatant fluid (25 μL) was injected onto the analytic column (Chromolith SpeedRod RP-18e, 50 × 4.6 mm monolithic column; Merck KGaA). An API 3000 triple quadrupole mass spectrometer with an APPI photospray ion source from Applied Biosystems (Darmstadt) was used as detector. The ratios of phytosterols and lanosterol to cholesterol were calculated to estimate intestinal cholesterol absorption and endogenous cholesterol synthesis.

Statistical analysis

Calculations were performed with IBM PASW Statistics software (version 18.0.3) and R (version 2.12.1; http://cran.r-project.org/). The study population was split along diet groups (low-fat, Mediterranean, and low-carbohydrate) and HOMA-IR groups (HOMA terciles 1–3 and patients with diabetes). Percentages of change are given as medians (2.5th to 97.5th percentiles). The statistical significance of the differences between groups was assessed by using the Kruskal-Wallis test, and Tukey’s honestly significant difference test was used for multiple comparisons (25). Intragroup differences in the time course of the study were evaluated by using the Friedman test for all 3 time points and the Friedman post hoc test for differences between time points. Correlations were computed as multivariate correlation estimators (p) according to the method proposed by Zhu et al (26) with time points as replicates.

RESULTS

Baseline characteristics

The dietary subgroups (low-fat, Mediterranean, and low-carbohydrate) did not differ significantly at baseline in age, HOMA-IR, or in blood concentrations of LDL cholesterol, fasting triglycerides, apo B-100, apoA-I, and noncholesterol sterols (eg, lanosterol and campesterol). HDL cholesterol was higher in the Mediterranean diet group than in the other groups (Table 1).
There were no significant diet-group differences. From baseline, \( P = 0.001 \) (paired Wilcoxon’s signed-rank test with continuity correction). There were no significant diet-group differences.

Noncholesterol sterols in the dietary subgroups

To eliminate the effects of varying cholesterol content in lipoproteins, all plasma noncholesterol sterols and their changes were expressed in terms of \( 10^2 \times \text{mmol/mol} \) of cholesterol (referred to as “ratio” in the text), dividing the concentrations of noncholesterol sterols by cholesterol obtained from the same liquid chromatography–tandem mass spectrometry run (4, 27) (Figure 2).

During the initial phase of maximum weight loss (0–6 mo), phytosterols increased significantly [campesterol (median: +16.8%; 2.5th to 97.5th percentiles: −23.7% to +87.6%)], which was followed by a slight but significant decrease [campesterol: −6.2%; −34.5% to +33.0%] in the maintenance phase (7–24 mo). Lanosterol showed a reciprocal reaction with a significant decrease (−16.5%; −67.1% to +81.6%) during the first 6 mo and a significant increase during the following 18 mo (+43.7%; −50.3% to +267.7%). Compared with baseline, plasma concentrations of the noncholesterol sterols at the end of the trial (24 mo) showed a persistent and significant increase in campesterol (+11.6%; −35.9% to +103.6%) and lanosterol (+28.0%; −55.9% to +210.6%). The ratios of lanosterol to cholesterol and of campesterol to cholesterol were both correlated with the BMI (see Supplemental Figure 1 under “Supplemental data” in the online issue).

Noncholesterol sterols in the total group

To eliminate the effects of varying cholesterol content in lipoproteins, all plasma noncholesterol sterols and their changes were expressed in terms of \( 10^2 \times \text{mmol/mol} \) of cholesterol (referred to as “ratio” in the text), dividing the concentrations of noncholesterol sterols by cholesterol obtained from the same liquid chromatography–tandem mass spectrometry run (4, 27) (Figure 2).

During the initial phase of maximum weight loss (0–6 mo), phytosterols increased significantly [campesterol (median: +16.8%; 2.5th to 97.5th percentiles: −23.7% to +87.6%)], which was followed by a slight but significant decrease [campesterol: −6.2%; −34.5% to +33.0%] in the maintenance phase (7–24 mo). Lanosterol showed a reciprocal reaction with a significant decrease (−16.5%; −67.1% to +81.6%) during the first 6 mo and a significant increase during the following 18 mo (+43.7%; −50.3% to +267.7%). Compared with baseline, plasma concentrations of the noncholesterol sterols at the end of the trial (24 mo) showed a persistent and significant increase in campesterol (+11.6%; −35.9% to +103.6%) and lanosterol (+28.0%; −55.9% to +210.6%). The ratios of lanosterol to cholesterol and of campesterol to cholesterol were both correlated with the BMI (see Supplemental Figure 1 under “Supplemental data” in the online issue).

**Weight loss in the total group**

Participants in the low-fat, Mediterranean, and low-carbohydrate diet groups experienced significant mean weight reductions \( P < 0.001 \) of 5%, 6%, and 10% after 6 mo and 4%, 5%, and 8% after 24 mo, respectively (Figure 1). The phase of maximum weight loss (0–6 mo) was followed by a maintenance phase (7–24 mo), which was characterized by a slight weight rebound of 1%, 1%, and 2%, respectively. These data were similar to the overall results of DIRECT (19). Serum lipid concentrations at baseline and during the study are displayed in Table 2.

**Noncholesterol sterols in the total group**

To eliminate the effects of varying cholesterol content in lipoproteins, all plasma noncholesterol sterols and their changes were expressed in terms of \( 10^2 \times \text{mmol/mol} \) of cholesterol (referred to as “ratio” in the text), dividing the concentrations of noncholesterol sterols by cholesterol obtained from the same liquid chromatography–tandem mass spectrometry run (4, 27) (Figure 2).

During the initial phase of maximum weight loss (0–6 mo), phytosterols increased significantly [campesterol (median: +16.8%; 2.5th to 97.5th percentiles: −23.7% to +87.6%)], which was followed by a slight but significant decrease [campesterol: −6.2%; −34.5% to +33.0%] in the maintenance phase (7–24 mo). Lanosterol showed a reciprocal reaction with a significant decrease (−16.5%; −67.1% to +81.6%) during the first 6 mo and a significant increase during the following 18 mo (+43.7%; −50.3% to +267.7%). Compared with baseline, plasma concentrations of the noncholesterol sterols at the end of the trial (24 mo) showed a persistent and significant increase in campesterol (+11.6%; −35.9% to +103.6%) and lanosterol (+28.0%; −55.9% to +210.6%). The ratios of lanosterol to cholesterol and of campesterol to cholesterol were both correlated with the BMI (see Supplemental Figure 1 under “Supplemental data” in the online issue).
the first 6 mo in all diet groups \((P = 0.008)\). During the maintenance phase, lanosterol showed a strong and significant increase in all diet groups \((P < 0.001)\). Over the complete trial period (0–24 mo), a significant increase of lanosterol could be observed in all diet groups \((P = 0.016)\).

### Apolipoproteins in the total study group

Circulating apo A-I concentrations were not correlated with any of the noncholesterol sterols over all time points. In contrast, circulating apo B-100 concentrations showed positive correlations with lanosterol \((\rho = 0.299, \, P = 0.020)\) and negative correlations with campesterol \((\rho = -0.105, \text{NS})\).

### Correlation between noncholesterol sterols and the insulin resistance–associated factors BMI and HOMA-IR

Exemplary for phytosterols, campesterol concentrations were negatively correlated with BMI \((\rho = -0.31; \, P < 0.001)\), and lanosterol concentrations were positively correlated with BMI \((\rho = 0.30; \, P < 0.001)\) and HOMA-IR \((\rho = 0.09; \, P = 0.001)\) (see Supplemental Figure 1 under “Supplemental data” in the online issue).

### DISCUSSION

Our investigation of cholesterol metabolism in participants of the 2-y DIRECT showed the following new insights: 1) a dietary long-term weight loss induces a characteristic response of the cholesterol metabolism: an initial upregulation of the markers of cholesterol absorption and downregulation of the markers of cholesterol synthesis during the first 6 mo is followed by a partial rebound of the markers of cholesterol absorption and an excessive rebound of the markers of cholesterol synthesis in the course of the following 18 mo; 2) the 3 diet regimens evaluated in this trial had a similar effect on cholesterol metabolism, which suggests that the effects we observed were related to weight changes and their associated metabolic responses rather than to diet composition; 3) whereas apo B-100 correlated with variables of cholesterol absorption and synthesis, apo A-I seems to be independent of them.

Disturbances of cholesterol metabolism are associated with insulin resistance states, such as obesity, the metabolic syndrome, and type 2 diabetes and significantly affect the risk of CVD \((1, 7, 28, 29)\). Insulin resistance itself markedly influences cholesterol metabolism, which is reflected in upregulated cholesterol synthesis and down-regulated cholesterol absorption \((1, 5, 7, 28, 30–33)\). We confirmed this well-known interrelation in this study. The participants of our study, at baseline, were obese, had fasting insulin activities in high-normal ranges, and had a HOMA-IRs suggestive of insulin resistance \((34)\). Absolute campesterol concentrations were in the low-normal range \((35)\). Relative campesterol concentrations were comparable with those reported for patients with the metabolic syndrome \((32)\), which points to reduced baseline sterol absorption probably secondary to increased cholesterol synthesis \((5)\). Previous clinical studies in subjects with insulin resistance indicated that a short-term dietary weight loss may lead to a partial remission of the associated changes in cholesterol metabolism \((5, 6, 17, 18)\). However, it is not known to what extent long-term dietary weight loss influences cholesterol metabolism and whether various diet regimens have a differential effect on it. Our study group showed an initial phase of maximum weight loss (0–6 mo) followed by a maintenance phase (7–24 mo) with a partial rebound or a plateau phase (19). In parallel with these weight changes, the first 6 mo were characterized by a significant upregulation of the markers of cholesterol absorption and a significant downregulation of the markers of cholesterol synthesis. These results are comparable with the previously described acute effects of weight reduction on cholesterol metabolism \((17, 36)\). The downregulation of cholesterol synthesis...
during the first 6 mo might have been primarily attributable to changes in insulin resistance after weight reduction (31), although downregulation of hydroxymethylglutaryl CoA reductase could be also effective (37).

In the following 18 mo, however, we observed a previously undescribed rebound of the initial effects, wherein the significant downregulation of cholesterol synthesis was particularly reversed. Interestingly, the various diet regimens had no significant differential effect on cholesterol metabolism. The rebound of cholesterol synthesis might be functionally related to the depletion of cellular cholesterol, which activates sterol regulatory element-binding protein 2 and leads to increased cholesterol synthesis via an insulin-independent pathway (31). The increase of sterol absorption and the associated elevated phytosterols may additionally induce the liver X receptor (38), which in turn might enhance cholesterol synthesis via sterol regulatory element-binding protein 2 (32).

Circulating apolipoprotein concentrations also changed in a characteristic manner. The initially favorable decrease ($P < 0.001$) in the ratio of apo B-100 to apo A-I, from 0.66 (0.36–0.96) to 0.60 (0.35–0.92), which is an accepted predictor of CVD risk (39, 40), was attenuated parallel to the rebound in cholesterol metabolism during the maintenance phase (data not shown). Nevertheless, the ratio of apo B-100 to apo A-I remained significantly ($P = 0.04$) decreased at the end of the trial. The changes in apolipoprotein metabolism might be related to liver X receptor–induced changes in reverse cholesterol transport (41–44).

Concentrations of apo A-I showed no significant correlation with any of the noncholesterol sterols, whereas apo B-100 concentrations were positively correlated with the markers of cholesterol synthesis and negatively with the markers of cholesterol absorption. We assume that cholesterol synthesis exerts stronger effects on the apolipoprotein ratio than does cholesterol absorption, because of the stronger correlation of apo B-100 with lanosterol than with campesterol. Equally, HOMA-IR correlates significantly with lanosterol, but not with campesterol. This effect was previously shown for type 2 diabetes patients in a short-term weight-reduction trial (17) and in patients with the metabolic syndrome (27). The clinical significance of the overall long-term increase in cholesterol absorption and synthesis regarding the CVD risk is still unknown. Such changes have been shown to be detrimental in other clinical studies, such as the 4S (10, 45) and a recent investigation in patients without diabetes mellitus (46). On the other hand, the favorable effects of weight loss on the ratio of apo B-100 to apo A-I probably counteract these effects. Thus, additional clinical studies of the overall balance are warranted.

Our investigation is limited because the monitoring of cholesterol metabolism by plasma noncholesterol sterols does not allow a quantitative analysis of the cholesterol absorption and synthesis compared with labeled isotope techniques (2). Moreover, cholesterol metabolism has a strong heritable component (45, 47–50). Thus, further clinical studies of the effect of dietary weight loss on cholesterol metabolism should also focus on its association with genetic polymorphisms.

In conclusion, a 2-y dietary weight-loss intervention was associated with a characteristic response of cholesterol metabolism, distinguished by an initial increase in the markers of cholesterol absorption and a decrease in the markers of cholesterol synthesis during the initial 6 mo of active weight loss, which was followed by a previously undescribed pronounced rebound in cholesterol synthesis during the following weight-maintenance/partial-regain period. We found no significant differential effect of the various dietary regimens on cholesterol metabolism. The changes in cholesterol metabolism had an effect on apolipoprotein ratios. Whereas the ratio of apo B-100 to apo correlated with markers of cholesterol synthesis and absorption, apo A-I concentrations seem to be independent of them. Further clinical studies are required to evaluate the effect of these dietary induced long-term effects of cholesterol metabolism on CVD risk prediction and therapeutic cholesterol-lowering strategies.

The authors’ responsibilities were as follows—ABL: designed and computed the statistical analyses and wrote the manuscript; CH and UC: performed the liquid chromatography–tandem mass spectrometry experiments; YG, DS, and RG: collected the data; IS and YH: designed and performed the study, provided important advice concerning the data analysis, and wrote the manuscript; MIS: contributed significantly to the data interpretation and revised the manuscript; MB and MS: contributed to the study and analytical design; JT: provided...
scientific advice; and GMF: conceived the study and wrote and redacted the manuscript. All authors stated that they had no conflicts of interest. The Dr. Robert C. and Veronica Atkins Research Foundation was not involved in any stage of the design, conduct, or analysis of the study and had no access to the study results before publication.

REFERENCES