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Serum sphingomyelin levels are related to the clearance of postprandial remnant-like particles

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Abstract **It is known that sphingomyelin (SM) content is higher in apolipoprotein B-containing particles (BLps) than in high density lipoproteins and that BLp levels, including chylomicrons and their remnant particles, are positively related to atherosclerosis. To evaluate the relationship between serum SM and postprandial remnant particle levels, we determined SM, triglyceride (TG), and cholesterol levels in serum and in remnant-like particles (RLPs) before and 3, 5, 7, and 10 h after a high-fat meal in 31 healthy subjects. We found that serum SM, like serum TG, was increased to its maximum 3 h after fat loading and then gradually decreased to basal levels after 10 h. More important, we determined that SM and TG levels in RLPs were parallel. Serum SM was positively correlated with serum TG ($P < 0.001$), RLP SM ($P < 0.001$), RLP TG ($P < 0.001$), and RLP cholesterol ($P < 0.001$) levels. It is our conclusion that serum SM is a marker for the clearance of RLPs.—**Schlitt, A., M. R. Hojjati, H. von Gizycki, K. J. Lackner, S. Blankenberg, B. Schwaab, J. Meyer, H. J. Rupprecht, and X-C. Jiang. **Serum sphingomyelin levels are related to the clearance of postprandial remnant-like particles. *J. Lipid Res.* 2005.** 46: **196–200.**

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Sphingomyelin (SM) is the most abundant sphingolipid in plasma membranes, organelle membranes, and lipoproteins. Abnormal SM metabolism has been associated with atherosclerosis and other diseases (1, 2). It is well known that the SM content is much higher in apolipoprotein B-containing particles (BLps) or triglyceride-rich lipoproteins (TRLs) than in HDLs (3). Increased plasma levels of BLps, including chylomicrons, VLDLs, and their remnants, as well as LDLs, are acknowledged risk factors for cardiovascular disease (4, 5).

It has been suggested that retention of BLps on the subendothelial matrix and subsequent aggregation triggers macrophage foam cell formation and atherogenesis (6). BLp aggregation in the vessel wall may result from enzymatic modification of BLps, induced by locally produced sphingomyelinase (7).

In a case-control study (279 cases and 277 controls), we have previously shown that plasma SM levels are an independent risk factor for coronary artery disease and also demonstrated a significant correlation with remnant cholesterol levels (8). Based on these results, we hypothesized that SM could be a marker for the clearance of TRL remnants and that enrichment of SM on the remnants could be atherogenic. In the present study, we investigated the following: *1*) postprandial serum SM and remnant-like particle (RLP) SM levels after high-fat diet loading, and *2*) a possible predictive value of serum SM for RLP metabolism. We found that SM is a clearance marker for RLP and also that prolonging the metabolic rate of those particles might have atherogenic consequences.

MATERIALS AND METHODS

Study population

The group studied consisted of 31 subjects with no clinical or anamnestic evidence of atherosclerosis. All subjects were of German nationality and were inhabitants of the Rhein-Main area. This study was approved by the Ethics Committee of the University of Mainz. Participation was voluntary, and each study subject gave written informed consent.

Laboratory methods

Blood samples were taken from all subjects under standardized conditions after an overnight period of fasting. After this first blood sample was taken, subjects ate, in a period of approxi-

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Abbreviations: apoA-I, apolipoprotein A-I; BLp, apolipoprotein B-containing particle; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; RLP, remnant-like particle; SM, sphingomyelin; TG, triglyceride; TRL, triglyceride-rich lipoprotein.

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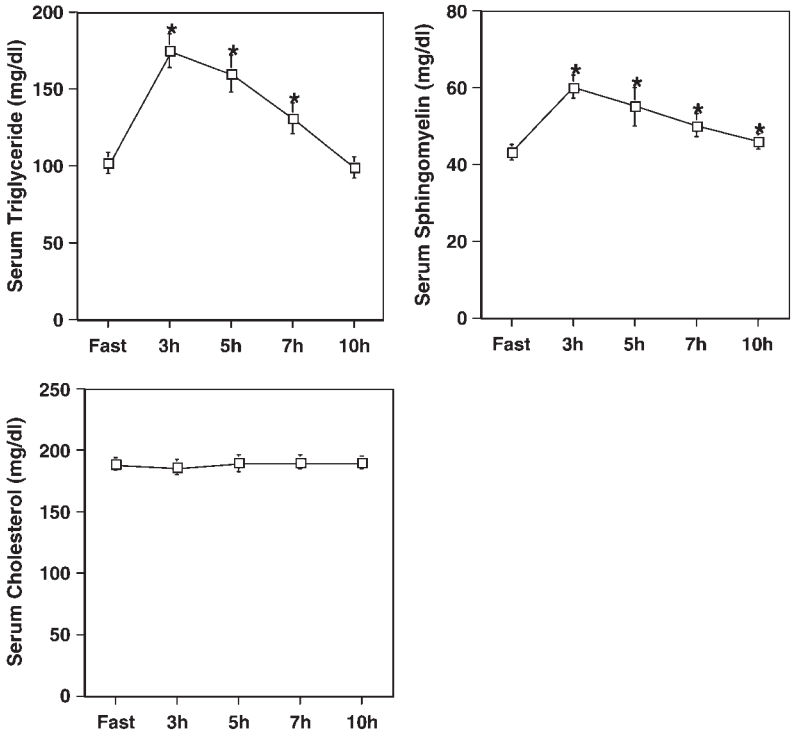

Fig. 1. Postprandial triglyceride (TG), sphingomyelin (SM), and cholesterol levels. The serums were prepared and the TG, SM, and cholesterol concentrations were determined as described in Materials and Methods. All results are presented as averages ± SEM. Statistical analysis was based on the comparison of fasting levels with 3, 5, 7, and 10 h levels by ANOVA. * $P < 0.05$.

mately 15 min, a meal high in fat, consisting of 1,265 kcal/m^2 body surface area (105 g of fat, consisting of 52 g of saturated fat and 300 mg of cholesterol, with 48 g of carbohydrates and 32 g of protein). Subjects were instructed not to eat any other food until after the last blood sample. Further blood samples were taken after 3, 5, 7, and 10 h. These were immediately centrifuged at 4,000 rpm for 10 min and divided into aliquots. All aliquots for the detection of total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), apolipoprotein A-I (apoA-I), apoB, and triglyceride (TG) were refrigerated until analysis. Samples for the detection of SM and RLP were immediately frozen at −80°C until analysis.

Serum SM levels were measured as previously described (8). RLP cholesterol, RLP SM, and RLP TG levels were determined using a modification of an RLP assay system (Japan Immunoresearch Laboratories Co., Ltd., Takasaki, Japan). Briefly, 20 μl of serum was incubated with 400 μl of gel containing apoB-100 and apoA-I antibodies, according to the kit guidelines. The supernatant (∼350 μl) was separated by centrifugation at 14,000 rpm for

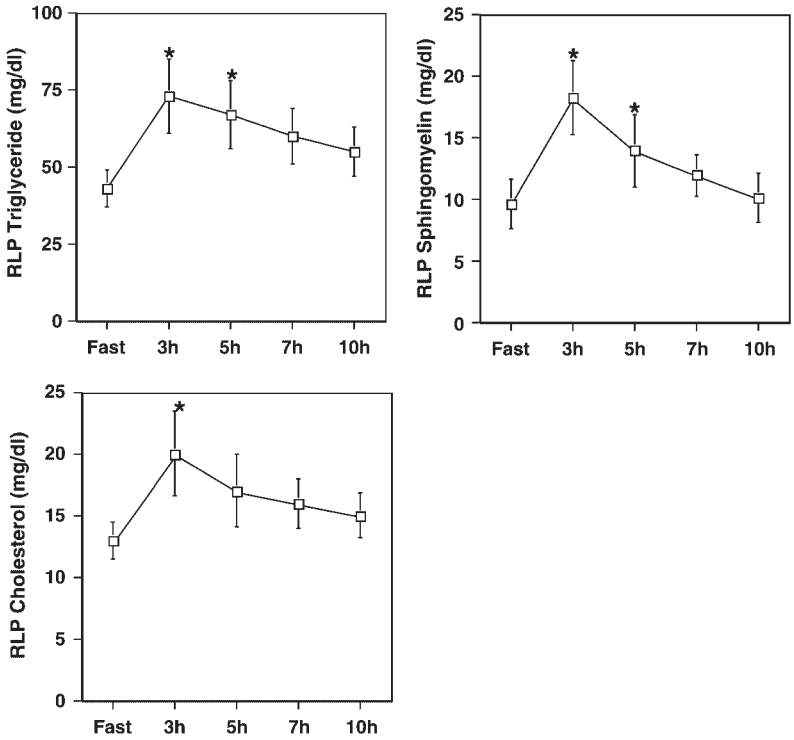

Fig. 2. Postprandial remnant-like particle (RLP) TG, RLP SM, and RLP cholesterol levels. The RLPs were prepared and the RLP TG, RLP SM, and RLP cholesterol concentrations were determined as described in Materials and Methods. All results are presented as averages ± SEM. Statistical analysis was based on the comparison of fasting levels with 3, 5, 7, and 10 h levels by ANOVA. * $P < 0.05$.

TABLE 1. Fasting and postprandial lipid and apolipoprotein parameters

Parameter	Fast	3 h	5 h	7 h	10 h
LDL cholesterol (mg/dl)	112 ± 7	98 ± 7	101 ± 7	110 ± 6	115 ± 26
HDL cholesterol (mg/dl)	54 ± 2	53 ± 2	53 ± 2	55 ± 3	58 ± 2
Phosphatidylcholine (mg/dl)	137 ± 18	139 ± 21	135 ± 17	137 ± 22	137 ± 19
Apolipoprotein A-I (g/l)	1.36 ± 0.05	1.37 ± 0.05	1.36 ± 0.05	1.37 ± 0.05	1.37 ± 0.04
Apolipoprotein B (g/l)	0.89 ± 0.05	0.88 ± 0.05	0.88 ± 0.05	0.89 ± 0.05	0.90 ± 0.05

All results are presented as means ± SEM.

10 min. The RLP-containing supernatant was lyophilized until 100 μl of solution remained. Cholesterol, SM, and TG levels were then determined in the supernatant. The intra-assay coefficients of variation for all three assays were less than 10%. The RLP cholesterol values determined by this modified method were comparable to the kit guidelines.

The total choline-containing phospholipid in plasma was assayed by an enzymatic method (Wako Pure Chemical Industries, Ltd). Phosphatidylcholine concentration was obtained by subtracting SM from total phospholipid concentration. Serum lipid levels were determined immediately (cholesterol, Roche Diagnostics; HDL-C, Rolf Greiner Biochemica; LDL-C, calculated according to the Friedewald formula; TG, Roche Diagnostics). ApoA-I and apoB concentrations were determined by immunoturbidimetric assays (Tina-quant; Roche Diagnostics).

Statistical analysis

Estimating the correlation between lipid values, which were repeatedly measured at different time points from the same subject, is outside the scope of standard correlation (i.e., Pearson *r*) and linear regression analysis, because the observations are not independent. Therefore, a general linear mixed model was used to perform repeated-measures regression in predicting time-dependent lipid measures. From this model, we could estimate correlation values. Here, we chose serum SM or serum TG as predictors for the dependent measure of other lipid parameters. Because a general linear mixed model does not produce a multivariate *R* term, an analog of *R* was estimated. This was done by calculating the correlation of the predicted model values and the actual values of RLP SM, RLP TG, and RLP cholesterol. All analyses were carried out using SPSS 11.0.

RESULTS

RLP isolation was based on the removal of apoA-I-containing particles (HDLs) and most apoB-containing particles (LDLs, nascent VLDLs, and nascent chylomicrons) using an immunoseparation technique (Japan Immunoresearch Laboratories), as previously described (9). This has been shown to leave particles characteristic of the previously mentioned chylomicron and VLDL remnants in the unbound fraction (10).

We found that mean serum concentrations of TG, SM, RLP cholesterol, RLP TG, and RLP SM were low at fasting. These levels were increased 3 h after the high-fat meal and then returned toward pretreatment levels during the subsequent 7 h study period (**Figs. 1**, **2**). The differences between the fasting and 3 h points were statistically significant for all parameters, as compared by ANOVA. The differences between the fasting and 5 h points remained significant for serum TG, serum SM, RLP TG, and RLP SM. The differences between the fasting and 7 h or 10 h points remained significant for serum TG or serum SM (Figs. 1, 2), respectively. As presented in **Table 1**, serum concentrations of LDL-C, HDL-C, phosphatidylcholine, apoA-I, and apoB were nearly stable during the study period.

To evaluate further the predictive value of serum SM for other lipid parameters, we created a general linear mixed model. This model, implying the average correlation across time between SM and other parameters, indicated that serum SM has significant predictive value on serum TG ($R = 0.552$, $P < 0.001$), RLP SM ($R = 0.658$, $P < 0.001$), RLP TG ($R = 0.535$, $P < 0.01$), and RLP cholesterol ($R = 0.647$, $P < 0.001$) (**Table 2**). We also used this model to correlate between serum TG and other variables, finding that serum TG also has significant predictive value on serum SM ($R = 0.521$, $P < 0.001$), RLP cholesterol ($R = 0.369$, $P < 0.01$), RLP SM ($R = 0.327$, $P < 0.01$), and RLP TG ($R = 0.96$, $P < 0.001$) (**Table 3**).

DISCUSSION

In the present study, we have demonstrated the following for the first time: *1*) that postprandial serum SM and RLP SM levels, like serum TG levels, have a bell-shaped curve with a peak at 3 h; *2*) that serum SM levels have a

TABLE 2. Correlation between serum SM and other variables

Variable	Serum TG[a]	RLP Cholesterol[a]	RLP SM	RLP TG
Serum SM				
R	0.552[b]	0.647[b]	0.658[b]	0.535[b]
N	152	65	65	65

RLP, remnant-like particle; SM, sphingomyelin; TG, triglyceride. A general mixed linear model was used to estimate the multivariate *R* for serum SM with serum TG, RLP SM, RLP TG, and RLP cholesterol.

[a] Log-transformed variables were used for skewed distribution.

[b] $P < 0.001$.

TABLE 3. Correlation between serum TG and other variables

Variable	Serum SM	RLP Cholesterol[a]	RLP SM	RLP TG
Serum TG[a]				
R	0.521[b]	0.369[b]	0.327[b]	0.960[b]
N	152	65	65	65

A general mixed linear model was used to estimate the multivariate *R* for serum TG with serum SM, RLP SM, RLP TG, and RLP cholesterol.

[a] Log-transformed variables were used for skewed distribution.

[b] $P < 0.01$.

statistically significant predictive value for serum TG, RLP SM, RLP TG, and RLP cholesterol; and *3*) that serum TG levels also have a statistically significant predictive value for serum SM, RLP SM, RLP TG, and RLP cholesterol. These results suggest that SM is one of the RLP clearance markers.

Recent data imply that impaired postprandial lipoprotein metabolism may contribute to, or be a marker for, the development and progression of atherosclerotic diseases (11). Patients with ischemic heart disease have delayed clearance of TRLs after consumption of a high-fat meal, compared with controls (11, 12). Several lines of evidence suggest that such remnants are particularly atherogenic (13). RLPs show a marked increase and remain high even 8 h after fat loading, especially in patients with coronary artery disease or diabetes mellitus, indicating that the postprandial state persists for almost the whole day in these patients (14). Immunohistochemical studies indicate colocalization of anti-apoB-48 receptor antibody in human atherosclerotic lesion foam cells, implying that the apoB-48 receptor may contribute to foam cell formation and atherosclerosis (14).

Remnants are related to intima media thickness, an early marker for atherosclerosis and an important prospective marker for patients with coronary artery disease (15). Studies have also demonstrated the association of delayed postprandial fat clearance with abnormalities in the lipoprotein profile. Delayed clearance is seen in conjunction with hypertriglyceridemia, low levels of HDL-C (primarily the anti-atherogenic HDL_2 subfraction), and the presence of small dense LDLs (i.e., a pattern typical of insulin resistance) (16). Patients carrying the apoε2 allele also have slower postprandial fat clearance (17). Plasma SM tends to become enriched in atherogenic remnants of TRL (18). Based on our results (Figs. 1, 2), plasma (or serum) and RLP SM measurements could act as markers for atherogenic remnant accumulation.

Several studies have suggested that postprandial SM and cholesterol-rich remnants may cause intracellular lipid loading. Van Lenten et al. (19) demonstrated that RLPs are taken up by monocyte-derived macrophages in the arterial wall, contributing to atherosclerotic plaque formation. Gianturco et al. (20) found that RLPs from human serum caused cholesterol accumulation in fibroblasts. Van Lenten et al. (21) demonstrated that RLPs induced foam cell formation in macrophages, whereas Georgopoulos, Kafonek, and Raikhel (22) found that postprandial TRLs from diabetic subjects produced cholesterol accumulation in macrophages. Yu and Mamo (23) provided specific evidence that remnants of postprandial lipoproteins (i.e., chylomicrons) are capable of inducing foam cell formation in human monocyte-derived macrophages, notably in the absence of significant oxidative modification.

In animal studies, it has been further confirmed that plasma SM is a risk factor for atherosclerosis. ApoE deficiency is a well-known mouse model for atherosclerosis (24), and the accumulation of RLPs in the circulation that are enriched in cholesterol (24) and SM (18) is the distinctive factor. These mice develop atherosclerotic lesions spontaneously at the age of 2 months without any dietary fat or cholesterol induction at all (24).

TG is a well-known RLP clearance marker (12–14). In this study, we do not emphasize that RLP SM is better than RLP TG as a marker for RLP metabolism, but we emphasize that RLP SM levels have their own specialty, in terms of atherogenicity. SM is not degraded in plasma; rather, it becomes enriched in remnants of TRL (3). SM removal from plasma is dependent on hepatic clearance mechanisms, such as the LDL receptor, the LDL receptor-related protein, or proteoglycan pathways. Substantial evidence now supports the role of lipoprotein SM and arterial sphingomyelinase in atherogenesis (6–8). SM carried into the arterial wall on atherogenic lipoproteins is acted on by an arterial wall sphingomyelinase, leading to an increase in ceramide content and promoting lipoprotein aggregation (6, 7).

As humans, we spend most of our lives in the postprandial state. It is entirely possible that postprandial SM, which resides on RLP, has an impact on atherosclerosis. If these SM-rich particles accumulate in the circulation, they clearly have more opportunity to be deposited on the arterial wall and to aggregate there as a result of sphingomyelinase activity (6). We cannot, of course, overlook other possibilities: for example, that BLp is modified in the arterial wall by processes such as oxidation, leading to the formation of macrophage foam cells and initiating atherosclerosis (25). At this juncture, however, it seems to us that the likelihood lies strongly in the direction of SM.

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