Original Article

Mannose-Binding Lectin in Obesity with Different Degrees of Metabolic Syndrome Abnormalities: Association with Atherogenic and Metabolic Traits

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***Aim*: In subjects with metabolic syndrome (MetS) endothelial dysfunction is a very consistent finding. Processes leading to endothelial dysfunction and atherosclerosis involve the altered control of subclinical inflammation by innate immune defenses that possibly include mannose-binding lectin (MBL). We investigated the associations of MBL with traits of MetS and early atherosclerosis in obese subjects before and after marked weight reduction.**

***Methods*: In a prospective longitudinal study, MBL concentrations of 96 severely obese subjects with and without MetS (Ø BMI with MetS 41.0 ± 7.9 kg/m^2, Ø BMI without MetS 39.4 ± 7.7 kg/m^2) were examined in association with markers of insulin resistance, dyslipidemia, adipokines, and subclinical atherosclerosis before and after marked weight loss (Ø weight loss 20 ± 8 kg after 3 months of participation in a standardized weight reduction program), in addition to the study of 25 seemingly healthy lean subjects (BMI 20-25 kg/m^2).**

***Results*: MBL concentrations did not differ between healthy lean and severely obese subjects independently of the presence of metabolic abnormalities. In severely obese subjects there was no significant difference concerning the cardiovascular risk profile, apolipoproteins, inflammatory and metabolic parameters, and markers of endothelial dysfunction and atherosclerosis between subjects with functional MBL deficienct (MBL < 778 ng/mL) and MBL sufficient (MBL ≥ 778 ng/mL) obesity. Marked weight loss did not influence MBL levels.**

***Conclusions*: Our findings suggest that plasma levels of MBL did not differ between healthy lean and severely obese subjects. MBL did not affect cardiovascular risk factors, or markers of endothelial dysfunction and early atherosclerosis in severely obese patients before and after marked weight loss.**

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Key words; Mannose-binding lectin, Metabolic syndrome, Atherosclerosis, Weight reduction

Introduction

Adipose tissue secretes a large number of bioactive substances, which may be involved in a variety of pathologic processes. Evidence has accumulated indicating that obesity is associated with a state of chronic, low-grade inflammation, suggesting that inflammation may be a potential mechanism whereby obesity leads to insulin resistance[1)]. Moreover, the interaction of proinflammatory cytokines, adipokines and hypofibrinolytic factors might lead to increased oxidative stress and endothelial dysfunction, promoting atherosclerosis in humans with metabolic syndrome (MetS)

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and with its distinctive cardiovascular risk profile[2-4]. Accordingly, in subjects with MetS, endothelial dysfunction is a very consistent finding.

Processes leading to endothelial dysfunction and atherosclerosis also involve the altered control of subclinical inflammation by innate immune defence that possibly include mannose-binding lectin (MBL). As an acute phase protein, MBL activates the complement system via MBL-associated serine proteases, initiating the lectin pathway of complement activation, and could aggravate systemic inflammation[5, 6]. Many complement components play an important role in the progression and maturation of atherosclerotic lesions[7, 8], and the complement component C3 is additionally related to insulin resistance[9, 10]. Common variant MBL genotypes, coding for markedly diminished levels of MBL, have been shown to be predictive of coronary artery disease (CAD) and severe atherosclerosis, even after adjustment for traditional cardiovascular risk factors[11-13]. MBL deficiency also appeared to be associated with venous bypass graft occlusions in patients with coronary heart disease[14].

However, in contrast to these findings, it has also been documented that a high MBL level is a risk factor for acute coronary syndromes[15] and future coronary artery disease[16], and that functional MBL deficiency contributes to reduced mortality in patients with acute myocardial infarction[17]. Moreover, high MBL was associated with higher all-cause mortality in patients with type 2 diabetes[18]. Thus, the effect of MBL on atherosclerosis and coronary artery disease phenotypes remains highly controversial.

Studies investigating whether MBL is linked to insulin resistance, plasma markers of atherosclerosis, and risk factors in subjects with chronic inflammatory states, such as obesity and MetS, are scarce[19, 20].

We hypothesize that circulating MBL levels in subjects with obesity and MetS are associated with a distinctive cardiovascular risk profile. Thus, we evaluated in our study 1) MBL levels in healthy lean controls compared to severely obese humans with and without MetS; 2) the association of plasma MBL levels with cardiovascular risk profile and several markers of early atherosclerosis in the obese; and 3) the influence of substantial therapeutic weight loss on MBL concentrations. Moreover, in the present study we introduce the "Obesity Weight Reduction and Remodeling Study".

Methods

Study Population: "Obesity Weight Reduction and Remodeling Study"

The rationale of the ongoing "Obesity Weight Reduction and Remodeling Study", a prospective longitudinal study conducted since 2007 at the University Hospital of Regensburg, Germany, is to evaluate excessive body fat for its pathogenic potential in terms of cardiometabolic diseases and to assess the effects of considerable weight reduction on interactions in systems biology. In order to establish this, we are currently building a study cohort to determine patterns of numerous metabolic and lipid/apolipoprotein abnormalities, lipidomics, adipokines, inflammatory markers, oxidative stress parameters and adhesion molecules, hormones of energy homeostasis, as well as subclinical atherosclerosis traits in the obese with and without characteristics of MetS, by extensively phenotyping very obese subjects before, during, and after a standardized weight reduction program, in addition to healthy lean subjects.

Obese patients intending to participate in a weight reduction program are offered enrollment in this study prior to the start of the program. Patients are eligible for enrollment if they participate either in the standardized multimodal Optifast-52 weight reduction program (Nestlé HealthCare Nutrition GmbH, Germany) provided by the Department of Psychosomatic Medicine at the University of Regensburg, or in a combined exercise and diet weight reduction program offered by a local fitness gym; however, for the present study, only "Optifast participants" were considered.

Patients were eligible for enrollment if they were 18-59 years old, had a BMI >30 kg/m^2 and a constant body weight in the last 3 months, and if they signed a declaration of consent. Patients were excluded if they had one or more of the following: more than 10% reduction of body weight in the last 6 months, cancer, pregnancy, therapy with steroids or thyroid hormones, known heart disease, known diabetes, known arterial hypertension or dyslipidemia using medications, known inflammatory bowel, rheumatiod or systemic diseases, known chronic renal failure, known liver diseases, mental disorders or addiction to drugs or alcohol.

For comparison, healthy normal weight control subjects (BMI 20-25 kg/m^2) of similar age and gender distribution are also studied. They are recruited by flyers, advertisements and friend referrals.

The study was approved by the local Ethics Committee. All subjects had given their informed

consent to study participation.

Standardized Weight Reduction Program

Optifast-52 (Nestlé HealthCare Nutrition GmbH) is a 52-week serious medical weight loss program encompassing diet, lifestyle changes, counseling, and exercise. The success of the program is documented and not only shows an average weight loss (52 pounds in 22 weeks), but a decrease in cholesterol, blood glucose and blood pressure[21, 22]. Optifast is administered through clinics staffed with physicians trained in obesity management and is intended for use in patients that need to lose 50 or more pounds safely. Optifast users receive ongoing medical monitoring during the initial phase to assess progress, primarily due to the quick loss of a significant amount of weight. They also receive guidance and support in nutrition, behavior and exercise. During the initial 3 months "Active Weight Loss Phase", which is portion controlled, calorically precise, and nutritionally complete, patients consume only meal replacements supplied by Optifast, and water. These come in the form of shakes, nutritional bars and soups. It is an 800-calorie per day program that contains all the vitamins and minerals recommended by the USDA. The low carbohydrate and fat content encourage a shift to fat breakdown and ketosis and the high protein content prevents the severe negative nitrogen balance associated with starvation and preserves lean body mass.

After the Active Weight Loss phase the following "Transition Period" will last 6 weeks, where meal replacements are gradually replaced by self-prepared meals. Menus and food recommendations are supplied to help during this transitional phase. Once the Transition is complete, the "Long Term Weight Management Program" begins. This will be the basis of a healthy lifestyle, utilizing a diet rich in produce, grains and low-fat proteins. Customized activity plans appropriate to the fitness level are part of the Optifast program and are considered to be essential to successful weight loss management.

Phenotyping

All subjects were studied after a 12-hour overnight fast between 7:00 and 9:00 a.m. All anthropometric measurements were performed by specially trained staff using standard techniques. Blood pressure (BP) and the patient's heart rate were taken after sitting for 5 minutes. We measured blood pressure 3 times in both arms with an appropriately sized blood pressure cuff (Welch Allynn®) with at least 60 seconds between the measurements.

Height and weight were recorded with participants wearing lightweight clothing and no shoes using a centimetre scale with attached stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference was measured midway between the lowest rib and the iliac crest with the subject standing at the end of gentle expiration, and the hip circumference was taken at the level of the widest diameter around the gluteal protuberance. Waist-hip-ratio was calculated as waist circumference in centimetres divided by hip circumference in centimetres.

Body composition was determined by *bioelectrical impedance analysis (BIA)* using Nutriguard-M (Data Input GmbH Darmstadt, Germany). It is used for measuring body fluid volumes, fat-free mass (FFM) and body cell mass. Impedance parameters are applied in the supine position with four gel-type electrodes, two voltage and two current, placed on the right foot and wrist. The BIA variables considered were resistance (R) and reactance (Xc). The bioimpedance index (BI) was calculated as the ratio height^2/resistance[23]. The instrument was regularly checked with resistors of known values.

Carotid Artery Studies

Ultrasonographic scans of the carotid artery were performed by expert sonographers who were specifically trained to perform the prescribed study examination. Ultrasound studies were performed using high-resolution B-mode ultrasound mainframes (Philips iE33) with the L11-3 MHz linear array transducer. Both common carotid arteries were scanned following a standardized protocol. The image was focused on the posterior (far) wall, and gain settings were used to optimize image quality. A resolution box function (zoom) was used to record an image 25 mm wide and 15 mm high. The intima media thickness (IMT) was measured as the distance between 2 parallel echogenic lines corresponding to the blood-intima and media-adventitia interface on the posterior wall of the artery. A moving scan with a duration of 5 seconds, which included the beginning of the carotid bifurcation and the common carotid artery, was recorded and stored in digital format on optical disks for subsequent off-line analysis. Digitally stored scans were manually analyzed by a reader blinded to participants' details. From the 5-second clip image, the best-quality end-diastolic frame was selected. From this image, measurements of the common carotid far wall were taken approximately 10 mm proximal to the bifurcation to derive maximal carotid IMT.

Ankle-Brachial Index (ABI)

The ABI was measured using the boso ABI 100 system (Bosch and Sohn, Germany), which allows blood pressure to be measured simultaneously in all four limbs. This simultaneous measurement produces a precise and reliable calculation of the ABI. The system measures oscillometrically without a Doppler probe or other sensor. Variations in individual measuring times are minimized by an intelligent inflation system and regulation of the deflation rate. Once the measurements have been taken, the results are transferred via a USB interface to a PC, where the application software calculates the ABI automatically for both sides. The lower ABI reading was used for further analysis.

Indirect Calorimetry

The resting metabolic rate was measured by indirect calorimetry using a DeltatracTM system (Datex Ohmeda) in a quiet environment. In the supine position, oxygen consumption and carbon dioxide production were determined for 30 min. Energy expenditure was derived from CO_2 production and O_2 consumption. The apparatus was calibrated with gas mixtures of known composition with 95% CO_2 and 5% O_2 before each test and the instrument was routinely checked.

Adipokines and Inflammatory Markers

Fasting plasma adiponectin, leptin and resistin were measured using a commercially available enzyme-linked immunoassay (ELISA) kit (Bio Vendor) and MBL was detected performing using the Sanquin ELISA kit. The concentration of highly sensitive C-reactive protein (hsCRP) was determined by nephelometry (Nephelometer BN ProSpec; Siemens). Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured by chemiluminescence detection (Immulite; Siemens).

Adhesions Molecules and Markers of Early Atherogenesis

Soluble CD40 ligand (sCD40 ligand), matrix metallopeptidase 9 (MMP-9), selectin, soluble intercellular cell adhesion molecule (sICAM) and soluble vascular cell adhesion molecule (sVCAM) were gauged using ELISA kits (R&D Systems), while oxidized low density lipoprotein cholesterol (oxLDL) was measured performing using the commercially available ELISA kit from Mercodia.

Glucose, Insulin and Lipids

Fasting plasma glucose, triglycerides (TG), high density lipoprotein cholesterol (HDL), low density lipoprotein (LDL) and free fatty acids (FFAs) were analyzed on an automated analyzer (ADVIA; Siemens), while apolipoprotein A1 (Apo-A1), apolipoprotein A2, apolipoprotein B (Apo-B) and lipoprotein (a) were measured by nephelometry (Nephelometer BN ProSpec; Siemens). Fasting serum insulin was determined using chemiluminescence (ADVIA Centaur; Siemens). Insulin sensitivity in the fasting state was assessed with homeostasis model assessment (HOMA) and calculated with the following formula: fasting serum glucose (nmol/L) × fasting serum insulin (mU/L) 22.5, as described by Matthews *et al.*[24].

Definitions

Metabolic Syndrome

MetS was diagnosed according to the NCEP Adult Treatment Panel Ⅲ (ATP Ⅲ)[25]. It requires the presence of central obesity with waist circumference ≥ 102 cm in men and ≥ 88 cm in women, dyslipidemia with triglycerides ≥ 150 mg/dL, and HDL-cholesterol < 40mg/dL in men and < 50 mg/dL in women. Hypertension and hyperglycemia were diagnosed for blood pressure ≥ 130/85 mmHg and fasting plasma glucose ≥ 110 mg/dL. MetS was diagnosed when at least three out of five metabolic abnormalities were determined.

Mannose-Binding Lectin Deficiency

MBL concentrations among healthy Caucasians vary from undetectable up to 10 000 μg/L with a median around 1 000 μg/L[26, 27]. Single base mutations within exon 1 and several mutations in the promoter region of the MBL gene result in interindividual differences in serum MBL levels. MBL deficiency is generally defined as serum < 500 ng/mL, but some groups have defined severe MBL deficiency as < 50 ng/mL and partial MBL deficiency from 50 ng/mL up to 1000 ng/mL[17, 28-30]. As there is no unique definition of functional MBL deficiency and clinical relevance may vary in different diseases, MBL < 778 ng/mL was chosen for the differentiation in MBL-deficient and MBL-sufficient obesity in our study. The cut-off value 778 ng/mL was determined as the lower 95% confidence interval in our lean healthy control subjects. The median plasma MBL level in MBL deficient obesity in our study was 245 ng/mL (interquartile range 59-599), while the median concentration in MBL sufficient obesity was 1704 (1218-2341) ng/mL.

Statistical Analysis

Continuous variables are presented as the means ± standard deviations, if normally distributed, or medians with interquartile intervals (25 and 75 per-

Table 1. Baseline characteristics in healthy, lean controls, and obese subjects with and without MetS

	Controls ($n=25$)	Obese-no MetS ($n=55$)	Obese-MetS ($n=41$)
Sex n (% women)	15 (60)	38 (69)	24 (59)
Age (years)	38 ± 13	42 ± 14	45 ± 12
BMI (kg/m^2)	22.3 ± 2.0	39.4 ± 7.7***	41.0 ± 7.9***
Waist (cm)	78 ± 8	120 ± 19***	121 ± 18***
Hip (cm)	99 ± 5	131 ± 14***	132 ± 17***
Waist/Hip	0.79 ± 0.06	0.91 ± 0.10***	0.92 ± 0.10***
Fatmass (%)	21.5 ± 6.5	42.2 ± 8.7***	41.1 ± 7.5***
RMR (cal./d)	1539 ± 221	1973 ± 522***	2045 ± 459***
HDL chol. (mg/dL)	67 ± 12	58 ± 15***	43 ± 8$^{***, \#\#\#}$
LDL chol. (mg/dL)	105 ± 26	113 ± 34	122 ± 36
Triglyc. (mg/dL)	73 ± 27	97 ± 35	170 ± 83$^{***, \#\#\#}$
Diabetes n (%)	0	1 (2)	10 (24)
Fasting Gluc. (mg/dL)	84 ± 5	92 ± 11	125 ± 55$^{***, \#\#\#}$
Fasting Insulin (mU/L)	5.9 ± 3.0	18.2 ± 14.8**	26.4 ± 16.9$^{***, \#}$
HOMA-IR	1.2 ± 0.6	4.3 ± 3.9	8.8 ± 7.9$^{***, \#\#\#}$
Systolic BP (mmHg)	123 ± 14	137 ± 19**	144 ± 13***
Diastolic BP (mmHg)	77 ± 9	83 ± 12	86 ± 9**

RMR, resting metabolic rate; data are the means ± SD; $^{*}p<0.05$ vs. Controls, $^{**}p<0.01$ vs. Controls, $^{***}p<0.001$ vs. Controls, $^{\#}p<0.05$ vs. Obese, no MetS, $^{\#\#}p<0.01$ vs. Obese, no MetS, $^{\#\#\#}p<0.001$ vs. Obese, no MetS.

centiles), otherwise.

Continuous variables were compared using one-way Anova for normally distributed variables (if significant, Student's *t*-test for the comparison of 2 groups, or the Tukey-Kramer post-hoc test for multiple pairwise comparisons. For non-normally distributed variables we used the Kruskal-Wallis test, and the Dwass-Steel post-hoc test was used to make all possible pairwise comparisons, accounting for multiple testing. Categorical variables were compared using Fisher's exact test. Linear correlation analysis was applied to examine the association between parameters of MetS and MBL levels.

The parametric paired *t*-test and the non-parametric Wilcoxon signed rank test were used to compare follow-up values of means from the same subjects, i.e. before and after weight reduction. Statistical significance was considered at the 0.05 level. All analyses were conducted using JMP, Version 9 (SAS Institute Inc., Cary, NC).

Results

Patient Characteristics at Baseline (Before Weight Reduction)

Baseline characteristics in healthy, lean controls, and obese subjects with and without MetS are presented in **Table 1**. Obese subjects with and without MetS did not differ statistically significantly with respect to age, gender, and the severity of obesity. By definition, obese subjects with MetS had lower HDL cholesterol and higher triglyceride levels, as well as higher glucose, insulin, and HOMA-IR than obese subjects without MetS.

Baseline adipocytokines, inflammmatory markers, and markers of atherosclerosis, and apolipoproteins are compared in **Table 2**. Obese subjects with MetS had lower adiponectin levels, higher levels of oxidized LDL cholesterol, higher homocystein levels, lower apolipoprotein A1 and A2 levels, higher apolipoprotein B levels, and higher IMT than obese individuals without MetS. Whereas hsCRP levels were much higher in obese subjects than in lean controls, no difference could be obeserved between obese subjects with and without MetS with respect to the inflammatory parameters hsCRP, TNFα, or IL-6, as well as IMT.

The levels of resistin, sCD40, serotonin, and free fatty acids were comparable across groups.

MBL concentrations did not differ between normal weight and severely obese subjects independently of the presence of MetS (**Fig. 1**).

By performing linear correlation analysis there was no statistically significant association between MBL levels and parameters of MetS, cardiovascular risk factors, or early atherosclerosis (data not shown).

Table 2. Baseline adipocytokines, inflammmatory markers, and markers of atherosclerosis, and apolipoproteins in healthy, lean controls, and obese subjects with and without MetS

	Controls ($n=25$)	Obese-no MetS ($n=55$)	Obese-MetS ($n=41$)
Adiponect. (μg/mL)	12.6 (10.2-18.0)	10.6 (9.1-13.6)*	9.5 (7.1-11.1)**
Leptin (μg/L)	8 (2-12)	48 (31-65)*	52 (30-65)***
Resistin (ng/mL)	4.7 (4.2-7.6)	5.3 (4.3-7.3)	5.1 (4.2-7.2)
Ghrelin (pg/mL)	75 (32-138)	11 (5-31)**	18 (8-39)*
TNFα (pg/mL)	7.0 (5.7-9.2)	7.7 (6.8-10.5)	10.0 (6.8-11.4)
hsCRP (mg/L)	0.5 (0.3-1.9)	4.2 (2.6-11.0)***	5.2 (2.6-8.7)***
IL-6 (pg/mL)	2.3 (2.0-3.3)	3.6 (2.2-4.7)	3.6 (2.4-5.4)
MMP9 (ng/mL)	354 (265-565)	532 (391-789)*	461 (357-627)
oxLDL (U/L)	46 (40-56)	47 (38-62)	57 (51-74)$^{**,\#}$
sCD40 (pg/mL)	6639 (5609-8670)	8040 (6300-10140)	7425 (6084-9340)
Homocyst (μmol/L)	10.2 (8.8-11.1)	9.3 (7.7-11.4)	10.5 (9.2-13.0)$^{\#}$
Serotonin (ng/mL)	107 (63-132)	95 (70-148)	94 (73-126)
Selectin (pg/mL)	28 (21-37)	38 (26-55)*	43 (35-60)***
sICAM (ng/mL)	245 (203-264)	239 (188-318)	294 (235-347)**
sVCAM (ng/mL)	635 (571-863)	633 (503-759)	654 (559-849)
IMT (mm)	0.50 ± 0.16	0.70 ± 0.22***	0.74 ± 0.23***
ABI	1.15 ± 0.13	1.08 ± 0.25	1.04 ± 0.13
FFA (mmol/L)	0.8 (0.6-1.5)	0.8 (0.6-1.1)	0.8 (0.6-1.0)
Apo A1 (mg/dL)	183 (159-196)	164 (146-188)	143 (130-158)$^{***,\#\#\#}$
Apo A2 (mg/dL)	35 (31-41)	34 (30-40)	29 (26-32)$^{***,\#\#\#}$
Apo B (mg/dL)	80 (73-89)	91 (74-105)	110 (86-127)$^{***,\#\#}$
Lp a (mg/dL)	22 (15-53)	31 (20-60)	19 (12-34)

IMT, intima media thickness; ABI, ankle-brachial index; data are the means ± SD if normally distributed or medians (interquartile range); $^{*}p<0.05$ vs. Controls, $^{**}p<0.01$ vs. Controls, $^{***}p<0.001$ vs. Controls, $^{\#}p<0.05$ vs. Obese, no MetS, $^{\#\#}p<0.01$ vs. Obese, no MetS, $^{\#\#\#}p<0.001$ vs. Obese, no MetS.

The cardiometabolic risk profile in MBL-sufficient and MBL-deficient obese subjects is given in **Table 3**. Analogously, detailled blood biomarkers, IMT, and ABI in both groups are shown in **Table 4**. The median MBL plasma level in MBL-deficient obesity was 245 ng/mL (59-559 ng/mL) and in MBL-sufficient obesity 1704 ng/mL (1218-2341 ng/mL), $p<0.0001$).

Anthropometrical data, cardiovascular risk profile, adipocytokines, inflammatory parameters, and markers of early atherosclerosis were almost identical between patients with low or high MBL levels in obese subjects.

Effect of Substantial Weight Loss on Plasma MBL Levels and Cardiometabolic Parameters

The effect of weight loss in the severely obese on plasma MBL levels is depicted in **Fig. 2**. For comparison, the MBL levels are also shown in healthy, lean controls with constant weight at the corresponding time intervals. Weight loss achieved by the hypocaloric "active weight loss phase" was substantial (−19.6 ± 7.6 kg, $p<0.0001$, data not shown); however, the most prominent changes achieved by the diet could be detected for leptin, adiponectin, hsCRP, selectin, and HOMA-IR (**Fig. 3**). In contrast, MBL levels were not influenced at all by weight loss (**Fig. 2**).

We additionally divided the obese subjects into subgroups according to increasing tertiles of different components of MetS or plasma biomarkers and compared the MBL levels before and after weight loss (**Fig. 4**). In each subgroup MBL levels were similar before and after weight loss irrespective of high or low baseline HOMA-IR, high or low HDL cholesterol, adiponectin, hsCRP, etc., suggesting that weight loss has no effect on MBL levels in the obese.

Discussion

Chronic low-grade inflammation, as found in obesity and particularly in MetS, may influence different components of the innate immune system that in

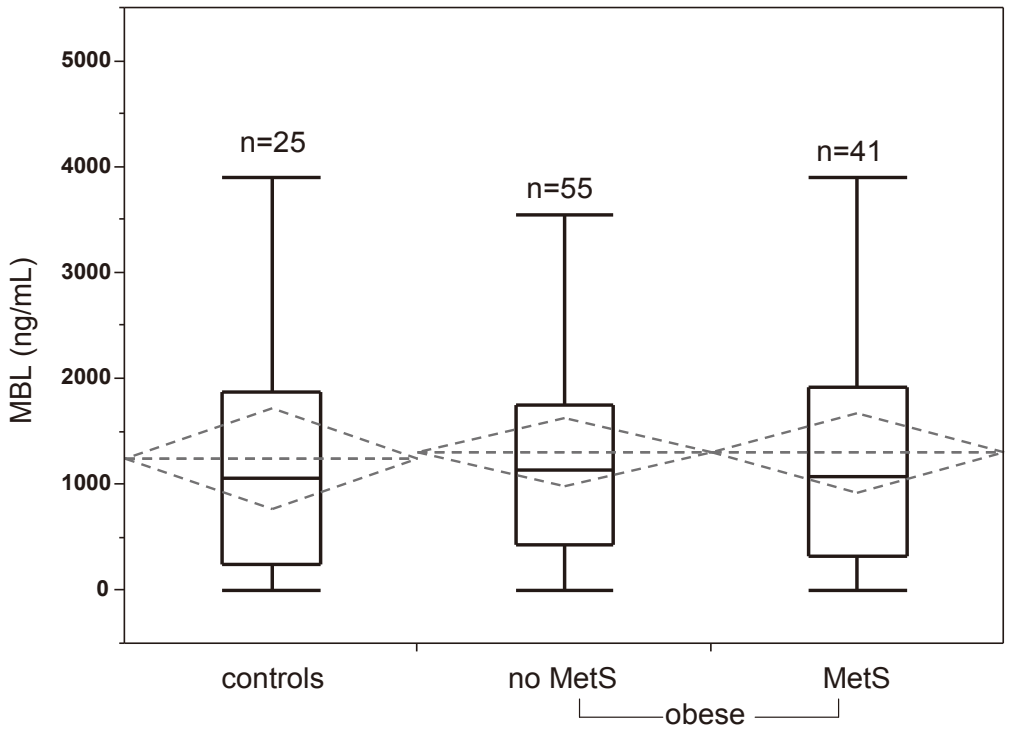

Fig. 1. Plasma MBL concentration in lean controls and obese patients without and with MetS at baseline. Beside the box-and-whisker plots representing the 10th, 25th, 50th, 75th and 90th percentiles, the means with its 95% confidence intervals are shown (dotted triangles).

Table 3. Cardiometabolic risk profile in MBL sufficient and MBL deficient obesity

	MBL-deficient obesity[§] ($n=40$)	MBL-sufficient obesity ($n=56$)	p value
Sex n (% women)	12 (30)	22 (39)	0.392
Age (years)	46 ± 13	44 ± 14	0.470
BMI (kg/m^2)	38.5 ± 7.4	41.2 ± 7.9	0.099
Waist (cm)	118 ± 16	122 ± 20	0.257
Hip (cm)	129 ± 13	134 ± 16	0.129
Waist/Hip	0.92 ± 0.08	0.92 ± 0.11	0.958
Fatmass (%)	41.4 ± 9.7	42.2 ± 6.7	0.664
RMR (cal./d)	1968 ± 527	2024 ± 468	0.605
HDL chol. (mg/dL)	51 ± 13	53 ± 15	0.494
LDL chol. (mg/dL)	111 ± 33	121 ± 36	0.163
Triglyc. (mg/dL)	130 ± 74	127 ± 69	0.847
Diabetes n (%)	3 (8)	8 (14)	0.351
Fasting Gluc. (mg/dL)	105 ± 43	107 ± 37	0.756
Fasting Insulin (mU/L)	20.1 ± 16.5	22.9 ± 16.0	0.400
HOMA-IR	5.2 ± 4.7	6.9 ± 7.2	0.193
Systolic BP (mmHg)	139 ± 18	140 ± 16	0.826
Diastolic BP (mmHg)	83 ± 11	85 ± 11	0.457

RMR, resting metabolic rate; data are the means ± SD; MBL levels in MBL-deficient obesity 245 ng/mL (59-559 ng/mL) vs. MBL-sufficient obesity 1704 ng/mL (1218-2341 ng/mL), $p<0.0001$; [§]MBL deficiency, MBL < 778 ng/mL (corresponding to the lower 95% CI in controls).

Table 4. Adipocytokines, inflammmatory markers, markers of atherosclerosis and apolipoprotein in MBL-sufficient and MBL-deficient obesity

	MBL-deficient obesity[§] ($n=40$)	MBL-sufficient obesity ($n=56$)	p value
Adiponectin (μg/mL)	10.5 (8.0-14.0)	9.9 (7.0-12.1)	0.349
Leptin (μg/L)	53 (30-65)	49 (32-66)	0.891
Resistin (ng/mL)	5.4 (4.4-7.5)	5.0 (4.3-7.1)	0.627
Ghrelin (pg/mL)	12 (7-46)	16 (5-29)	0.857
TNFα (pg/mL)	8.8 (6.5-10.8)	8.7 (6.9-11.3)	0.546
hsCRP (mg/L)	5.1 (2.7-12.7)	5.2 (2.5-8.8)	0.809
IL-6 (pg/mL)	3.6 (2.2-5.4)	3.2 (2.2-5.2)	0.923
MMP9 (ng/mL)	481 (353-810)	501 (394-699)	0.932
oxLDL (U/L)	53 (36-63)	56 (44-75)	0.080
sCD40 (pg/mL)	7364 (5999-9939)	8193 (6632-9701)	0.251
Homocystein (μmol/L)	10.2 (8.8-12.2)	9.6 (8.0-11.4)	0.209
Serotonin (ng/mL)	98 (73-132)	95 (68-138)	0.876
Selectin (pg/mL)	41 (32-56)	41 (24-59)	0.752
sICAM (ng/mL)	266 (190-330)	266 (206-335)	0.806
sVCAM (ng/mL)	643 (505-836)	647 (544-769)	0.973
IMT (mm)	0.75 ± 0.26	0.70 ± 0.20	0.345
ABI	1.03 ± 0.17	1.06 ± 0.23	0.474
FFA (mmol/L)	0.8 (0.6-1.0)	0.8 (0.7-1.1)	0.139
ApoA1 (mg/dL)	152 (137-179)	153 (137-182)	0.997
Apo A2 (mg/dL)	31 (27-35)	32 (27-37)	0.429
Apo B (mg/dL)	96 (76-115)	97 (82-121)	0.688
Lp a (mg/dL)	25 (12-36)	32 (18-60)	0.148

IMT, intima media thickness; ABI, ankle-brachial index; data are the means ± SD if normally distributed or medians (interquartile range); MBL levels in MBL-deficient obesity 245 ng/mL (59-559 ng/mL) vs. MBL-sufficient obesity 1704 ng/mL (1218-2341 ng/mL), $p<0.0001$; [§]MBL deficiency, MBL < 778 ng/mL (corresponding to the lower 95% CI in controls).

turn lead to insulin resistance and type 2 diabetes, dyslipidemia, endothelial dysfunction and atherosclerosis. Although the correlation between increasing BMI and the incidence and prevalence of these chronic diseases is well documented, a part of the obese population has remarkably normal insulin sensitivity and cardiovascular risk profile, and even an improved cardiovascular prognosis[31-33]. Here, we investigated the link between severe obesity as a state of subclinical inflammation and mannose binding lectin (MBL) as part of innate immunity. We investigated obese patients with and without MetS separately in addition to healthy, lean control subjects to investigate whether MBL levels differ between these groups. Moreover, we studied the effects of a standardized weight reduction program on MBL concentrations in the obese. Our results show that MBL levels did not differ between normal weight and severely obese subjects independendly of presenting with or without MetS. Separating individuals with low and high MBL levels did also not reveal an association with the parameters of MetS or markers of atherosclerosis. Moreover, although the weight loss program resulted in marked changes not only in the body mass index, but also with respect to adipocytokines, such as a marked decrease in leptin and an increase in adiponectin levels, as well as improvements in hsCRP-, HOMA-IR, selectin and triglyceride levels, it did not influence MBL levels. Even detailed subgroup analyses did not reveal a group of obese in whom weight loss influenced MBL clearly; therefore, plasma MBL levels do not seem to be related to body weight or MetS, and the concentrations are not affected by weight loss and associated cardiometabolic improvements.

Contrary to these findings, a recent study presented an association among MBL, BMI and insulin resistance, whereby MBL levels were positively correlated with insulin action[19], although the patients in this study did not have fully developed MetS. In contrast to our findings, the study also showed an association between BMI and MBL serum concentration in a secondary longitudinal analysis comprising 10 obese

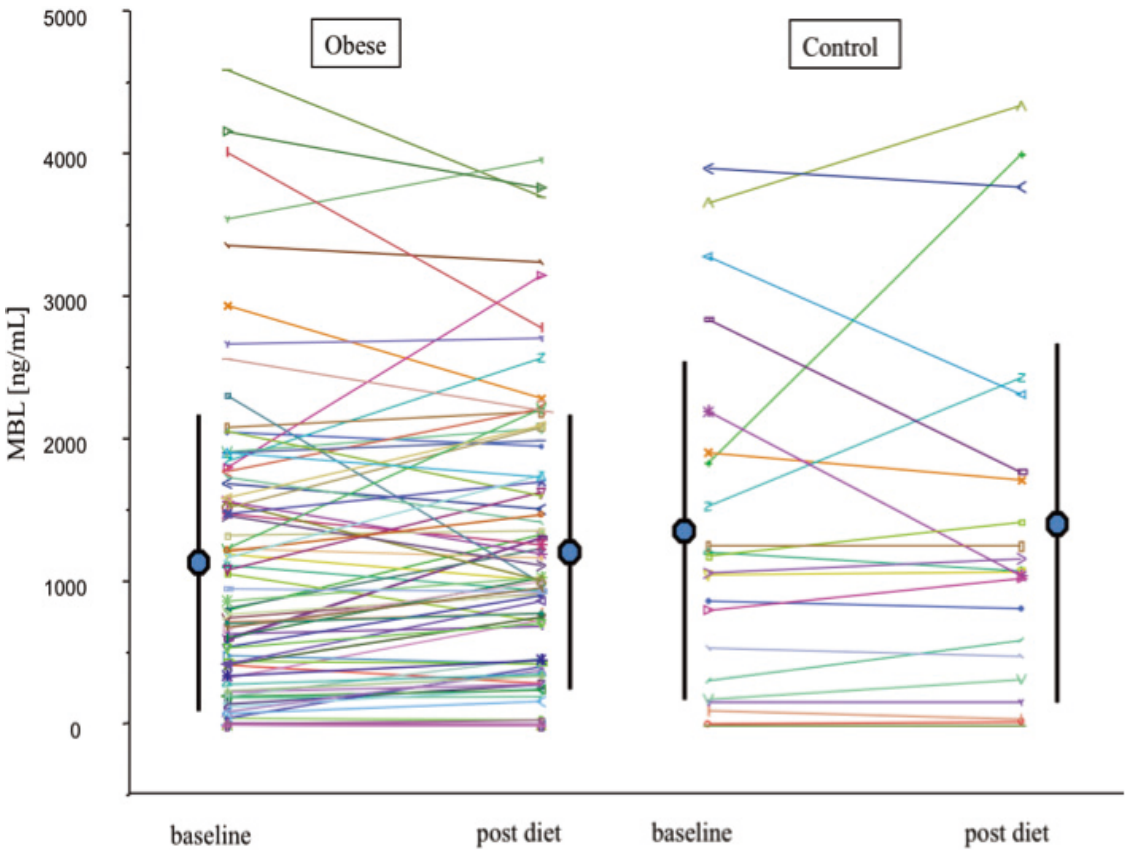

Fig. 2. The individual effect of weight loss on serum MBL levels in severely obese subjects compared to subsequent measurements in healthy lean controls.

patients[19]. With respect to the latter finding, the constrained power in a longitudinal analysis of only 10 subjects should be recognised.

Notably, our results are also in accordance with other reports showing that marked weight loss of 53% excess body weight after surgical intervention did not influence the MBL serum concentration[20]. Moreover, in a large study from Iceland with almost 1000 study participants, MBL levels did not correlate with diabetes mellitus, total serum cholesterol, systolic or diastolic blood pressure, body mass index or the erythrocyte sedimentation rate[26].

Investigating plasma concentrations in healthy persons, the individual MBL level is very stable over time and inter-individual differences in MBL depend primarily on the MBL genotype[30, 34]. The missing link between obesity and MBL could be explained by the fact that MBL is synthesized in hepatocytes, but not in human adipose tissue[35]; however, since it has been reported that individual MBL concentrations vary considerably during acute phase reactions[36] and may rise manifold, it may be speculated that chronic inflammation associated with obesity and MetS affect MBL concentrations. Potentially, altered gene expression or other post-transcript mechanisms, hormonal regulation of MBL production, altered granular hepatic storage and/or release, or altered metabolism may affect MBL concentrations in subjects with obesity and MetS. However, our data do not imply that individual MBL concentrations are influenced to a large extent by such mechanisms associated with obesity or MetS and may not be modifiable by lifestyle interventions.

Contradictory data for MBL are also available with respect to atherosclerosis and coronary disease. Common variant MBL genotypes, coding for markedly diminished levels of MBL, have been shown to be predictive of coronary artery disease and severe atherosclerosis, even after adjustment for traditional cardiovascular risk factors[11-13]. Contrary to these findings, it has been documented that a high MBL level may be a risk factor for acute coronary syndromes[15] and future coronary artery disease[16], and that functional MBL deficiency contributes to reduced mortality in patients

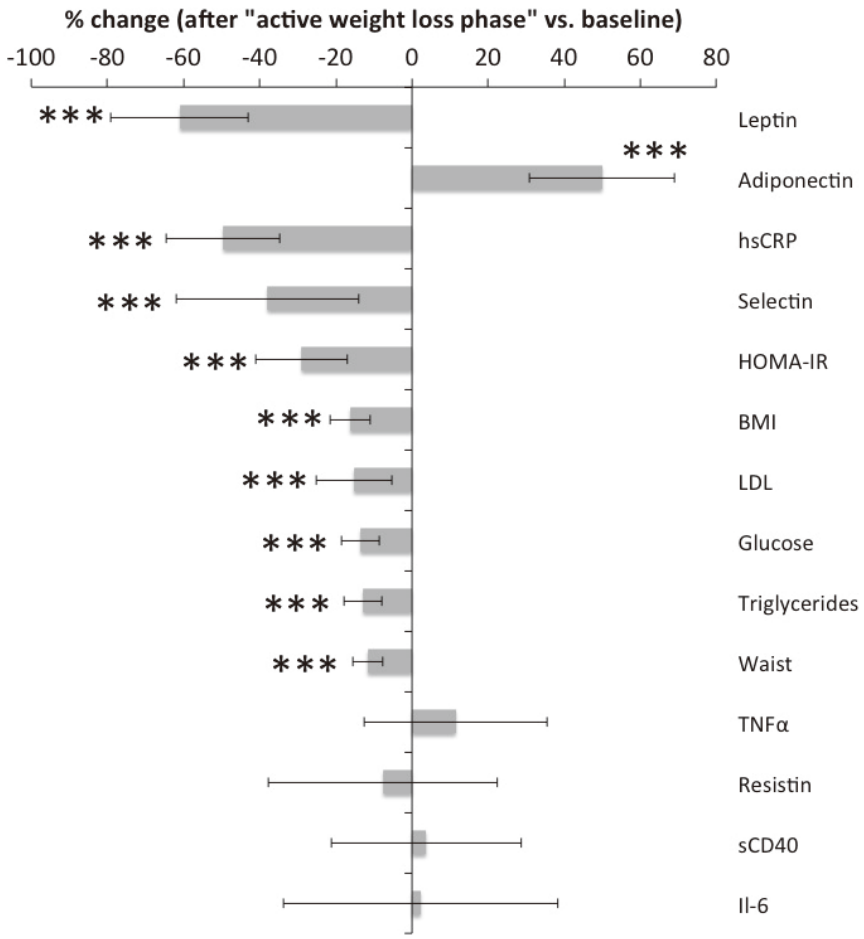

Fig. 3. Changes in selected anthropometrical data, cardiovascular risk profile, adipocytokines, inflammatory parameters, and markers of early atherosclerosis achieved by dieting in obese patients.

with acute myocardial infarction[17]. Moreover, in a prospective study of patients with severe carotid atherosclerosis undergoing eversion endarterectomy, subjects with the normal MBL genotype (alleles A/A) and consecutive higher MBL serum concentrations were at higher risk for experiencing restenosis than those with MBL2 variant genotypes[37].

Experimental data support the importance of complement in the development of atherosclerosis. It could be demonstrated in a mouse model that MBL was abundantly present in developing atherosclerotic lesions, whereas only small amounts of MBL were found in advanced atherosclerotic lesions and no MBL was seen in healthy vascular tissue[38]. In human atherosclerotic lesions, MBL deposition was detected in ruptured lesions within the enlarged intima along necrotic segments of atherosclerotic plaque[38].

As complement activation via the lectin pathway occurs following oxidative stress, low serum MBL levels or the inhibition of MBL have been associated with a favourable outcome in settings of ischemia/reperfusion injury[39, 40] and reduced mortality in patients with STEMI after PCI[17, 39].

To our knowledge, the present study is the first to correlate MBL concentrations with markers of early atherogenesis, oxidative stress parameters, adhesions molecules, or atherogenic lipoproteins, in addition to traditional components of MetS; however, no correlations were found between these markers and serum MBL concentrations and no differences regarding these parameters could be observed between MBL-deficient and MBL-sufficient obesity.

Various arbitrary cutoff levels have previously been used to define insufficient MBL levels[41, 42]. Several previous studies are based on MBL genotyping[37, 43, 44], but DNA samples were not available in

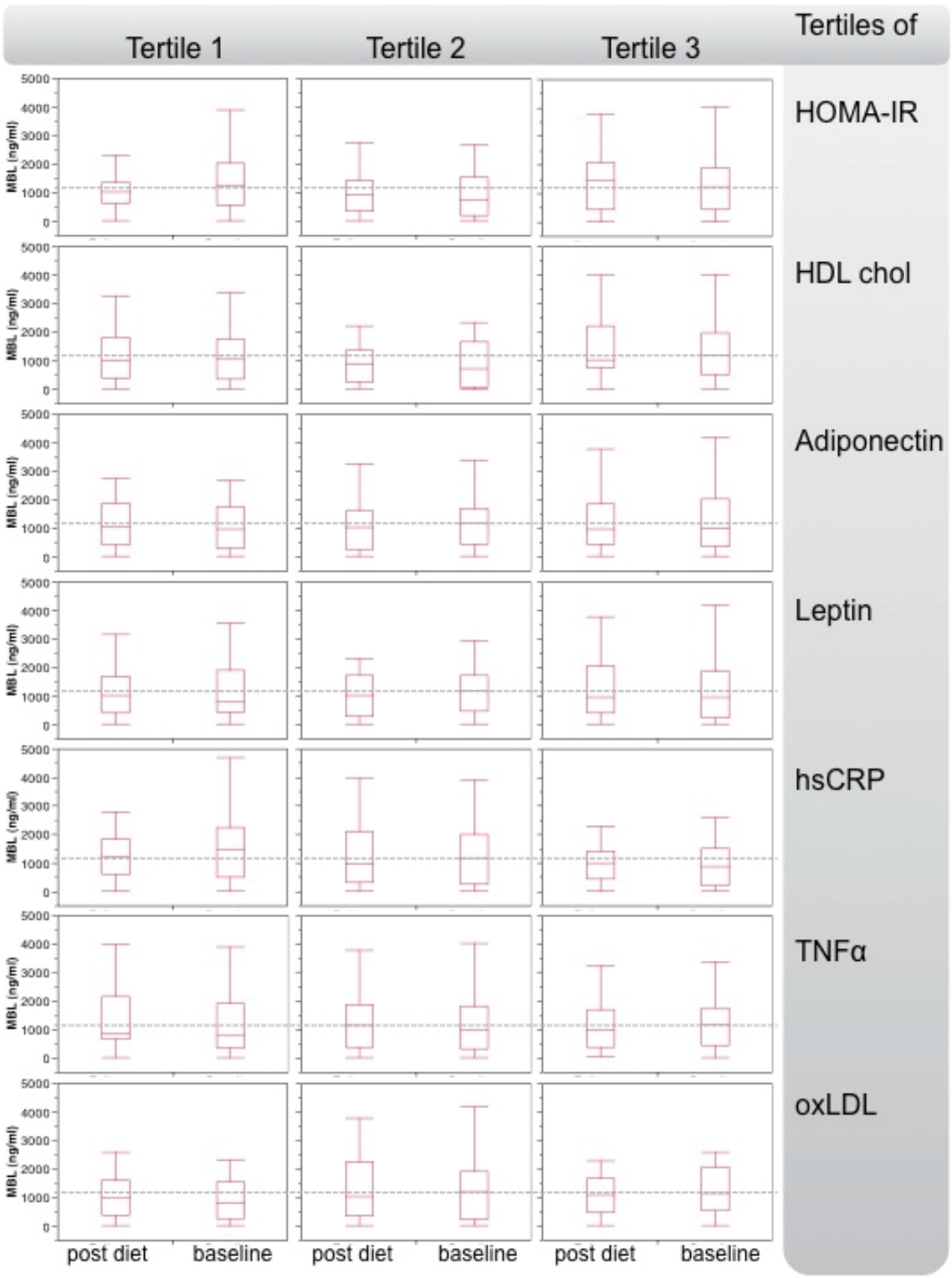

Fig. 4. Serum MBL concentration in severely obese subjects at baseline and post-diet, whereby subjects were divided into subgroups according to increasing tertiles of different components of metabolic traits (right border). In each subgroup, MBL levels were similar before and after marked weight loss.

this study. Our cutoff was determined statistically based on the MBL distribution in healthy control subjects, and approximates other reports[17, 29, 43]. Analysis of continuous MBL values and tertiles as well as evaluation of the 500 and 1,000 g/L cutoff gave similar results (data not shown) to those presented in this paper.

We are aware of the limitations of this study. We examined serum MBL levels in association with markers of metabolic disorders and subclinical atherosclerosis in severely obese patients with and without metabolic syndrome before and after marked weight loss. Thus, the role of circulating MBL in the development of cardiovascular disease was not directly investigated and remains uncertain; however, in light of the existing contradictory clinical endpoint data we feel that our investigations add new data to this field.

The strengths of the present study include its prospective design with detailled phenotypic characterization of severely obese and normal weight healthy controls, as well as considerable changes in the cardiometabolic risk profile achieved by the long-term weight loss.

In summary, our findings suggest that MBL levels did not differ between normal weight and severely obese subjects. Also, MBL did not influence cardiovascular risk factors, metabolic parameters and markers of endothelial dysfunction and early atherosclerosis in severely obese patients before and after marked weight loss.

Further research is needed to describe the complex interplay of metabolic pathways and subclinical inflammation influencing the initiation and progression of arterial diseases in obesity.

Acknowledgment

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