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THE ASSOCIATION OF MANNOSE-BINDING LECTIN WITH PARAMETERS OF METABOLIC SYNDROME AND EARLY ATHEROSCLEROSIS MARKERS

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Structure:

1. Introduction

1.1 The definition of metabolic syndrome and its role in cardiovascular disease

Metabolic syndrome is a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes. It affects one in five people and the prevalence increases with age. Some studies estimate the prevalence in the USA to be up to 25% of the population.

The pathophysiology of metabolic syndrome is extremely complex and has been only partially elucidated. The most important contributing factors are weight, genetics, aging and sedentary lifestyle, i. e. low physical activity and excess caloric intake.

The symptoms and features of metabolic syndrome are:

- Fasting hyperglycaemia, diabetes mellitus type 2 or impaired fasting glucose, impaired glucose tolerance or insulin resistance.
- High blood pressure
- Central (also known as visceral) overweight with fat deposits mainly around the waist
- Decreased HDL cholesterol and
- Evaluated triglycerides.

Various institutions offer different diagnostical criteria for the metabolic syndrome. One of the major definitions currently used was provided by International Diabetes Federation (IDF):

Central obesity (defined as waist circumference with ethnicity specific values) and any two of the following:

- Raised triglycerides: > 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality.
- Reduced HDL cholesterol: < 40 mg/dL (1.03 mmol/L) in males, < 50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality

- Raised blood pressure: systolic BP > 130 or diastolic BP >85 mm Hg, or treatment of previously diagnosed hypertension.
- Raised fasting plasma glucose: (FPG)>100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes. If FPG >5.6 mmol/L or 100 mg/dL, an oral glucose tolerance test (OGTT) is strongly recommended but is not necessary to define presence of the syndrome.

[#] If BMI is >30 kg/m², central obesity can be assumed and waist circumference does not need to be measured.

The World Health Organization(WHO) criteria (1999) requires for the presence of diabetes mellitus, impaired glucose tolerance, impaired fasting glucose or insulin resistance, AND two of the following:

- Blood pressure: ≥ 140/90 mmHg
- Dyslipidaemia: triglycerides: ≥ 1.695 mmol/L and high-density lipoprotein cholesterol (HDL) ≤ 0.9 mmol/L (male), ≤ 1.0 mmol/L (female)
- Central obesity: waist:hip ratio > 0.90 (male); > 0.85 (female), and/or body mass index > 30 kg/m^2
- Microalbuminuria: urinary albumin excretion ratio ≥ 20 mg/min or albumin:creatinine ratio ≥ 30 mg/g.

NCEP

The US National Cholesterol Education Program Adult Treatment Panel III (2001) requires at least three of the following:

- central obesity: waist circumference ≥ 102 cm or 40 inches (male), ≥ 88 cm or 36 inches(female)
- dyslipidemia: TG ≥ 1.7 mmol/L (150 mg/dl)
- dyslipidemia: HDL-C < 40 mg/dL (male), < 50 mg/dL (female)
- blood pressure ≥ 130/85 mmHg
- fasting plasma glucose ≥ 6.1 mmol/L (110 mg/dl)[2)]

Various studies have shown that metabolic syndrome is associated with subsequent development of type 2 diabetes mellitus and cardiovascular disease (CVD).

Cardiovascular disease and all-cause mortality are increased in men with the metabolic syndrome, even in the absence of baseline CVD and diabetes.[3)]
A recent study with 12089 participants has demonstrated that men and women with the metabolic syndrome are 1.5 and 2 times more likely to develop coronary heart disease than control subjects after adjustment for age, smoking, LDL cholesterol, and race/ARIC center.[4)]

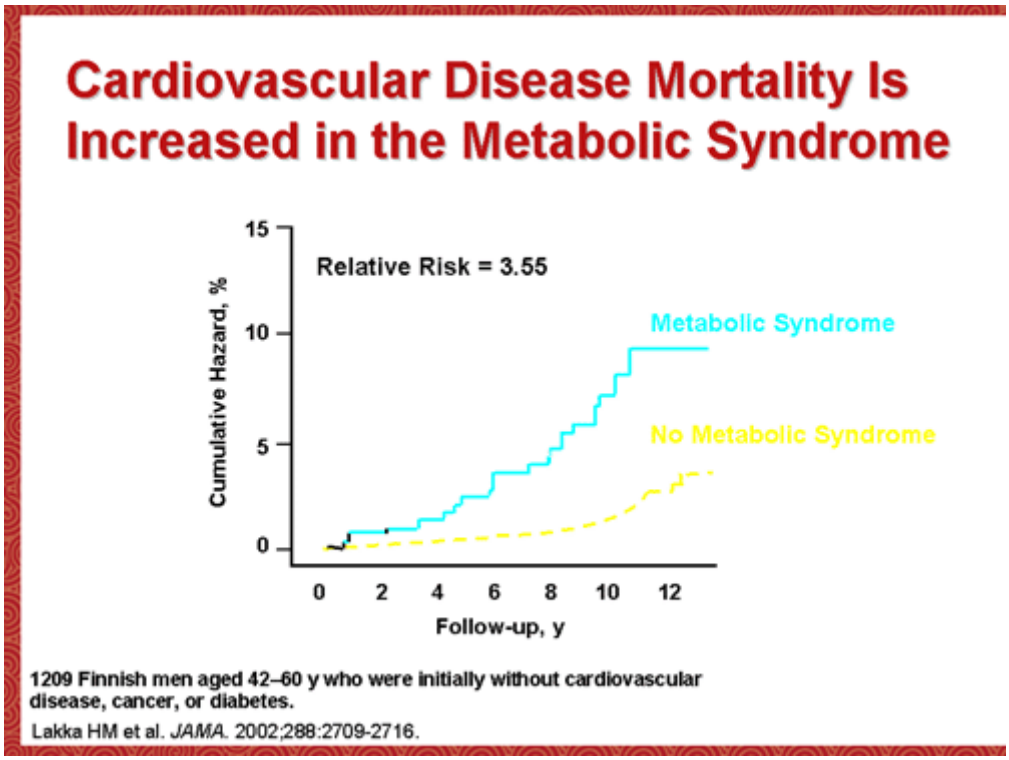

Figure 1: Association of metabolic syndrome with cardiovascular disease mortality[3)]

Another study exposed a 24-fold risk of developing diabetes in men having 4 or 5 features of metabolic syndrome according to ATP III of NCEP compared with men with none.[5)]

Obesity, one of the main components present in the metabolic syndrome is associated with low grade inflammation of adipose tissue, resulting from chronic activation of innate immune system.[6)]

1.2 Innate immune system and it's component: mannose-binding lectine

The innate immune system is considered as the first line of host defence against infectious agents, which have penetrated the mechanical barriers of human organism. It is comprised of soluble and membrane bound proteins with a predefined specifity, in many cases involving carbohydrate moieties.[7)]

The complement system plays a crucial role in the innate defense against common pathogens. Activation of complement leads to robust and efficient proteolytic cascades, which terminate in opsonization and lysis of the pathogen as well as in the generation of the classical inflammatory response through the production of potent proinflammatory molecules.[8)]

The human collectin mannose binding lectine (MBL) is one of the important components of innate immunity. It provides first-line defence by its ability to bind sugar residues on the bacterial surface through its carbohydrate recognition domain and activates the complement pathway leading to lysis of bacteria independent of antibody.[9)]
There is increasing evidence that MBL has a complex role in many diseases.[10)]

1.2.1 MBL: structure

Mannan-binding lectine is a plasma glucoprotein of the collectin-family, members of which bind to carbohydrates on the surfaces of microorganisms and particulate materials, including altered host material, such as apoptotic sells via their C-terminal lectin domains. MBL is produced by the liver; its serum levels are extremely variable, ranging from almost 0 to >5μg/mL in healthy humans. MBL deficiency is common (5% or more of the population, depending on the threshold concentration used to define deficiency).[11)] It is associated with severe and repeated infections in infants.

The polypeptide chain of secreted MBL is 228 amino acids long, not including the 20-residue signal peptide. It consists of a cystein-rich cross-linking region, collagen-like-region, a neck region and a C-terminal calcium-dependent carbohydrate-binding lectin domain. The neck region forms an α-helical coiled-coil structure which is supposed to promote trimerization of three polypeptides to form the subunit. The trimer is stabilized by hydrophobic interactions and inter-chain disulphide bonds within the N-terminal cystein-rich region. The commonest oligomeric form in humans appears to be a six-unit with an overall molecular mass of 18×25000 Da (see fig. 1).[11)]

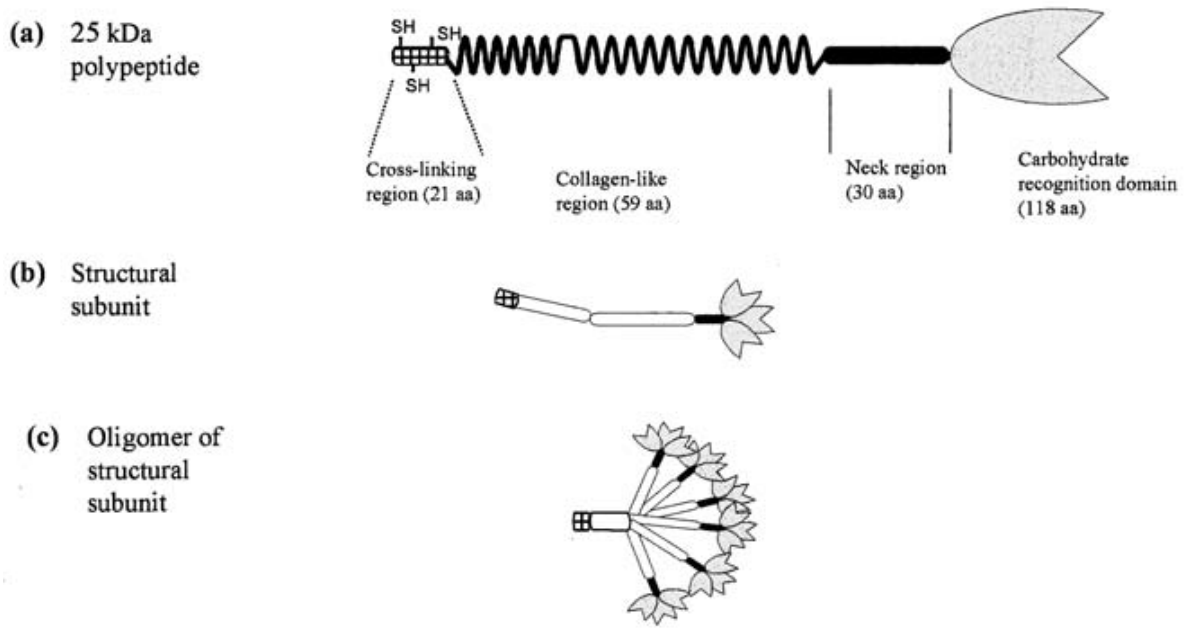

Figure 2: structure and organization of MBL

1.2.2 MBL: binding specifity

MBL shows selective and calcium (Ca^{2+})-dependent binding to terminal sugars D-mannose, L-fructose and N-acetyl-D-glucosamine (GlcNAc), but not to D-galactose and sialic acid. This selectivity relies on the presence of conserved amino acid residues within their CRDs. The CRD of MBL contains an EPN (Glu-Pro-Asn) amino acid motif that provides a preference for binding to sugars with equatorial 3- and 4-hydroxyl (OH) groups such as D-

mannose, L-fructose, and GlcNAc. All these sugars are commonly found on the surface of many microorganisms.[12)]

MBL leads to opsonisation and phagocytosis by polymorphonuclear cells. This binding also results in activation of the complement system in an antibody in C1q independent manner and requires MBL associated serine proteases MASP-1, MASP-2, MASP-3. Bound MBL associated with MASP-2 is able to promote C4 deposition and to form C3 convertase that leads to formation of membrane attack complex (MAC) and lysis of bacteria.[9)]

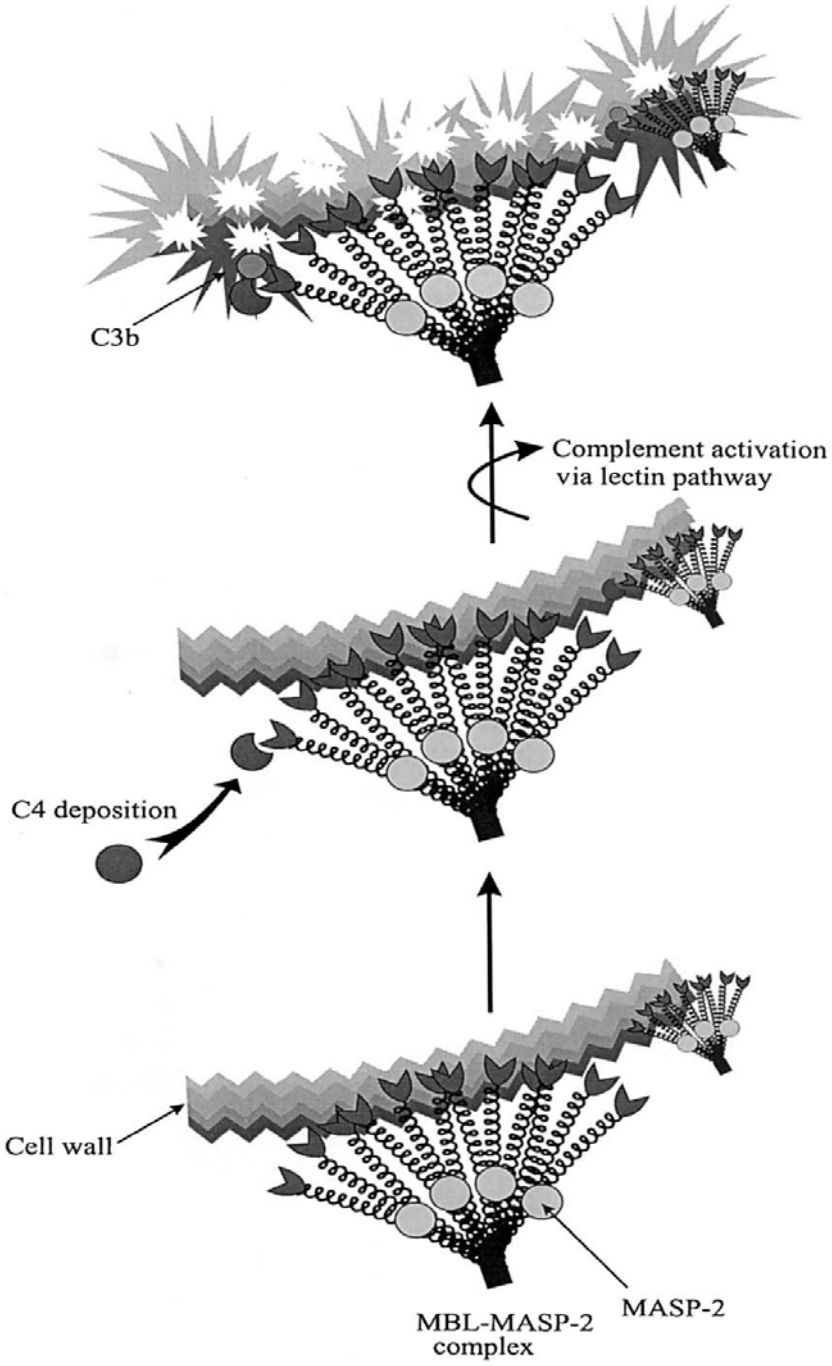

Figure 3: Mannose-binding lectin (MBL) and MBL-associated serine protease 2 (MASP-2) forming a complex that binds to sugar groups on microbial structures. Complement activation through the lectin pathway is initiated by C4 deposition and results in C3b formation and damage to microorganisms.[13)]

1.2.3 MBL: genetics

MBL belongs to a family of collectin proteins, which are generally located on chromosome 10 (q21-24). There are two MBL genes: MBL-1 and MBL-2. MBL-1 is a pseudo-gene and does not code for an active MBL protein. MBL- 2 consists of 4 exons and codes for the active MBL protein. The MBL-2 gene has three known mutations, all found on exon 1: allele B at codon 54, allele C at codon 57, and allele D at codon 52. These mutations lead to structural abnormalities in the collagenous triple helix and eventually failure to form a biological functional MBL protein and therefore they cause MBL deficiency. The wild-type is allele A. In addition to mutations in the promoter region of the MBL-2 gene, there are two mutations in the promoter region at -550(H/L, rs 11003125) and -221(Y/X, rs 7096206), which result in a decreased synthesis of the protein. The alleles interact with each other to form MBL secretor haplotypes, producing high, intermediary and low MBL levels. The combination of structural gene and promoter polymorphisms results in a dramatic variation of MBL concentration in apparently healthy individuals of up to 1000 fold.[9)]

The three *MBL2* variant alleles appear with different frequencies in different populations around the world. The *B* allele is virtually absent in Sub-Saharan West-Africa and occurs with low frequency in northern East-Africa, but is quite common among Caucasians (allele frequency 0.12–0.14) and Asians (allele frequency 0.10–0.25). The frequency in South American Indians may exceed 0.50. By contrast, the *C* allele is very common in Sub-Saharan Africans (0.10–0.30), rare among Caucasians (less than 0.03), and absent among Asians and American Indians. The frequency of the *D* allele is less frequent than the other two and appears to be confined to the Caucasians and northern East-African populations (allele frequency 0.06–0.08).[14)]

1.2.4 MBL: serum levels

MBL is primarily synthesized in the liver. Although it predominantly circulates as a serum protein, MBL has also been detected at various sites, for examples, in middle ear fluid, in synovial fluid of an inflamed joint, in amniotic fluid from 26 weeks gestation, and in nasopharyngeal secretion.[11)] MBL concentrations in umbilical cord blood are generally lower than in the circulation of adults, but there is a rapid rise during the first week of life. [15)]

The combination of structural gene polymorphisms results in a dramatic variation in MBL concentration in apparently healthy individuals of up to 1000 fold (Caucasian<20-10000ng/ml).[9] The median level of MBL within a Danish Caucasian population was reported at 1.2µg/ml plasma for individuals homozygous for the wild type allele.[7]

Currently, there is no definition of low MBL level.

Unlike the archetypal acute phase proteins such as serum amyloid P- and C-reactive protein (CRP), whose levels can increase by 10-1000 fold during inflammation, MBL serum levels remain relatively constant in individuals and determined by gene polymorphisms described above. Circulating levels of MBL only increase by 2-3 fold upon infectious and inflammatory challenge. However, during inflammation loss of vascular integrity results in leakage of MBL from the circulation into the inflamed site and hence it is reasonable to assume that more significant changes in the local MBL concentration might occur during inflammation states.[12]

1.2.5 Association of variant MBL serum levels with various diseases

Various recently made studies have investigated correlations between varying MBL plasma serum levels on autoimmune and infectious diseases, metabolic and cardiovascular disorders. Both high and low MBL concentrations seem to have positive effects and disadvantages depending on the nature of the disease that the host is exposed to.

Studies have demonstrated the importance of MBL in the anti bacterial defense. Pediatric patients with infections and with suspected immunodeficiencies have increased frequencies of MBL variant alleles.[17] A strong clinical association between MBL variant alleles and meningococcal disease susceptibility has been shown in consecutive pediatric patients seen in a London hospital and in a United Kingdom-wide study of patients with meningococcal disease. Bacteriemia and pneumonia were significantly associated with decreased MBL levels (<500 µg/L) in chemotherapy-treated Danish hematology patients.[13]

Because of the high frequency of variant MBL alleles in different populations it has been speculated whether MBL deficiency could protect against certain infectious diseases. Based on the high frequency of MBL deficiency found in 56 Kenyan Africans, the authors suggested that low circulating levels of MBL may protect against infections with intracellular organisms

that display MBL ligands on their surface. The presence of MBL on the surface of parasites might allow for cellular uptake either directly via an MBL receptor or via receptors for complement fragments deposited on the parasite as a result of complement activation by the MBL pathway. In support to the protective effect of MBL deficiency, it was found that Ethiopians infected with M. leprae had a significant higher MBL than non-infected controls. [7)]

Several reports have presented evidence for the involvement of MBL in systemic lupus erythematodes (SLE). A significant correlation between the serum level of MBL and the risk acquiring SLE was also found. [7)]

In the study made in Glostrup University Hospital, in Copenhagen, Denmark, the presence of MBL variant alleles was found to be associated with the development of sepsis, severe sepsis, and septic shock.[18)]

MBL 2 structural variant genotypes were found to be significantly associated with BMI of >25 and hypercholesterolemia. MBL2 exon 1 variant allele genotypes (A/O or O/O, where O indicates any of the variant alleles D, B or C) were more common among combined overweight and obese group (BMI >25.0) than among the normal weight group (38% vs. 20%; $p = 0.021$.[19)]

MBL seems to play dual role in occurrence and proliferation of cardiovascular disorders. While some studies propose cardioprotective role of high MBL serum circulation levels reducing the risk of myocard infarction, others claim that lectin pathway of complement activation leads to tissue damage in myocardial ischaemia/reperfusion (I/R) injury, and therefore, low MBL levels may be favorable in patient with cardiovascular disorders.

In patients undergoing primary percutaneous coronary intervention, functional deficiency of complement MBL (serum MBL levels of or below 100 ng/mL) was associated with reduced mortality. [20)] In a resent in-vivo study made on test animals monoclonal antibodies were raised against rat MBL. Rats were subjected to 30 minutes of left coronary artery occlusion and 4 hours of reperfusion. A pretreatment with the produced antibodies effected postischemic myocardial reperfusion injury positively: significantly reduced myocardial creatine kinase loss (48%), infarct size (39%), and neutrophil infiltration (47%) were observed.[21)]

On the other hand, MBL deficiency seems to be associated with bypass graft occlusion in patients with coronary heart disease.[22] Furthermore, in a study with 76 Norwegian patients undergoing coronary-artery bypass, coronary-valve replacement surgery, or both and 100 blood-donor controls, an association of MBL deficiency with severe atherosclerosis was observed.[23] Variant MBL alleles with lowered MBL serum levels were associated with increased carotid plaque area.[24]

High MBL may predict decreased likelihood of myocardial infarction. In a population-based Reykjawik study including 19381 participants, a cross-sectional group was selected from the original cohort (n=987). High MBL (>1,000ng/L) was associated with a greatly lowered odds ratio for MI (0.64, $P<0.001$). In the next cohort sample (n=1309), high MBL was associated with greatly decreased MI risk in diabetic ($P=0.02$) or hypercholesterolemic individuals ($P=0.004$**)**. Also, the results of the study propose that hypercholesterolemic individuals with high MBL had enhanced risk of MI compared with participants with normal cholesterol levels, but the risk was much lower than that of hypercholesterolemic individuals with low MBL in both study groups[25]

According to a study with patients who attended the Steno diabetes center in Gentofte, Denmark, the mortality in type 2 diabetes was significantly higher among patients with MBL levels greater than or equal to 1000µg/L than among patients with levels less than 1000µg/L.[26] In contrast to this, low mannose-binding lectin genotype was found to be associated with future cardiovascular events in type 2 diabetic South Asians.[27]

MBL deposition was also found in human ruptured atherosclerotic plaques: Atherosclerotic lesions, stable and ruptured were collected during surgical procedures (carotid eversion endarterectomy) from individuals suffering end-stage symptomatic occlusive vascular disease. MBL deposits were located within the enlarged intima, along ill-defined necrotic segments of the atherosclerotic plaque.[28]

In a recent report authors emphasized a dual role of mannose-binding lectine in relation to carotid intima-media thickness in patients with rheumatoid arthritis. They found a strong association of quadratic term of serum MBL with intima-media thickness of the common carotid artery in 114 rheumatoid arthritis patients, reflecting a U-shaped relation. This finding suggests that both high and low MBL levels may be associated with increased subclinical atherosclerosis within the same diseased population.[29]

2. Hypothesis

While metabolic syndrome and its feature increased visceral fat deposit is connected to chronic activation of innate immune system and MBL is a central player in the innate immune response, we assumed that mannan-binding-lectine might be an important diagnostic parameter of metabolic syndrome.

We hypothesize that circulating MBL levels in subjects with obesity and the 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 the 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.

3. Methods

3.1. 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 February 2006 at the University Hospital of Regensburg, Germany, is to evaluate excessive body fat for its pathogenic potential in terms of cardiometabolic diseases and to evaluate the effect of a considerable weight reduction on interactions in systems biology. In order to establish this, we are currently building up this 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 obese with and without characteristics of the 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 (Nestle Healthcare®) 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, present with a BMI>30 kg/m2 and a constant body weight in the last 3 months, and if they signed 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 month, cancer, pregnancy, therapy with steroids or thyroid hormones, known heart disease, 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/m2) 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 their participation in the study.

3.2 Standardized weight reduction program

Optifast 52 (Nestle Healthcare) 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 as 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.

3.3 Phenotyping

3.3.1 Anthropometric analysis

All subjects were studied after a 12 hour overnight fast between 7:00 and 9:00 a.m.
To measure waist circumference, the abdominal region of participants was cleared of closing or accessories to remove any pressure on the abdomen. If tight or restrictive clothing was worn, the belt was unlatched and their pants were loosed, the garment was lowered sufficiently. Participants were asked to stand with their feet shoulder-width apart and to cross their arms over their chest in a relaxed manner. Their hips were palpated in order to locate the top of the iliac crest. The measuring tape was placed on the level of the uppermost border of the iliac crest horizontally around the subject´s abdomen. The tape was kept horizontal from left to right and from back to front. (A mirror was used to facilitate this procedure). The tape was tightened around the abdomen gently without depressing the skin. The subjects were asked to relax and breathe normally. Subjects took 3-4 breaths before the measurement was taken. Measurement was taken from the 0 to the nearest millimeter at the end of a normal expiration.
To measure hip circumference, all clothing and accessories except light underwear were removed. Participants were asked to stand with their feet about 15cm apart and their weight equally distributed on each leg. Participants were asked to breathe normally; the tape was tightened gently around the greatest hip circumference without depressing the skin. Subjects took 3-4 breaths before the measurement was taken. The reading of the measurement was taken at the end of gentle exhaling.

To measure the weight of the probands correctly, shoes and bulky clothing were removed, pockets were emptied. The scale was balanced before participants stepped on it. Participants were asked to stand with their both feet in the center of the platform. After the scale had been in balance for 3-4 seconds, the weight was recorded to the nearest 0.1 kg.

Height of the participants was measured with a standard SECA stadiometer. The distance from the floor of the platform to the highest point on the head was measured Participants were asked to remove their shoes and bulky clothes, to put their feet together and arms by their sides. Heels, buttocks and upper back of the participants were in contact with the wall. While the measurement was taken the subjects were facing directly ahead.

Using the data gained above, waist-to-hip-ratio and body mass index (BMI) of the probands were evaluated. Waist-to-hip-ratio was calculated by dividing the waist circumference in centimeters with the hip circumference in centimeters of the subject. BMI was gained by dividing the individual's body weight by the square of his or her height.

Blood pressure and the patient’s pulse have been taken after a sitting period of 5 minutes. The patient’s pulses (bilateral radial and dorsalis pedis arteria) were documented respecting rhythmen and character.

In conclusion with the American heart association we measured blood pressure 3 times at both arms with an appropriate blood pressure cuff Welch Alynn with at least 60 seconds between the measurements.

3.3.2 Body composition analysis

Body composition was determined by *bioelectrical impedance analysis (BIA)* using Nutriguard-MTM (Data Input GmbH Darmstadt). It was used for measuring body fluid volumes, fat-free mass (FFM) and body cell mass. Impedance meters are applied in a supine position with four geltype electrodes, two voltage and two current ones, 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 height2/resistance.[30)] The instrument was regularly checked with resistors of known values.

3.3.3 Serological Analysis

Adipokines and inflammatory markers. Fasting plasma adiponectin, leptin and resistin were measured using a commercially available enzyme-linked immunoassay (ELISA) kits (Bio Vendor®) and MBL was detected performing the ELISA kit Sanquin®.

The concentration of high sensitive C-reactive protein (hsCRP) was determined by means of 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 the commercially available ELISA 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) x fasting serum insulin (mU/L) divided by 22.5, as described by Matthews et al.[31)]

3.3.4 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.

3.3.5 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 on 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 time are minimised 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.

3.3.6 Analysis of a resting metabolic rate via respiratory exchange ratio

Resting metabolic rate (RMR) was measured by *indirect calorimetry* using a DeltatracTM system (DatexOhmeda) in a quiet environment. In supine position oxygen consumption and carbon dioxide production were determined for 30 min. Energy expenditure was derived from CO2 production and O2 consumption. The apparatus was calibrated with gas mixtures of known composition with 95% CO2 and 5% O2 before each test and the instrument was routinely checked.

3.3.7 Evaluation of patient`s physical fitness

A six-minute walking test (6-MWT) was performed as a measure of functional capacity. The 6-MWT was conducted following a standardized protocol. A flat, obstacle-free corridor, with chairs placed on one side was used. Patients were instructed to walk as far as possible, turning 180º every 35 m in the time of 6 minutes. Patients walked unaccompanied so as not to influence walking speed, and they were asked to walk as far as possible within 6 minutes. Patients were permitted to rest, if needed. At the end of the 6 minutes, the observer measured the total distance walked by the patient and noted the number and length of breaks, if breaks have been taken. The patient's pulse was taken before and after the 6-minutes walk test.

3.4 Definitions

3.4.1 Definition of MBL deficiency

MBL concentrations among healthy Caucasians vary from undetectable up to 10 000 µg/L with a median around 1 000 µg/L.[25), 32)] Single base mutations within exon 1 and several mutations in the promoter region of the MBL gene result in the interindividual differences in serum MBL levels. MBL deficiency is generally defined as serum levels < 500 ng/mL, but some groups have defined severe MBL deficiency as levels < 50 ng/mL and partial MBL deficiency from 50 ng/mL up to 1000 ng/mL.[20), 33);34)]

As there is no unique definition of functional MBL deficiency and clinical relevance may vary in different diseases, MBL levels <778 ng/mL were chosen for the differentiation in MBL deficient and MBL sufficient obese 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 obese in our study was 245ng/mL (interquartile range 59-599), while the median concentration in MBL sufficient obese was 1704 (1218-2341) ng/mL.

3.4.2 Definition of metabolic syndrome

MetS was diagnosed according to the Adult Treatment Panel III (ATP III).[2)] It requires presence of central obesity with waist circumference ≥102 cm in men and ≥ 88 cm in women and dyslipidemia with values of triglycerides of ≥150 mg/dL, and HDL-cholesterol of <40mg/dL in men and < 50 mg/dL in women. Hypertension and hyperglycemia were diagnosed for values of blood pressure ≥ 130/85 mmHg and values of fasting plasma glucose ≥ 110mg/dL. The MetS was diagnosed when at least three out of five metabolic abnormalities were determined.

3.5 Statistical analysis

Continuous variables are presented as mean ± standard deviation or median with its interquartile intervals (percentiles 25 and 75), when appropriate.

Categorical variables were compared using the Fisher's exact test. Continuous variables were compared using oneway anova for normally distributed variables (if significant, the Student's t-test for the comparison of 2 groups, or the Tukey-Kramer post hoc test for multiple pairwise comparisons were used. 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.

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.

4. Results

4.1 Patients characteristics at baseline

According to the definition of metabolic syndrome (see above) we evaluated with the gained data the number of obese patients without (n=55) and with fully developed metabolic syndrome (n=41).

The baseline characteristics of both obese participants with and without metabolic syndrome, as well as oft the lean control group are presented in table 1. Obese participants without and with MetS were of a similar age, had similar BMI (average of 39,7 without MetS vs 41,0 with MetS), waist/hip-ratio (0,91 and 0,92 respectively) and fatmass (42,2% and 41,4% respectively). By definition, obese subjects with MetS had lower HDL cholesterol and higher triglyceride levels, as well as higher glucose, insulin, and HOMA-IR levels than obese without the MetS and lean controls.
As expected, the percentage of subjects with diabetes among those with MetS was considerably higher than among obese participants without MetS (24% vs 2%).

Table 1: Baseline characteristics in healthy, lean controls, and obese subjects with and without the 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/m2)	22.3±2.0	39.4±7.7 c)	41.0±7.9 c)
Waist (cm)	78±8	120±19 c)	121±18 c)
Hip (cm)	99±5	131±14 c)	132±17 c)
Waist/Hip	0.79±0.06	0.91±0.10 c)	0.92±0.10 c)
Fatmass (%)	21.5±6.5	42.2±8.7 c)	41.1±7.5 c)
RMR (cal./d)	1539±221	1973±522 c)	2045±459 c)
HDL chol. (mg/dL)	67±12 5	8±15 c)	43±8 c), f)
LDL chol. (mg/dL)	105±26	113±34	122±36
Triglyc. (mg/dL)	73±27	97±35	170±83 c), f)
Diabetes n (%)	0	1 (2)	10 (24)
Fasting Gluc. (mg/dL)	84±5	92±11	125±55 c), f)
Fasting Insulin (mU/L)	5.9±3.0	18.2±14.8 b)	26.4±16.9 c), d)
HOMA-IR	1.2±0.6	4.3±3.9	8.8±7.9 c), f)
Systolic BP (mmHg)	123±14	137±19 b)	144±13 c)
Diastolic BP (mmHg)	77±9	83±12	86±9 b)

RMR, resting metabolic rate; data are means ± SD; a): $p<0.05$ vs. Controls, b): $p<0.01$ vs. Controls, c): $p<0.001$ vs. Controls, d): $p<0.05$ vs. Obese, no MetS, e): $p<0.01$ vs. Obese, no MetS, f): $p<0.001$ vs. Obese, no MetS

Baseline adipocytokines, inflammmatory markers, and markers of atherosclerosis, and apolipoproteins in healthy lean controls, and obese subjects with and without the MetS are exposed at table 2.

It can be observed below, that adiponectin levels in obese subjects were generally lower in both obese subgroups, with and without MetS, then in control group. Additionally, adiponectin was lower in the subgroup of obese with MetS, then in the obese subgroup without. Contrary could be observed in leptin concentration: it was significantly lower in controls, but also obese subjects with MetS had higher leptin serum levels then obese participants without MetS. Next, both subgroups of obese had statistically significant increase in hsCRP and intima media thickness in comparison with controls. Obese subjects with MetS expressed higher oxLDL , Apo A1, Apo A2 and ApoB serum levels then both control cohort and obese group without MetS.

Table 2: Baseline adipocytokines, inflammmatory markers, and markers of atherosclerosis, and apolipoproteins in healthy lean controls, and obese subjects with and without the 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) **a)**	9.5 (7.1-11.1) **b)**
Leptin (μg/L)	8 (2-12)	48 (31-65) **a)**	52 (30-65) **c)**
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) 2)	18 (8-39) **a)**
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) **c)**	5.2 (2.6-8.7) **c)**
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) **a)**	461 (357-627)
oxLDL (U/L)	46 (40-56)	47 (38-62)	57 (51-74) **b), d)**
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) **d)**
Serotonin (ng/mL)	107 (63-132)	95 (70-148)	94 (73-126)
Selectin (pg/mL)	28 (21-37)	38 (26-55) **a)**	43 (35-60)
sICAM (ng/mL)	245 (203-264)	239 (188-318)	294 (235-347) **b)**
sVCAM (ng/mL)	635 (571-863)	633 (503-759)	654 (559-849)
IMT (mm)	0.50±0.16	0.70±0.22 **c)**	0.74±0.23 **c)**
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) **c), f)**
Apo A2 (mg/dL)	35 (31-41)	34 (30-40)	29 (26-32) **c), f)**
Apo B (mg/dL)	80 (73-89)	91 (74-105)	110 (86-127) **c), e)**
Lp a (mg/dL)	22 (15-53)	31 (20-60)	19 (12-34)

IMT = intima media thickness;

a): p<0.05 vs. Controls, b): p<0.01 vs.Controls, c): p<0.001 vs. Controls, d): p<0.05 vs. Obese, no MetS, e): p<0.01 vs. Obese, no MetS, f): p<0.001 vs. Obese, no MetS

Figure 4 exposes plasma MBL concentration in lean controls and obese patients without and with MetS at baseline. Beside the box-and-whisper plots representing the 10th, 25th, 50th, 75th and 90th percentiles, the means with its 95% confidence intervals are shown (dotted triangles).

Mean MBL serum levels did not differ statistically significant in controls, obese subjects without and with MetS.

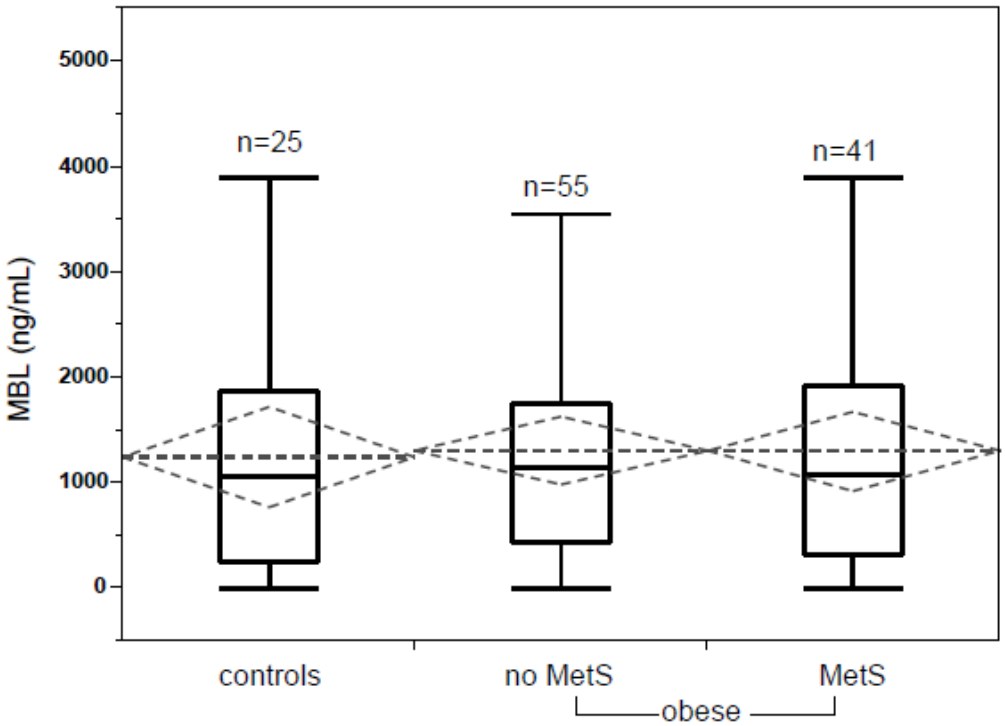

Figure 4. Plasma MBL concentration in lean control participants, obese subjects without metabolic syndrome and with present metabolic syndrome according to the definition of ATP III

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

Table 3: Cardiometabolic risk profile in MBL sufficient and MBL deficient obese

	MBL deficient obese* (n=40)	MBL sufficient obese (n=56)	P Value
Sex n (% women)	12 (30)	22 (39)	0.392
Age (years)	46±13	44±14	0.470
BMI (kg/m2)	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 means ± SD; MBL levels in MBL deficient obese 245ng/mL (59-559 ng/mL) vs. MBL sufficient obese 1704 ng/mL (1218-2341 ng/mL), $p<0.0001$; *MBL deficiency, MBL<778 ng/mL (corresponding to the lower 95% CI in controls).

In both MBL sufficient and deficient obese, mean fatmass, waist/hip ratio, triglucerid levels and fasting glucose were almost identical. MBL deficient obese subjects expressed even slightly lower HOMA-IR (5,2±4,7) than MBL sufficient cohort (6,9±7,2).

Table 4: Adipocytokines, inflammmatory markers, markers of atherosclerosis and apolipoproteins in MBL sufficient and MBL deficient obese

	MBL deficient obese* (n=40)	MBL sufficient obese (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 medians (interquartile range) or means ± SD; MBL levels in MBL deficient obese 245 ng/mL (59-559 ng/mL) vs. MBL sufficient obese 1704 ng/mL (1218-2341 ng/mL), $p<0.0001$; *MBL deficiency, MBL<778ng/mL (corresponding to the lower 95% CI in controls).

Observing the table 4, it can be recognized that MBL sufficient and deficient obese subjects express only minor differences in their cardiometabolic risk profile. The markers of atherosclerosis were almost identical in both subgroups (as example, hsCRP serum levels were at 5,1 and 5,2 in MBL deficient and sufficient obese respectively), no statistically relevant differences could be observed in apolipoproteins. Among the markers of atherosclerosis, solely the IMT was slightly higher in MBL deficient obese subjects compared to participants with no MBL deficiency (0,75 mm and 0,70 mm respectively).

4.2 Evaluation of patients parameters at a 3-months-folow up

The changes in selected anthropometrical data, cardiovascular risk profile, adipocytokines, inflammatory parameters, and markers of early atherosclerosis achieved by diet in obese patients after substantial weight loss in percentage in comparison to the baseline investigation are presented in figure 5. The most prominent changes achieved by the diet could be detected for leptin (>60%), adiponectin (>50%), hsCRP, selectin, and HOMA-IR.

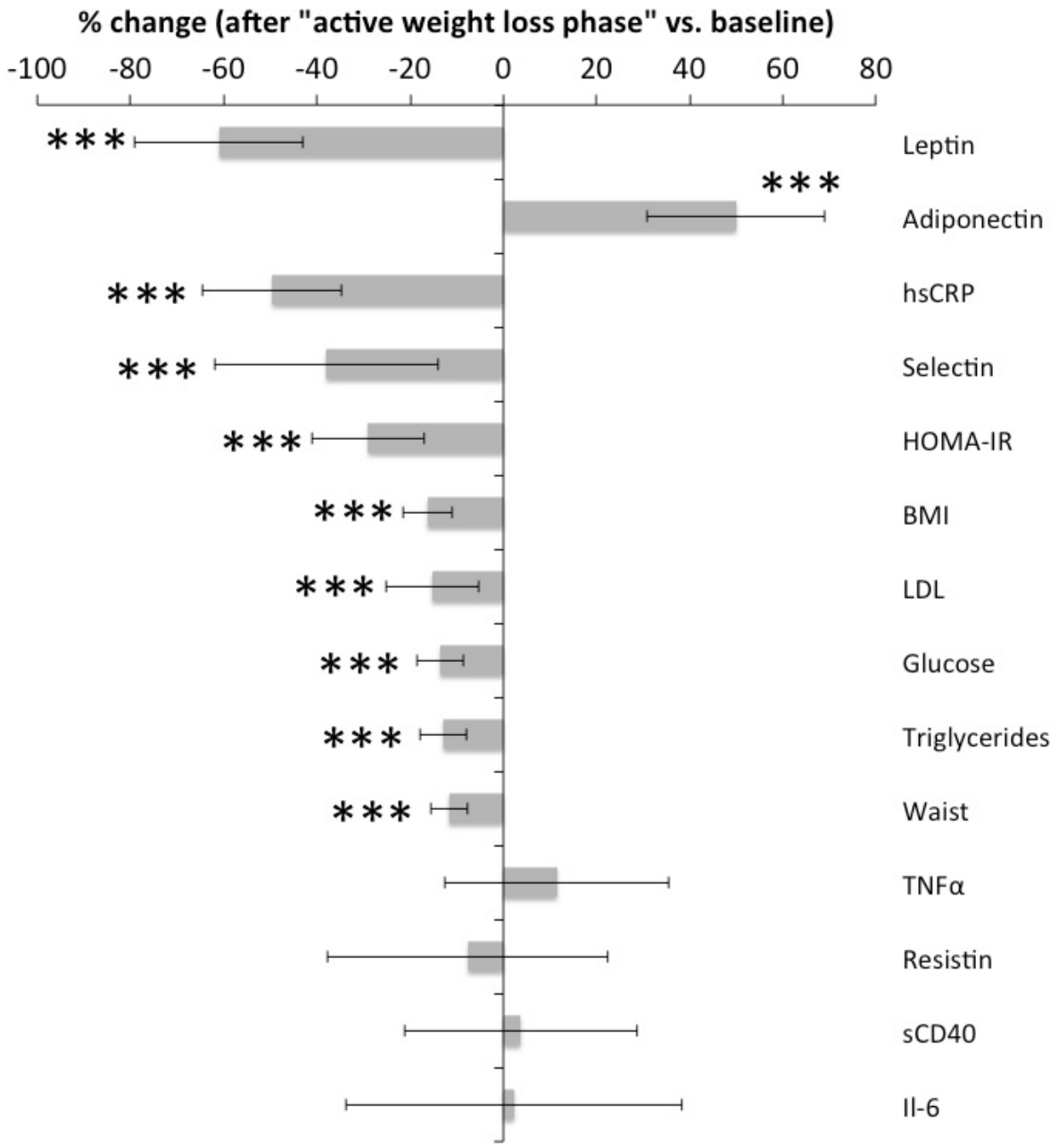

Figure 5. Changes of selected anthropometric parameters, adipocytokines, inflammatory parameters achieved in the group of obese patients by diet are exposed in % in comparison with baseline investigation.

For more detailed analysis of obese subjects we subdivided the group in three nearly equal subgroups according increasing tertiles of different components of metabolic traits. The group in the left column presents lower, the group in the right column correspondingly, higher concentrations of various parameters of metabolic syndrome and atherosclerosis.

We observed the 3 subgroups at a subject of MBL level in association to different serum parameters of MetS. Results can be observed at figure 5. In each subgroup MBL levels were similar before and after marked weight loss.

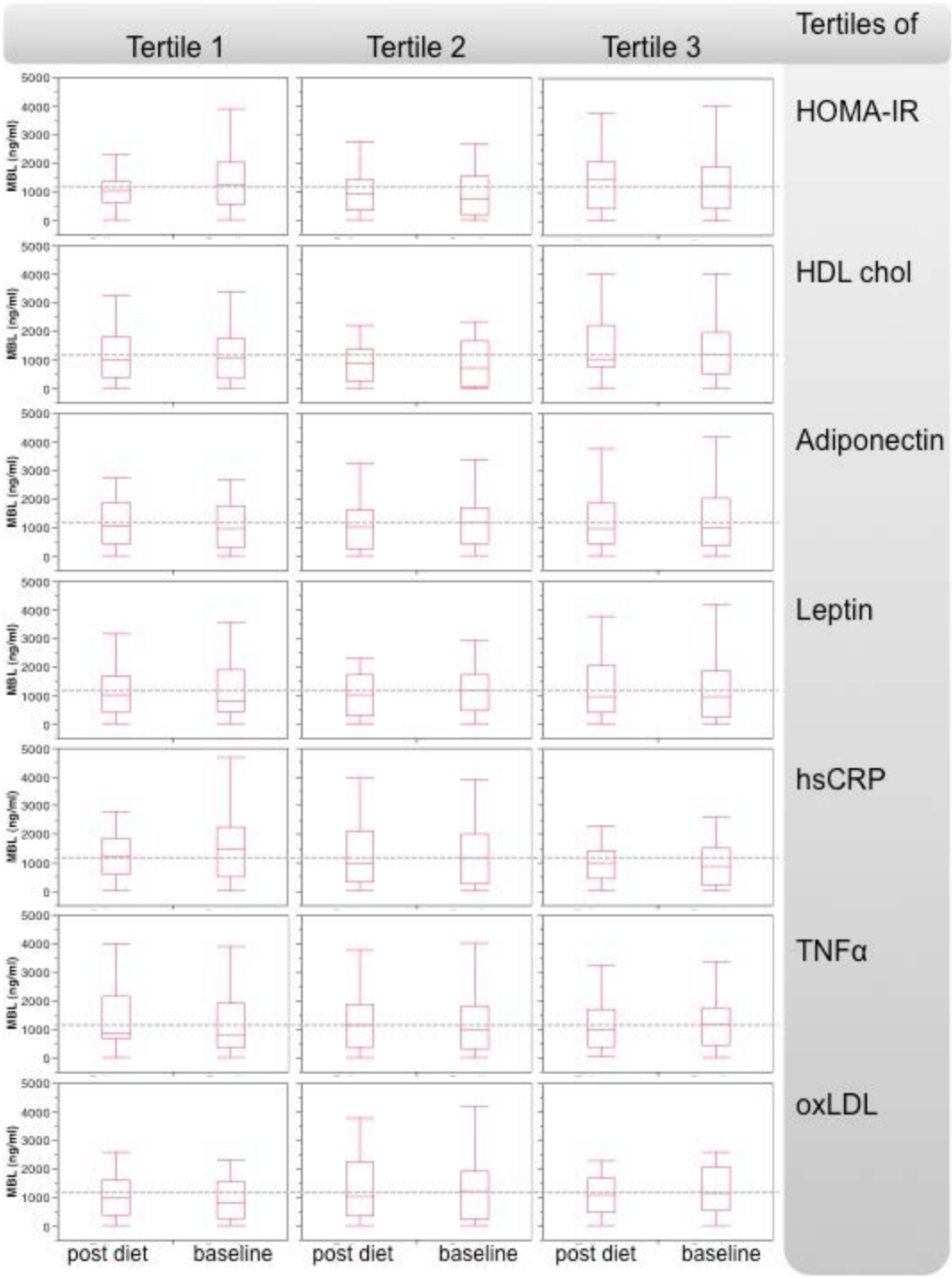

Fig. 6. Serum MBL concentration in severely obese subjects at baseline and post diet, whereby subjects were divided into subgroups according to increasing tertiles of different components of metabolic traits. In each subgroup MBL levels were similar before and after marked weight loss

It can be recognized that MBL is not associated to any of the chosen parameters: HOMA-IR, HDL cholesterol, adiponectin, leptin, hsCRP, TNFα, oxygenized LDL.

Figure 7 exposes the MBL levels of both obese and control groups at a baseline in comparison to those attending a 3-month follow up investigation. The mean MBL concentration in obese participants at a baseline was 1132,93 ±1047,30, while at the 3-month follow up 1207,94±961,25 (p=0,6397). Analogously, in lean controls, the concentrations at baseline and at a 3-month follow up were 1370,73±1182,08 and 1413,70±1267,71. It is recognizable that despite of various changes in MBL concentrations in single individuals, the mean MBL level remained nearly unchanged before and after dietary intervention in the obese group, as well as in the control group without a dietary intervention.
Furthermore, the MBL serum levels do not differ significantly between the obese and lean participants.

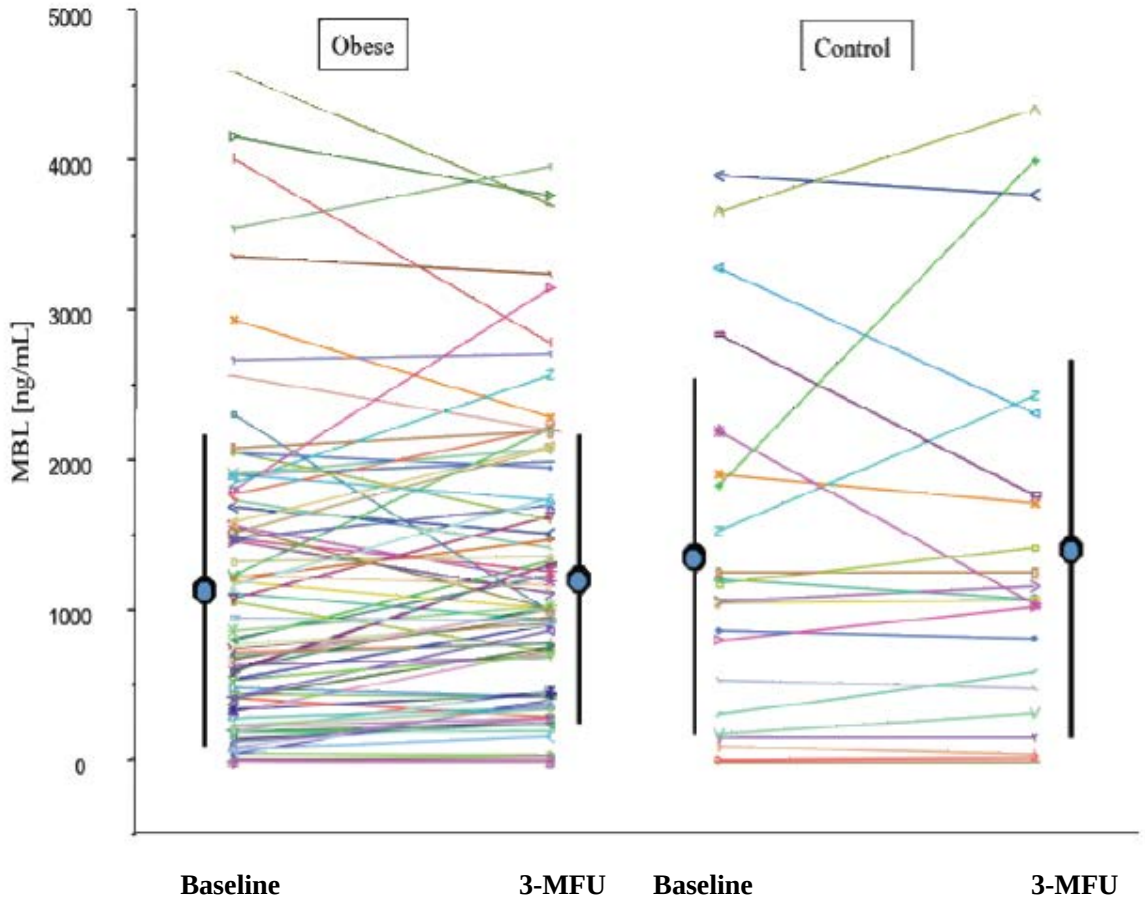

Fig. 7. The individual effect of weight loss on serum MBL levels in severely obese compared to subsequent measurements in healthy lean controls.

5. Discussion

Mannose-binding lectin is a serum protein that plays a central role in the innate immune response.[12)]
The genetic inheritance has the largest influence on circulating MBL concentration. MBL serum levels are relatively constant.

However, several studies claimed a 2-3 fold increase of MBL serum levels during an acute phase response.[35)]

Obesity appears to be associated with a low-grade inflammation of white adipose tissue. Therefore we hypothesized that obesity may be associated to an activation of native immune system and the MBL circulating levels differ from those of lean subjects. However, in the population of obese participants with or without evidence of metabolic syndrome we quantified similar serum MBL concentrations to those of the control group.

Furthermore, our study has shown that MBL serum level can not be affected by dietary intervention and weight reduction: MBL concentration was not associated to weight loss in the population of obese participants.

The reason why differing MBL concentrations were not observed in obese subjects is that MBL increase may conduct when a certain degree of inflammation has been reached and a considerable activation of the innate immune system is required. Increased MBL levels after a major surgery described by Thiel in [35)] can be probably explained as exactly the type of challenge for a native immune system of its host.

MBL insufficiency has been linked to many specific disorders, although the findings of similar studies have sometimes been contradictory.

Whereas in early childhood and in immunodefficient patients a native immune system and its major component MBL undoubtedly plays a crucial role and therefore MBL deficiency may lead to severe health disorders, the role of MBL in other diseases is controversial. Both high and low serum MBL concentrations may be advantageous depending on the disease and the type of pathogen the host is exposed to.

One popular hypothesis or assumption is that MBL insufficiency is only clinically relevant in the context of another coexisting immune deficiency. This view has not been conclusively confirmed or refuted, but enough data have been collected to support it as a sensible working hypothesis. Firstly, it is undoubtedly true that the majority of individuals in the general population with low MBL levels, or even very low MBL levels, are healthy. Secondly, clinical symptoms ascribed to MBL insufficiency have sometimes been found in combination with a specific laboratory-defined defect: IgG subclass deficiency (Aittoniemi et al., 1998)[36], a chemotaxis defect (Ten et al., 1999)[37] and neutropenia induced by the chemotherapy (Neth et al., 2001[38], Peterslund et al., 2001[39]) are examples.[16]

The comparison of results on the baseline investigation and in the 3-month-follow-up investigation in the group of obese subjects reveals a significant improvement both of anthropometric and serologic parameters. Furthermore, in the interviews with observers the majority of obese participants claimed to notice an improvement in their health related quality of life after weight loss.

Using factor analysis to reveal correlations of MBL serum levels and various serum parameters of a metabolic syndrome, we have found no associations with the most parameters: insulin, fasting serum glucose, HDL, total adiponectin, selectin, triglycerides.

While no significant differences in MBL circulating levels in obese and lean subjects were found, we observed in the control group associations between low MBL and cardioprotective parameters such as adiponectin, fasting glucose, HDL, LDL, $ApoA_2$, ApoB and resistin.

Furthermore, we divided the obese subjects into subgroups according to increasing tertiles of different components of the MetS or plasma biomarkers and compared the MBL levels before and after weight loss. No statistically relevant changes of MBL plasma concentrations were observed both in the subgroup without and with fully developed MetS.

Considering the fact that MBL serum concentration was similar in obese population and in the control group on the baseline and expressed no significant changes after significant improvement of anthropometric and serologic parameters in obese group associated with the

weight loss, we summarize that MBL cannot be used as a diagnostical parameter of metabolic syndrome.

Although the MBL concentrations in the whole population of obese patients and controls remained nearly unchanged, in single cases MBL levels varied in a same person on a baseline and on 3-month-follow-up considerably. During the observation period our participants were not exposed to any major surgeries and seemingly did not have severe inflammation that can explain MBL serum level variations. Therefore we suppose that other factors than inflammation in acute phases are able to influence the MBL serum concentration in individuals. Long-term measurements of MBL serum levels in participants with and without medicamental intervention may be of a great interest in order to evaluate factors other than genetic expression that may be able to modify the MBL levels in individuals.

Contrary to our findings a recent study presented an association between MBL, BMI and insulin resistance, whereby MBL levels were positively correlated with insulin action[40)], despite the patients in this study (n=434) did not have a fully developed metabolic syndrome. The same study also assessed a correlation between weight loss (in consequence of biliopancreatic diversion) and insulin sensitivity in a secondary longitudinal analysis comprising 10 obese patients ; a correlation of serum MBL concentration change with insulin sensitivity, but not with changes in BMI was asserted. With respect to the latter findings, the constrained power in a longitudinal analysis of only ten subjects should be recognized.

Furthermore, our results are in accordance to another report, showing that a massive loss of excessive body weight did not affect serum MBL, visfatin and TNF-α, despite of significant changes in parameters of metabolic syndrome.[41)] Moreover, in a large study with 987 participants, MBL levels did not correlate with diabetes mellitus, BMI, or total serum cholesterol.[25)]

Another study undertaken by Aarhus University Hospital, Denmark included 36 non-diabetic obese subjects who received a low-calorie diet for 8 weeks. A common weight loss of 13.5 kg was achieved. Similar to our study, the relationship between the MBL serum level, obesity and insulin resistance was investigated. No correlations were found between weight loss and changes in MBL, nor between changes in insulin resistance and MBL.[42)]

To our knowledge the present study is the first correlating MBL concentrations with markers of early atherogenesis, oxidative stress parameters, adhesions molecules, or atherogenic lipoproteins, in addition to traditional components of the MetS.

Various arbitrary cutoff levels have previously been used to define insufficient MBL levels [15), 43)]. Several previous studies are based on MBL genotyping[44)-46)], but DNA samples were not available in this study. Our cutoff was determined statistically, based on the MBL distribution in healthy control subjects, and approximates other reports.[33), 45), 47)] We have also performed statistical analysis using the cutoffs generally found in the literature with MBL deficiency defined as serum MBL< 1000ng/mL and MBL< 500ng/mL and achieved similar results to those presented in our study (data are not shown).

In a recent study, MBL was detected using the ELISA kit Sanquin® ELISA kit for in vitro quantitative analysis of mannose binding lectin in human serum (or plasma). The kit has a minimum detection level of 9.0 ng/mL and possesses an intra-essay variation of 6.1%[48)]. We performed repeated determinations to ensure high quality of measurements and achieved a high conformity of results.

The strengths of the present study are detailed phenotypic characterization of participants including both anthropometric data, serological atherosclerosis and diabetes markers ECG, ECH and Carotid ultrasound imaging, and an achievement of a considerable changes in cardiometabolic risk profiles by valuable weight loss.

It is worth mentioning, that although MBL seems to be unsuitable as a prognostic factor of insulin resistance, our findings lead us believe that a combination of waist circumference above 129cm and low adiponectin are strongly associated with impaired insulin action in obese participants.

6. Summary

MBL is a major component of a native immune system. It plays a considerable role in the activation of the immune cascade. MBL deficiency is common and occurs in a high percentage of population.

A large number of frequent studies describe associations of circulating MBL serum levels to various diseases. Depending on the art of disorder, MBL deficiency seems play either negative, or, in some cases, protective role, preventing the excessive activation of the innate immune system.

Obesity is associated with low grade inflammation of adipose tissue, resulting from chronic activation of the innate immune system.

Chronic low-grade inflammation, as found in obesity and particularly in the MetS, may influence different components of the innate immune system that in turn lead to insulin resistance and type 2 diabetes, dyslipidemia, endothelial dysfunction and atherosclerosis. In a present study, we investigated the association of MBL serum levels with obesity the possibility to influence the MBL concentration via dietary intervention, the association of MBL with atherosclerotic parameters as well as metabolic syndrome and insulin resistance.

We enrolled 96 severely obese patients, with and without MetS, who participated in a standardized weight loss program “Optifast”, and 25 lean (20<BMI<25), seemingly healthy person as a control group.

MBL serum levels of participants were examined in association with markers of insulin resistance, dyslipidemia, adipokines, and subclinical atherosclerosis before and after marked weight loss (20 ± 8 kg after 3 months) in the obese group and in the control group with no dietary intervention. The results of both groups were analyzed and compared to each other.

The MBL serum levels among lean and obese participants did not differ on the baseline investigation independently on presence of a MetS. Furthermore, the MBL serum concentration remained nearly equal both in the control group and among the obese participants after dietary intervention in the 3-month-follow up investigation.

In severely obese subjects there was no significant difference concerning cardiovascular risk profile, apolipoproteins, inflammatory and metabolic parameters, and markers of endothelial dysfunction and atherosclerosis between subjects with functional MBL deficiency (MBL < 778 ng/mL) and MBL sufficient obese (MBL ≥ 778 ng/mL).

Our findings suggest that plasma levels of MBL do not differ between healthy lean and severely obese subjects. There seems to be no association between MBL serum concentration and markers of insulin resistance, dyslipidemia, adipokines, and early atherosclerosis in severely obese patients before and after marked weight loss.

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8. Abbreviations:

6-MWT	=	6-minute walking test
aa	=	amino acid
ABI	=	Ankle-brachial index
Apo-A1	=	apolipoprotein A-1
Apo-A2	=	apolipoprotein A-2
Apo-B	=	apolipoprotein B
ATP III	=	adult treatment panel
BIA	=	bioimpedance index
BMI	=	body mass index
BP	=	blood pressure
CRD	=	carbohydrate recognition domain
CRP	=	C-reactive protein
Da	=	Dalton
DNA	=	desoxyribonuclein acid
dL	=	decilitre
ECG	=	electrocardiography
ECH	=	echocardiography
ELISA	=	enzyme-linked immunoassay
FFAs	=	fat-free acids
FFM	=	fat-free mass
FPG	=	fasting plasma glucose
FPG	=	fasting plasma glucose
g	=	gram
GlcNAc	=	N-acetyl-D-glucosamin
hsCRP	=	high sensitive C-reactive protein
HDL	=	high density lipoprotein
HOMA	=	homeostasis model assessment
I/R	=	ishemia/reperfusion
IDF	=	International diabetes federation
IL	=	interleukin
IMT	=	intima media thickness

kcal	=	kilocalory
L	=	liter
LDL	=	low density lipoprotein
MAC	=	menbrane attack complex
MASP	=	mannan associated serum protease
MBL	=	mannan-binding lectin
MetS	=	metabolic syndrome
mg	=	milligram
mmHg	=	millimeters of mercury (pressure)
mmol	=	millimole
mU	=	milliunit
NCEP	=	National Cholesterol Education Program
ng	=	nanogram
oxLDL	=	oxydized low density lipoprotein
R	=	resistance
RMR	=	resting metabolic rate
RMR	=	resting metabolic rate
sCD 40	=	soluble CD40 ligand
sICAM	=	soluble intercellular cell adhesion molecule
SLE	=	systemic lupus erythematodes
sVCAM	=	soluble vascular cell adhesion molecule
TNF-α	=	tumor necrosis factor α
Xc	=	reactance
µg	=	microgram

Zusammenfassung

Das metabolische Syndrom ist eine Kombination von kardiovaskulären Risikofaktoren und ist charakterisiert durch die vier Hauptkomponenten, abdominelle Adipositas, Bluthochdruck (Hypertonie), veränderte Blutfettwerte (Dyslipidämie), und Insulinresistenz. Aufgrund des gleichzeitigen Vorhandenseins mehrerer atherogener Risikofaktoren gilt es als bedeutender Risikofaktor für kardiovaskuläre Erkrankungen, insbesondere für die koronare Herzerkrankung. Auch das Risiko, an einem manifesten Typ 2 Diabetes mellitus zu erkranken, ist um das vielfache erhöht. Frühzeitig sind bei Vorhandensein eines metabolischen Syndroms Zeichen einer endothelialen Dysfunktion nachweisbar.

Inzwischen ist bekannt, dass bei Vorhandensein eines metabolischen Syndroms im Fettgewebe proinflammatorisch wirksame Moleküle sezerniert werden, die möglicherweise eine bedeutende Rolle bei der Entwicklung einer Insulinresistenz sowie Atherosklerose spielen. Desweiteren sind in die Pathogenese der Atherosklerose auch Faktoren des angeborenen Immunsystems involviert.

Eines der Bestandteile des angeborenen Immunsystems ist das Plasmaprotein Mannose-bindendes Lektin (MBL). Als Akut-Phaseprotein ist es in der Lage, Komplement zu aktivieren und somit systemische Inflammation zu aggravieren.
Die MBL- Konzentration ist individuell hauptsächlich genetisch präterminiert und schwankt zwischen sehr geringen, nicht messbaren Werten bis über 5 µg/mL. Die Plasmaspiegel sind relativ konstant; sie können jedoch im Rahmen von Akut-Phasereaktionen auch um das 2-3 fache ansteigen. MBL könnte eine Rolle in der Interaktion zwischen Adipositas bzw. dem metabolischen Syndrom und kardiovaskulären Folgeerkrankungen spielen. Die Ergebnisse diesbezüglich bislang publizierter Untersuchungen zu kardialen Endpunkten sind allerdings widersprüchlich.

Das Ziel folgender Arbeit war festzustellen, ob bei Patienten mit morbider Adipositas und somit vorhandener chronischer „low-grade-inflammation“ ein Zusammenhang zwischen den Parametern des metabolischen Syndroms und der endothelialen Dysfunktion bzw. der frühen Atherogenese einerseits und der MBL-Plasmakonzentration andererseits besteht. Desweitern sollte der Frage nachgegangen werden, ob durch eine Verbesserung der metabolischen Situation, wie sie durch eine Lifestyle-Intervention bzw. durch eine Teilnahme an einem

standardisierten Gewichtsreduktionprogramm erreicht werden kann, die MBL-Plasmakonzentration beeinflusst werden kann.

Zu diesem Zweck wurde eine prospektive Untersuchung an 96 sehr adipösen Probanden durchgeführt, die an dem Gewichtsreduktionsprogramm „Optifast-52“ (Nestle Healthcare) teilnahmen. Zusätzlich wurden 25 vermeintlich gesunde, normalgewichtige Kontrollpersonen untersucht. Alle Teilnehmer wurden einer ausführlichen klinischen und laborchemischen Phänotypisierung unterzogen. Parameter des metabolischen Syndroms, der endothelialen Dysfunktion und der frühen Atherogenese wurden im longitudinalen Verlauf vor, während und nach Gewichtsreduktion mit den Plasma-MBL-Konzentrationen korreliert.

Dabei waren die MBL-Konzentrationen bei gesunden Kontrollpersonen mit denjenigen der sehr adipösen Probanden vergleichbar, und zwar unabhängig vom Vorhandensein eines metabolischen Syndroms. Desweiteren zeigte eine ausführliche klinische Charakterisierung der Studienteilnehmer keine wesentlichen Unterschiede bei adipöse Probanden mit hohen und niedrigen Plasma MBL-Konzentrationen. Detaillierte Subgruppenanalysen konnten ebenfalls keine statistisch signifikante Assoziationen zwischen den untersuchten Parametern und den MBL-Konzentrationen identifizieren.
Auch führte die die hypokalorische, die mit einem Gewichtsverlust von 20±8 kg verbunden war, zu keiner Änderung der MBL-Plasmaspiegel.

Zusammenfassend scheint es keinen Zusammenhang zwischen MBL und Parametern des metabolischen Syndroms sowie der Atherogenese zu geben. MBL beeinflusst weder kardiovaskuläre Risikofaktoren, noch hat eine Lifestyle-Intervention Auswirkung auf die MBL-Plasmaspiegel.

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