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Modest Diet-Induced Weight-Loss Reduces Macrophage Cholesterol Efflux to Plasma of Metabolic Syndrome Patients

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Abstract

BACKGROUND—Obesity-linked metabolic syndrome (MetS) is associated with a dyslipidemic profile that includes hypertriglyceridemia and low plasma high-density lipoprotein cholesterol (HDL-C). HDL initiates reverse cholesterol transport (RCT) via macrophage cholesterol efflux (MCE). Some hypothesize that dyslipidemic patients have impaired RCT. MCE to patient plasma, a metric of HDL function, inversely correlates with atherosclerotic burden. Paradoxically, MCE to plasma of hypertriglyceridemic subjects is higher than that to normolipidemic (NL) plasma.

OBJECTIVE—Although weight-loss reduces dyslipidemia, its effect on MCE to the plasma of obese, MetS patients is unknown. Thus, we tested the hypothesis that reducing dyslipidemia with weight-loss reduces the MCE capacity of MetS plasma to that of NL plasma.

METHODS—Cholesterol efflux (MCE) from THP-1 macrophages to plasma from NL controls and to obese, MetS patients before and after weight-loss was measured.

RESULTS—MCE to plasma of obese, MetS patients was higher than that to control plasma ($p=0.006$). Weight-loss in MetS patients (mean = -9.77 kg) reduced dyslipidemia, insulin resistance and systolic blood pressure. HDL-C was unchanged and apolipoprotein A-I decreased with weight-loss. Weight-loss in MetS patients normalized MCE ($p<0.001$) to that of NL subjects. MCE correlated with apolipoprotein B levels ($r^2 = 0.13 - 0.38$). Chromatography showed that macrophage cholesterol initially associates with HDL but accumulates in apolipoprotein B-containing lipoproteins at later times.

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CONCLUSIONS—While the initial acceptor of macrophage cholesterol efflux is HDL, the elevated apo B lipoproteins are a cholesterol sink that increase MCE in MetS patients. Weight loss results in decreased apo B-lipoproteins and decreased MCE to plasma of MetS patients.

Keywords

Metabolic Syndrome; Obesity; Weight Loss; Lipoproteins; Macrophage Cholesterol Efflux

INTRODUCTION

The prevalence of metabolic syndrome (MetS) with obesity in adults and children has increased over the past four decades, with a concomitant increase in its major comorbidity, cardiovascular disease (CVD). Although obesity-linked insulin resistance is a risk factor for atherosclerosis,^{1, 2} the mechanistic links between obesity, atherogenesis and its other risk factors remain poorly defined. Obesity predisposes to MetS, which is associated with a proatherogenic dyslipidemic phenotype—insulin resistance, elevated plasma triglycerides, low plasma high-density lipoprotein (HDL) cholesterol (-C), and the occurrence of small, dense low-density lipoproteins (LDL). HDL particles are dynamic multi-molecular complexes that function as vehicles for reverse cholesterol transport (RCT),³ i.e., the transfer of cholesterol from subendothelial macrophages to the liver for disposal or recycling. Initial RCT acceptors of macrophage-cholesterol efflux include apolipoprotein (apo) A-I and various forms of HDL.^{4–6} RCT, which removes the burden of excess cholesterol from arterial macrophages, underlies the putative antiatherosclerotic effects of HDL.

The most important positive determinants of cholesterol efflux are cellular lipid concentration and acceptor protein and lipid composition and charge. Cholesterol efflux is increased by increasing the phospholipid content of acceptors, including whole plasma or purified HDL.^{7–11} The kind of phospholipid is also important, with sphingomyelin, which is highly cholesterolophilic,¹² being a better acceptor than phosphatidylcholine.¹³ The cholesterol to phospholipid ratio of the acceptor is also important;¹⁴ as the cholesterol in reassembled HDL (rHDL) rises above 15 mol%, it becomes a net donor rather than an acceptor.¹⁰ Lastly, acceptor charge and size are also determinants of efflux; pre β -1 HDL, a small discoidal particle with less net negative charge than α -HDL particles,¹⁵ is a preferential acceptor of macrophage-cholesterol and is the major contributor to efflux to plasma that is independent of HDL-C.¹⁶

Insulin resistance alters cholesterol and triglyceride metabolism and predisposes to impaired anti-atherosclerotic mechanisms, including RCT. The conventional hypothesis is that individuals with atherogenic dyslipidemia have impaired RCT that leads to atherogenesis. However, macrophage cholesterol efflux (MCE) from adenosine triphosphate-binding cassette transporter A1 (ABCA1)-upregulated macrophage cell lines to sera of non-diabetic Type IV hypertriglyceridemic (HTG) subjects is actually higher than that to normolipidemic (NL) Controls.¹⁷ Recent studies measured MCE to apo B depleted serum, rather than whole serum, and showed that efflux to the whole HDL fraction was inversely correlated to the degree of atherogenic burden, as measured by carotid intima-media thickness as well as the

likelihood of angiographic coronary artery disease.¹⁸ While this inverse correlation of efflux to apo B depleted serum with prevalent coronary disease has been confirmed, the same study found a paradoxical association of enhanced cholesterol efflux with increased incident cardiovascular risk.¹⁹

The effect of weight-loss, which lowers plasma triglycerides, on MCE in obese, MetS individuals is unknown. Given the positive relationship between obesity and a high plasma triglyceride level and that MCE to plasma from Type IV HTG subjects is greater than that to NL Control plasma, we tested the hypothesis that MCE to the plasma of obese, MetS patients would be reduced to that of NL controls after diet-induced weight-loss, which lowers plasma triglyceride levels.

METHODS

Patient Recruitment

We recruited volunteers that included obese patients with a body mass index (BMI) >30 (MetS, $n = 24$) and NL Controls ($n = 24$) with a BMI between 20 and 25 kg/m². In this study, MetS was defined according to the American Heart Association (AHA) / National Heart Lung and Blood Institute (NHLBI) consensus definition, updated in 2005, in which the fasting glucose level was lowered from 6.11 to 5.55 mmol/L, based on the definition of MetS developed by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). At enrollment, 13/24 of the obese MetS patients had fasting glucose > 5.55 mmol/L and 3/24 were diabetic. The only exception to the AHA/NHLBI definition was the use of body mass index (BMI) ≥ 30 kg/m² as a surrogate for waist circumference. Age and gender were recorded for each patient. Anthropometric data included weight, height, blood pressure, and waist circumference. Recruitment of MetS patients was limited to individuals electively enrolled at The Methodist Hospital Medical Weight Management Program. Control subjects were recruited from Baylor College of Medicine personnel. Recruitment was conducted from January 2005 through May 2009. The study was approved by the Institutional Review Boards of Baylor College of Medicine and The Methodist Hospital. All participants were educated about the nature and duration of the study and provided written informed consent. Consideration for patient enrollment in the Weight Management Program included a prescreening of the patient's physical chart and laboratory data at an initial pre entry visit. Fasting glucose and lipid profile (total cholesterol, triglyceride, HDL-C and calculated LDL-C), liver function tests, blood urea nitrogen (BUN), creatinine, and serum insulin were measured. Insulin resistance was estimated on the basis of fasting glucose and serum insulin levels and the homeostatic model assessment of insulin resistance (HOMA-IR). The MCE assays, described in detail below, were performed on all samples. Both anthropometric and laboratory data were collected at the baseline visit, prior to entering the weight management program, and 4–6 weeks after entry. Exclusion criteria for patients and controls included the presence of a known eating disorder, active cancer diagnosis, use of lithium or steroids, type 1 diabetes mellitus, active inflammatory bowel disease, active gout, liver disease, cardiovascular event within the preceding 3 months, an endocrine cause for obesity, pregnancy or treatment with a diuretic.

Weight Management Program

The Medical Weight Management Program at The Methodist Hospital is located in a tertiary care medical center, and is a specialized medically-monitored nutrition-based program designed to accelerate weight-loss through a low-carbohydrate, high-protein liquid diet that limits caloric intake to 600–800 kcal/day. Patient enrollment occurs by either self-referral or referral by a healthcare provider. Evaluation prior to initiation of the program includes a comprehensive physical exam by a physician as well as an electrocardiogram and laboratory studies. Consultation with a registered dietician and psychological counseling are provided throughout involvement in the program. Weekly monitoring by a nurse includes 1 hour of education on the weight-loss program and assessment of weight and blood pressure; laboratory data are assessed every 4 weeks. Monthly examination by the medical director ensures careful attention to patients' progress and safety. Study participants were followed for the first 4 to 6 weeks after initiation of the weight management program.

MCE Assay

At each visit, 25 mL of whole blood was drawn, centrifuged (2400 rpm), and plasma was isolated and stored at -80°C within 2 hours of collection. Human monocyte-derived macrophage cell line THP-1 cells (American Type Culture Collection, Manassas, Virginia, USA) were maintained in RPMI 1640 medium supplemented with 10% FBS, 100 units/mL penicillin, 100 mg/mL streptomycin, 1 mmol/L sodium pyruvate, and 2 mmol/L glutamine. Tissue culture reagents were from Invitrogen (Carlsbad, California, USA). To activate cells and elicit the macrophage phenotype, phorbol myristate acetate (PMA) (Sigma, St. Louis, Missouri, USA) was added to the cell media to a final concentration of 100ng/mL for 72 h. Cholesterol efflux was assayed as described previously.^{20, 21} In brief, cells were seeded and grown for 24 h, then labeled in serum-containing medium with [$1\alpha, 2\alpha(n)$]- ^3H]cholesterol (specific radioactivity 1.81TBq/mmol, final radioactivity 74 KBq/mL) for 48 h at 37°C in a 5% CO_2 incubator at which time they were ~ 80% confluent. After labeling, cells were washed twice with $\text{Ca}^{2+}/\text{Mg}^{2+}$ free PBS to remove excess label, and incubated for an additional 18 h in serum-free medium (SFM) to ensure equilibration of labeled cholesterol into all intracellular pools. During this phase, ABCA1 and adenosine triphosphate-binding cassette transporter G1 (ABCG1) receptor expression was up regulated by adding 1 $\mu\text{mol/L}$ of the live X receptor (LXR) agonist (TO-901317) (Sigma, St. Louis, Missouri, USA). Efflux assays were initiated by adding the cholesterol acceptor, 3% plasma in SFM, to the cells and incubating for 2 hrs. After incubation, the medium was collected, centrifuged (5 min at 14,000 rpm) and the supernatant β -counted. Cell lipids were extracted and analyzed by β -counting. Cholesterol efflux was expressed as the percentage of total labeled cholesterol transferred from cells to the medium. Each assay was performed in triplicate. Initial kinetic studies established that cholesterol efflux to 3% plasma was linear for 2 hrs (Figure 2A). Methodological analyses included comparisons of fresh to frozen plasma samples and determination of assay variability.

Size Exclusion Chromatography (SEC)

Aliquots of selected samples were analyzed by SEC over Superose HR6 (GE Healthcare) to reveal the distribution of effluxed cellular cholesterol into the lipoprotein subclasses, VLDL,

LDL and HDL, of the acceptor plasma²¹. [³H]-Cholesterol labeled cells were incubated with 3% plasma in media for 10 min to 2 hr, media was collected, centrifuged and a 200 μ L aliquot was injected into SEC columns. One mL fractions were collected and β -counted. The percent of cholesterol associated with each lipoprotein fraction was calculated from the ratio of total counts in each lipoprotein peak to the total counts eluting from the column.

Statistical Analysis

Analysis of anthropometric and clinical parameters, and % Net Efflux between MetS and NL samples indicated that many of these parameters failed normality and equal variance tests, so the data for these parameters in Tables 1 and 2 are given as median values with 25% – 75% ranges. Values in the abstract and text are mean $\pm$ standard deviation (SD) unless otherwise indicated. The Mann Whitney Rank Sum test was used to determine if MetS and NL values were significantly different. Chi-square test with Yates correction was used to compare Gender (%F) in the two groups. Comparison of these parameters in MetS patients at baseline and after weight-loss was done with the Wilcoxon Signed Rank Sum Test for paired samples. While the number of samples was 24 for the majority of variables assessed in the NL to MetS at baseline comparison, and 22 paired samples for the MetS baseline to MetS after weight-loss comparison, not all analytes were determined for all samples, and the respective n values for these are indicated in the footnotes to Tables 1 and 2. Linear regression correlation coefficients and p values as well as the rank sum tests were calculated using SigmaPlot for Windows Version 11.0 (Systat Software, Inc.) and the Chi-square test for Gender (%F) was done with the Graph Pad calculator (<http://www.graphpad.com/quickcalcs/contingency1.cfm>).

RESULTS

Anthropometric and Clinical Lipid Parameters—MetS at baseline versus NL control

A total of 24 controls and 24 MetS patients at baseline were studied, and 22 paired MetS samples were reassessed 4 to 6 weeks after initiation of the weight management program. Table 1 contains the baseline characteristics of the participants. Comparison of anthropometric parameters shows that the NL and MetS groups were well-matched for age, gender and height, but as expected, MetS baseline values for weight, BMI, and waist, as well as blood pressure, were significantly higher than in the NL cohort. Blood lipid chemistry reflected the dyslipidemia in the MetS patients: Triglyceride, non-HDL-C, and apo B concentrations were significantly elevated, while HDL-C concentrations were low. However total cholesterol, LDL-C and apo A-I concentrations were not significantly different from those of the NL group. Glucose, insulin and HOMA-IR values were all elevated, consistent with insulin resistance in MetS patients.

Effects of Weight-loss on Clinical Parameters

The time each patient remained in the weight management program varied between 4 and 12 weeks. We continued to monitor patients who remained in the program longer as their diet transitioned from liquid-only to solid food. This transition was personalized to each patient's weight-loss rate according to the medical judgment of the supervising physician. Patients enrolled in our efflux study were reassessed between 4 and 6 weeks after initiation of the

very low calorie liquid diet, to ensure that the liquid-only, calorie-controlled intervention in the study was consistent for all study patients. At follow-up, the mean weight-loss was 9.77 ± 3.51 kg and despite this moderate weight-loss, the patients still satisfied the BMI criterion for obesity. Nevertheless, this weight-loss was associated with the improvement of numerous MetS risk factors (Table 2). Weight-loss reduced median BMI (–10%), waist circumference (–4%), systolic blood pressure (–6%) and plasma concentrations of apo B (–15%), triglyceride (–35%), non-HDL-C (–14%), total cholesterol (–12%), LDL-C (–12%), and serum insulin (–29%). According to the HOMA-IR algorithm, weight loss also reduced insulin resistance (–31%). Changes in HDL-C with weight-loss were not significant while plasma apo A-I levels decreased (–16%).

MCE—Assay Validation

For MCE, we used the macrophage cell line THP-1 because it expresses ABCA1 and ABCG1, the major cholesterol transporters in human monocyte-derived macrophages.^{22, 23} Efflux to 3% plasma was linear over the 2hr time used for this study (Figure 2A). We confirmed previous studies showing that storing human HDL at –80 °F in sucrose preserves much of its functionality.²⁴ The rates of cholesterol efflux to freshly isolated plasma and frozen plasma stored at –80°F were tested for 30 paired samples and were highly correlated ($r^2 = 0.966$) with an average coefficient of variance (CV) of $\pm 7.3\%$, confirming that assays of fresh and frozen plasma samples did not differ significantly. All the NL and MetS samples analyzed in this study were assayed in triplicate with an average with-in day CV of $\pm 11.7\%$. Repeat analysis of samples in separate assays gave an average day-to-day CV of $\pm 15.8\%$.

MCE—Comparison of Efflux to NL plasma and MetS Plasma before and after Weight Loss

Our baseline MCE data were like those of Fournier,^{25, 26} who studied HTG patients. We found that MCE to MetS patient plasma was greater than that to NL control plasma (median values of 7.14 vs. 6.11 %/2h, $p=0.006$; Figure 1). However, post weight-loss, efflux to MetS patient plasma “normalized” i.e., decreased to that observed with NL plasma (MCE = 6.25 %/2h; $p=0.001$ compared to MetS baseline, Figure 1).

Cholesterol effluxed to HDL transfers to apo B lipoproteins

Using size exclusion chromatography (SEC), we previously showed that MCE first appears in HDL,²¹ which has by far the highest plasma lipoprotein molarity,²⁷ i.e., many more particles, and then transfers to the apo B-containing lipoproteins that are more cholesterophilic.²⁸ This was confirmed to be the case under the conditions used in the present study, efflux to 3% plasma (Figure 2B and 2C). Extrapolation of the fitted data of Figure 2C to $t = 0$ showed that initially, 71% of MCE transfers to HDL. While most of the effluxed cholesterol is associated with HDL at early time points, by 2 hours most appears with the apo B lipoproteins. Of note, little if any of the effluxed cholesterol is associated with albumin or lipid free apo A-I, which elute 2 and 3 mL respectively after the HDL peak. This contrasts with the report that ~ 60% of effluxed cholesterol is associated with albumin and lipid-poor apo A-I when apo B depleted serum is used as the acceptor.¹⁹ Consistent with the occurrence of HTG among the MetS patients, these patients have elevated apo B (Table 1), thus one would expect them to have more apo B-containing lipoproteins, so we

hypothesized that the underlying cause for efflux differences was due to free cholesterol (FC) transfer to the apo B-containing lipoproteins. This hypothesis was supported by SEC data (Figure 3) showing that the proportion of [^3H]cholesterol associated with the apo B-containing lipoproteins (VLDL and LDL peaks of Figure 3) of the pre weight-loss MetS patients was higher than that of control but was reduced by weight-loss. The percent of total effluxed [^3H]cholesterol associated with (LDL + VLDL) from NL subjects (shaded curve) was less than that from MetS patients both pre and post weight-loss. In MetS patients the amount of [^3H]cholesterol associated with (LDL + VLDL) was reduced with weight-loss (white and black circles, respectively). The percent (VLDL + LDL)-associated [^3H]cholesterol, calculated from the sum of the radioactivity associated with VLDL and LDL was 67, 58, and 35% respectively for MetS patients pre and post weight-loss and for NL subjects.

MCE and its Correlation to Plasma Lipoprotein Parameters

We found that MCE correlated with the plasma levels of some lipoprotein analytes. Surprisingly, cholesterol efflux was only weakly positively correlated with NL plasma HDL-C, and weakly negatively correlated with HDL-C for MetS patients pre and post weight-loss (Figure 4 A – C respectively). Moreover, when the data were combined, there was virtually no correlation (Figure 4 D). Efflux was also disparately and weakly correlated with apo A-I (Figure 4 E – F); weight-loss restored the efflux-apo A-I correlation to the NL profile with a similar slope and correlation coefficient (Figure 4 E, G). Similarly, the combined efflux vs. apo A-I data (Figure 4 H) showed a weak positive correlation. Equally surprising, non HDL-C (Figure 4 M – P) and especially apo B (Figure 4 I – L) better predicted efflux than HDL-C or apo A-I. The correlations with apo B ($r^2 = 0.12 - 0.38$) were better than those with non-HDL-C ($r^2 = 0.04 - 0.12$), suggesting that apo B particle number is more important than apo B particle mass. Interestingly, the Y-intercepts for apo B and non HDL-C are ~5%, which represents the contribution of HDL to MCE in the absence of apo B-containing lipoproteins. These correlations suggest that in plasma apo B and non HDL-C levels account for differences in MCE rates in NL controls and in MetS patients, before and after weight-loss.

DISCUSSION

Modest Weight-loss among Morbidly Obese, MetS Patients Improves Diabetes and CVD Risk Factors

This is the first study of the effects of weight-loss among obese, MetS patients on biomarkers of insulin resistance, CVD and RCT. We showed that the initial RCT step, MCE, was higher at baseline in the obese, MetS patients compared to controls, and that weight-loss reduced the MCE to the levels of NL controls. Wang et al also found that MCE to whole and apo B-depleted plasma of obese type 2 diabetic patients is reduced following prolonged (16-week) caloric restriction.²⁹ Comparison of NL control volunteers and MetS patients showed that weight-loss brought glucose, insulin and HOMA-IR, and CVD risk factors (systolic blood pressure, apo B, triglycerides, total cholesterol, and LDL-C) within or closer to optimal levels. These improvements occurred in spite of only modest weight and BMI reductions, since the patients remained obese, with a median BMI of 36.7 kg/m². This

was a study of the effects of weight loss on patients with MetS, so cannot be generalized to obese patients without MetS; however, it suggests that even moderate weight loss improves not only BMI but individual components of MetS, i.e., hypertension, plasma lipid profiles, and insulin resistance.

HDL and the Apo B-Containing Proteins are MCE Acceptors

We previously showed and confirm here (Figure 2) that in whole plasma, HDL particles are the primary early acceptors of MCE but that with time, the MCE distribution profile among lipoproteins shifts to the apo B-containing lipoproteins (VLDL + LDL), which are the ultimate depots for most of the [^3H]cholesterol.²¹ Consistent with this, the %FC efflux is strongly correlated with plasma apo B and non-HDL-C content (Figure 4), and not with HDL-C or apo A-I. In a study of obese, diabetic patients, Wang et al also observed that % cholesterol efflux correlates with plasma non-HDL-C and not with HDL-C.²⁹ Within this context, Rothblat suggested that LDL is a sink for plasma cholesterol, and that albumin may well mediate cholesterol transfer from HDL to LDL. This process of cholesterol transfer from HDL to the apo B lipoproteins may be facilitated by various lipid components, such as cholesteryl ester transfer protein (CETP), which is elevated in obese subjects²⁹ and by albumin.^{30, 31} We suggest that efflux to whole plasma is more physiologically relevant than to apo B depleted plasma or serum,^{18, 19} in which the HDL component readily saturates and the effluxed cholesterol associates with albumin rather than the apo B lipoproteins as we observed for whole plasma.

There have been two important reports of the relationship between macrophage cholesterol efflux to apo B lipoprotein-depleted plasma, a surrogate for HDL, and cardiovascular disease. One found efflux correlated inversely with both carotid intima-media thickness and the likelihood of angiographically confirmed coronary artery disease, an observation that was independent of the HDL-C level.¹⁸ In contrast, the other reported that efflux magnitude was associated with increased prospective risk for myocardial infarction, stroke, and death, and moreover, that the majority of cholesterol efflux was destined for non HDL particles.¹⁹ Although criteria for patient selection were somewhat different, this would not be expected to produce contradictory findings. It is notable however, that the latter study revealed cholesterol efflux to both lipoprotein and non lipoprotein fractions, including serum albumin and apo A-I, as previously reported by Fielding.³⁰ In this context, perhaps it would be more appropriate to correlate plasma functionality, i.e., efflux to unfractionated plasma, with the severity of angiographically confirmed CVD.

Our data suggests that the lipoprotein distribution profile of cholesterol following MCE is controlled by kinetic factors at early efflux times and thermodynamic factors at longer efflux times. The number of HDL particles is nearly ten times that of all the other lipoproteins combined²⁷, therefore at early time points, the more numerous HDL particles are the more probable acceptor of MCE although the smaller numbers of LDL and VLDL are also acceptors. However, once bound to lipoproteins, the fate of cholesterol is determined by a different set of kinetics, that is, its rate of desorption from lipoprotein surfaces. Since the rate of lipid desorption from lipid particle surfaces varies inversely with the size of the particle, once having desorbed from that surface the rate of association with acceptor

particles is largely diffusion-controlled.³² Therefore, once associated, HDL-C is 15 times more likely than LDL-C to transfer to another lipoprotein, and that is also true for a comparison of HDL and VLDL. One would also expect the largest number of interparticle transfers to be silent, i.e., among the more abundant HDL. However, in parallel with the cholesterol transfer among HDL particles, a small fraction of cholesterol transfers to LDL and VLDL, where it is retained due to high LDL and VLDL cholesterophilicity, and released at much lower rates.^{28, 32} In the end, however, the slow rate of release of cholesterol from LDL and VLDL makes them the major sites of cholesterol accumulation.²⁷

Although some studies show that macrophage cholesterol efflux is atheroprotective, there is still debate about the importance of cholesterol efflux in CVD. Mouse models in which adenosine triphosphate-binding cassette transporters, ABCA1 and ABCG1, have been ablated present with foam cell formation and accelerated atherosclerosis.³³ Tangier disease, which results from various loss-of-function mutations in the ABCA1 transporter, is also associated with atherosclerosis.³⁴ The clinical importance of HDL function in cholesterol efflux capacity was demonstrated by Khera et al¹⁸ who reported that atherosclerosis as assessed by carotid artery intima-media thickness was negatively correlated with MCE to apo B depleted patient sera, i.e. to the whole HDL fraction. Our studies extend this work by demonstrating that while initial cholesterol efflux is primarily to HDL, the apo B lipoproteins in whole plasma support additional MCE by acting as a sink for the effluxed cholesterol, and that reducing plasma apo B, in this study by weight-loss, reduces MCE capacity.

A potential limitation of our study is that the patients were participating in an active weight loss program and their weight was not stable at the time of the second evaluation. As a result, HDL-C levels, which did not change significantly during the course of this study, were most likely not at a new steady state, and this could influence efflux results. We feel, however, that our results are valid since the efflux measure was strongly correlated with the change in atherogenic particle number (apo B and non-HDL-C), and not with HDL-C, and hence a steady state in HDL-C would not have made an appreciable difference.

CONCLUSION

These data and our study compel one to reconsider the atheroprotective value of MCE from a different perspective. In spite of the reported negative correlation between carotid artery intima-media thickness and MCE to the same patient's HDL fraction,¹⁸ maximizing cholesterol efflux to whole plasma is not necessarily the optimal therapeutic endpoint. Indeed a new report¹⁹ of association of enhanced MCE with increased incident cardiovascular risk begs further analysis of the interpretation of cholesterol efflux results. There is overwhelming evidence that reducing hypertension, total cholesterol, LDL-C, non HDL-C, apo B and HOMA-IR are atheroprotective. By the same token, reducing these risk factors by weight-loss (Table 2) must also be considered a good therapeutic strategy in spite of the decrease in MCE. The much higher CVD risk in diabetic and MetS patients compared to control non-diabetic subjects suggests that the reduction in cholesterol efflux to that of control plasma is not as important as reducing the traditional CVD risk factors, especially the number of apo B-containing lipoproteins. Thus, putative detrimental effects of weight

loss-associated decreases in MCE to total lipoproteins are outweighed by reduction of other risk factors. In the MetS population, other RCT steps beyond MCE, such as selective hepatic cholesterol uptake, may be more important.

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ABBREVIATIONS

ABCA1	Adenosine Triphosphate-binding cassette transporter A1
ABCG1	Adenosine Triphosphate-binding cassette transporter G1
AHA	American Heart Association
Apo	apolipoprotein
BMI	body mass index
BUN	blood urea nitrogen
-C	cholesterol
CETP	cholesteryl ester transfer protein
CV	coefficient of variance
CVD	cardiovascular disease
FBS	fetal bovine serum
FC	free cholesterol
HDL	high density lipoprotein
HOMA-IR	homeostatic model assessment of insulin resistance
HTG	hypertriglyceridemic
LDL	low density lipoprotein
LXR	liver X receptor
MCE	macrophage cholesterol efflux
MetS	metabolic syndrome
NCEP-ATP III	National Cholesterol Education Program Adult Treatment Panel III
NHLBI	National Heart Lung and Blood Institute
NL	normolipidemic
PBS	phosphate buffered saline
PMA	phorbol myristate acetate
RCT	reverse cholesterol transport

rHDL	reassembled HDL
SD	standard deviation
SEC	size exclusion chromatography
SFM	serum free medium
VLDL	very low density lipoprotein

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HIGHLIGHTS

Macrophage cholesterol efflux to plasma of metabolic syndrome patients is elevated

Initial efflux to HDL is followed by transfer to apo B lipoproteins

Amount of efflux correlates with plasma apo B levels

Weight loss decreases plasma apo B and cholesterol efflux

Figure 1.

Comparison of MCE to plasma from NL controls and obese, MetS patients, pre and post weight-loss as labeled. The horizontal bar denotes the median for each group. Wilcoxon rank sum p values are given in Tables 1 and 2.

MCE = macrophage cholesterol efflux; MetS = metabolic syndrome; NL = normolipidemic

Figure 2.

Kinetics and lipoprotein distribution of $[^3\text{H}]\text{FC}$ after MCE from THP-1 macrophage cells to 3% NL plasma. A. Time course for efflux. Efflux is linear over the time 0 – 2 hr. B. Percent of effluxed $[^3\text{H}]\text{FC}$ associated various plasma lipoproteins as a function of time after efflux from THP-1 macrophage cells. Time of efflux for sampling of the media for SEC was 10, 20, 30, 60 and 120 min respectively as indicated on each chromatogram from top to bottom. C. Plot of $\% [^3\text{H}]\text{FC}$ associated with the HDL peak as a function of time of efflux. A fit of the data to a single 3-parameter exponential curve gave $\% \text{HDL-}[^3\text{H}]\text{FC} = 29.1 + 45.5\exp(-0.060t)$; $r^2 > 0.87$. Extrapolation to zero time indicates that initially $\sim 75\%$ of effluxed cholesterol is associated with HDL.

HDL = high density lipoprotein; MCE = macrophage cholesterol efflux; NL = normolipidemic; SEC = size exclusion chromatography

Figure 3.

Distribution of [3H]-cholesterol among plasma lipoproteins after efflux from macrophage cells as labeled. Cell media was sampled after 2 hrs of efflux to 3% plasma, and SEC fractions were analyzed for [3H]-cholesterol. [3H]-cholesterol profiles of NL, MetS pre weight loss (pre WL) and post weight loss (post WL) are indicated by grey shading, black circles and open circles respectively. The fraction of total cholesterol associated with the apo B peaks (VLDL + LDL) was NL < MetS post WL < Met S pre WL.

apo = apolipoprotein; LDL = low-density lipoprotein; MetS = metabolic syndrome; NL = normolipidemic; SEC = size exclusion chromatography; WL = weight loss
VLDL = very low-density lipoprotein

Figure 4.

Correlation of MCE to plasma from control and MetS patients, pre and post weight-loss with plasma analytes. In the All Groups panels D, H, L and P symbols are Control (black), MetS pre weight-loss (white) and MetS post weight-loss (gray). Correlation coefficients are indicated in each panel. ANOVA analysis indicated significant correlations % efflux vs. apo B (panels I ($p=0.018$), K ($p=0.030$), L ($p<0.001$) and % efflux vs. non-HDL-C (Panel P, $p=0.003$).

ANOVA = analysis of variance; apo = apolipoprotein; HDL-C = high-density lipoprotein cholesterol; MCE = macrophage cholesterol efflux; MetS = metabolic syndrome

Table 1

Comparison of MetS Patients at Baseline to Normolipidemic Controls.

	NL Control		MetS Patients at Baseline		Rank Sum [‡]
	n=24		n=24		p value
	median	range	median	range	
		(25%–75%)		(25%–75%)	
Anthropometric Parameters					
Age (years)	46.0	(33.5–53.0)	46.0	(40.0–50.8)	0.918
Gender (%F)	62.5%		58.3%		0.768 [€]
Weight (kg)	63.5	(59.4–75.6)	122.9	(101.3–138.6)	<0.001
Height (cm)	168	(160–178)	170	(163–183)	0.563
BMI (kg/m ²)	23.3	(22.0–25.0)	40.6	(35.3–46.8)	<0.001
Waist (cm)	76.2	(73.7–86.4)	129.5	(114.3–134.6)	<0.001
Systolic blood pressure (mm Hg)	120	(112–126)	138	(130–148)	<0.001
Diastolic blood pressure (mm Hg)	76	(70–80)	80	(75–90)	0.037
Clinical Parameters					
Apo A-I* mg/dL)	128	(115–183)	119	(101–136)	0.103
Apo B* (mg/dL)	76	(56–94)	98	(91–122)	0.002
Triglyceride (mmol/L)	0.89	(0.61–1.10)	1.79	(1.08–2.18)	<0.001
Total cholesterol (mmol/L)	4.56	(3.83–5.31)	4.61	(4.35–5.18)	0.672
HDL-C (mmol/L)	1.55	(1.27–2.12)	0.98	(0.91–1.27)	<0.001
LDL-C (mmol/L)	2.49	(1.97–3.47)	2.82	(2.43–3.50)	0.163
Non-HDL-C (mmol/L)	3.08	(2.23–3.94)	3.73	(3.34–3.83)	0.043
Glucose (mmol/L)	5.22	(4.83–5.61)	5.72	(5.05–6.49)	0.030
AST [‡] (U/L)	18.0	(16.0–23.0)	18.5	(15.8–25.8)	0.487
ALT [‡] (U/L)	15.0	(11.0–21.5)	20.0	(15.8–37.3)	0.018
BUN* (mmol/L)	6.07	(4.93–6.43)	5.18	(5.00–5.71)	0.089
Creatinine* (μmol/L)	86.6	(74.3–98.1)	76.9	(70.7–89.3)	0.154
Insulin (μUI/mL)	4.00	(3.00–5.75)	15.5	(9.0–33.8)	<0.001
HOMA-IR	0.85	(0.60–1.25)	3.76	(2.23–9.81)	<0.001
% Net Efflux	6.11	(5.71–7.09)	7.14	(6.31–8.38)	0.006

Apo = apolipoprotein; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; BUN = blood urea nitrogen; F = female; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostatic model assessment of insulin resistance; LDL-C = low-density lipoprotein cholesterol; MetS = metabolic syndrome; NL = normolipidemic

* n=14.

[‡] n=21 for NL controls, n=22 for MetS Baseline.

[‡] Mann-Whitney Rank Sum Test p value.

[€] Chi-square with Yates correction p value.

Table 2

Comparison of MetS Patients at Baseline and after Weight Loss.

	MetS Patients at Baseline		MetS Patients after Weight Loss		Rank Sum [‡]
	n=22		n=22		p value
	median	Range (25%–75%)	median	Range (25%–75%)	
Anthropometric Parameters					
Weight (kg)	123	(104–135)	116	(94–124)	<0.001
BMI (kg/m ²)	40.6	(34.8–47.3)	36.7	(31.8–43.3)	<0.001
Waist (cm) [†]	127	(114–134)	121	(110–126)	<0.001
Systolic blood pressure (mm Hg)	138	(130–151)	130	(121–138)	<0.001
Diastolic blood pressure (mm Hg)	80	(74–92)	82	(77–86)	<i>0.899</i>
Clinical Parameters					
Apo A-I* (mg/dL)	117	(100–136)	98	(86–108)	0.002
Apo B* (mg/dL)	98	(93–125)	84	(71–104)	0.001
Triglycerides (mmol/L)	1.74	(1.03–2.23)	1.13	(0.70–1.54)	<0.001
Total cholesterol (mmol/L)	4.71	(4.38–5.31)	4.14	(3.50–4.92)	<0.001
HDL-C (mmol/L)	1.06	(0.91–1.27)	1.05	(0.86–1.18)	<i>0.231</i>
LDL-C (mg/dL)	2.85	(2.46–3.52)	2.51	(2.05–3.21)	0.027
Non-HDL-C (mmol/L)	3.73	(3.34–3.99)	3.21	(2.43–3.89)	0.002
Glucose (mmol/L)	5.55	(5.00–6.72)	5.22	(4.77–5.94)	<i>0.105</i>
AST [†] (U/L)	18.0	(15.5–24.0)	23.5	(18.5–29.8)	<i>0.820</i>
ALT [†] (U/L)	20.0	(15.5–33.0)	23.0	(17.3–32.5)	<i>0.424</i>
BUN* (mmol/L)	5.35	(5.00–5.71)	5.35	(5.11–6.43)	0.032
Creatinine* (μmol/L)	76.9	(71.6–89.3)	78.7	(73.4–92.8)	0.042
Insulin (μUI/mL)	15.5	(8.8–33.3)	11.0	(5.5–15.5)	0.010
HOMA-IR	3.76	(2.16–8.08)	2.61	(1.39–3.74)	0.014
% Net Efflux	7.14	(6.38–8.40)	6.25	(4.84–7.20)	0.001

Apo = apolipoprotein; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; BUN = blood urea nitrogen; HDL-C = high-density lipoprotein cholesterol; HOMA-IR = homeostatic model assessment of insulin resistance; LDL-C = low-density lipoprotein cholesterol; MetS = metabolic syndrome

* n=12 or 13.

[†] n=21 or 22 for MetS Baseline, n=12 for MetS after weight loss.

[‡] Wilcoxon Signed Rank Sum Test p value.