Increased plasma lipopolysaccharide-binding protein and altered inflammatory mediators in overweight women suggest a state of subclinical endotoxemia

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Abstract

Background: Over 65% of American women are overweight or obese. Obesity and the closely related metabolic syndrome increase the probability for developing several diseases, including cardiovascular disease (CVD). Chronic low-grade inflammation has been recognized as an underlying event linking obesity to CVD. However, inflammatory alterations in individuals who are overweight remain understudied. To provide insight, we performed a pilot study to determine the levels of key circulating biomarkers of endotoxemia and inflammation in overweight vs. lean

women with high cholesterol and/or high blood pressure - two important conventional risk factors for CVD.

Methods: Plasma samples from adult female subjects who were lean (n=20, BMI=22.4±1.6 kg/m^2) or overweight (n=20, BMI=27.0±1.5 kg/m^2) with similar ages (55.65±9.1 years and 59.7±6.1 years), and race/ethnicity, and self-reported high cholesterol and/or high blood pressure were analyzed and compared. Samples were obtained through the Northwell Health "Genotype and Phenotype, GaP" registry. Plasma levels of lipopolysaccharide-binding protein (LBP), CRP, IL-6, leptin, and adiponectin were analyzed using commercially available assay kits.

Results: Plasma levels of LBP (a recognized marker of metabolic endotoxemia in obesity) were significantly higher in the overweight group compared with the lean group (p=0.005). The levels of CRP, a general marker of inflammation, were also significantly higher in overweight subjects (p=0.01), as were those of the cytokine IL-6 (p=0.02) and the adipokine leptin (p=0.002), pro-inflammatory mediators associated with cardiovascular risk. Levels of adiponectin, an adipokine with anti-inflammatory and anti-atherogenic functions, were significantly lower in the overweight group (p=0.002). The leptin/adiponectin ratio, a preferential atherogenic marker was significantly increased in women who are overweight (p=0.02). Alterations in LBP, CRP, leptin, and adiponectin significantly correlated with BMI, but not with age. The absolute levels of these analytes were within the ranges reported for healthy subjects evaluated in larger clinical trials and thus can be classified as consistent with subclinical endotoxemia.

Conclusion: These results document the presence of a pro-inflammatory state in overweight compared with lean women and are of interest for further evaluation of evidence of inflammation in overweight individuals as an additional risk factor for cardiometabolic disease.

Introduction

Obesity and the closely related metabolic syndrome are associated with an increased risk for cardiovascular disease (CVD) and other debilitating and lethal disorders (1-6). Publicly available information on the World Health Organization (WHO) website states that in 2016 around 1.9 billion adults (people over 18 years of age) were overweight, and more than 600 million were obese and the expectations are that more than 2.16 billion people will be overweight and 1.12 billion will be obese by 2030. A major underlying factor driving the pathogenesis in obesity and metabolic syndrome is the presence of a chronic low-grade inflammation, which is characterized by increased circulating IL-6 and other cytokines, as well as altered levels of adipokines, such as leptin and adiponectin (3, 7-11). An important driver of the inflammatory state in obesity is *metabolic endotoxemia*, manifested by increased gut lipopolysaccharide (LPS)-containing microbiota and the consequent compromising of intestinal permeability that leads to increased circulatory LPS levels (12-15). Obesity-associated metabolic endotoxemia and chronic inflammation promote metabolic derangements and are associated with increased cardiovascular risk (9, 15-21).

In addition to obesity, there is evidence that overweight individuals may be at increased risk for CVD and other diseases (22, 23). CVD is the leading cause of death among women in the United States (6, 24). Increased cholesterol levels (hypercholesterolemia) and high blood pressure (hypertension) are important risk factors for CVD (6, 25). Elevated total cholesterol, hypertension, and excessive body weight have been linked to age-dependent increases of coronary heart disease incidence and mortality in both men and women, but to a larger extent in women (26). However, the underlying explanation for sex-specific differences in the CVD pathophysiology remain poorly understood (6). As recently summarized, “Cardiovascular disease in women remains understudied, under-recognized, underdiagnosed, and undertreated globally” (6).

While metabolic endotoxemia and inflammation have been documented in people with obesity and linked to CVD and other diseases, endotoxemia and inflammatory alterations in overweight individuals remain to be characterized. This is of specific interest for improved understanding of women's cardiovascular health. To generate insight, we profiled a panel of plasma biomarkers of endotoxemia and inflammation, previously associated with CVD in obesity, in a cohort of women who were overweight compared to those who were lean. As dyslipidemia and hypertension are recognized leading traditional risk factors for CVD in women (6, 25), we enrolled subjects having high cholesterol and/or high blood pressure in both groups. We observed that increased circulating levels of LBP, a marker of metabolic endotoxemia in parallel with elevated CRP, leptin, and IL-6 levels, and decreased adiponectin levels were found in overweight women.

Material and Methods

Human subjects

Frozen plasma was obtained from research subjects who consented to participate in the IRB-approved Genotype and Phenotype (GaP) registry (http://www.gapregistry.org/ IRB #09-081A), a living biobank of volunteers. Participants gave random blood samples and were chosen based on gender, BMI (lean: 18-24.9 kg/m^2 (N=20) vs. overweight: 25-29.9 kg/m^2 (N=20)), age, demographic information, and health/medical information (**Supplementary Table 1**). Subjects in both groups were relatively healthy, except they had self-reported hypertension (systolic $\geq$130mm Hg and diastolic >80 mm Hg) and/or high cholesterol (200 mg/dL) and minor conditions, including acne, eczema, gastroesophageal reflux disease (GERD), drug allergies, and other allergies, as well as osteoarthritis, osteopenia, osteoporosis. Excluded conditions were Lyme disease, cancer (solid and blood [leukemia, lymphoma, etc.]), anemia, pancreatitis, emphysema, asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (ulcerative colitis, Crohn's), lupus, rheumatoid arthritis, valvular disease, heart failure, HIV, excess alcohol use, diabetes

(types 1 and 2) and Alzheimer's disease and other neurological conditions that would impair the subjects' ability to consent, as well as those using steroids, insulin, metformin, or glyburide and those who smoke or vape.

Plasma sample analyses

All plasma samples were collected from consented GaP participants prior to the COVID-19 pandemic, aliquoted, and stored at -80°C in the Boas Center Biorepository. Just prior to analysis plasma samples were thawed and then assayed for numerous analytes (using dilutions optimized in prior studies) according to the manufacturer's guidelines: adiponectin using the adiponectin/Acrp30 ELISA (DY1065, R&D System, lower limit of detection [LLoD] 15.6pg/ml), C-reactive protein or CRP by ELISA (DY1707, R&D Systems, LLoD 15.6pg/ml); leptin by ELISA (DY398-05, R&D Systems, LLoD 31.2pg/ml); LPS binding protein or LBP by ELISA (DY870-05, R&D Systems, LLoD 0.8ng/ml); and IL-6 using the V-plex MSD platform (K151QXD-2, Meso Scale Discovery, LLoD 0.06pg/ml).

Statistical analysis

Data were analyzed using GraphPad Prism 9.5.1 software and applying an unpaired Student's t test with Welch's correction; $P<0.05$ was considered significant. The linear correlation for the data was analyzed using the Pearson's correlation coefficient (or Pearson's r). The strength of the correlations was assessed based on the r values and considered to be weak (0.2-0.39), moderate (0.40-0.59), or strong (0.6-0.79). Graphical representations were created using GraphPad Prism 9.5.1.

Results

There was no statistically significant difference in subjects' age (**Table 1**) The BMI of the subjects in the overweight group was significantly higher compared with the BMI of the lean group (**Table 1**).

Table 1. Study participants' age and BMI.

Subjects	Lean	Overweight	p value
Age (years)	55.65±9.1	59.7± 6.1	p>0.05
BMI (kg/m^2)	22.4±1.6	27.0±1.5	p<0.0001

LBP is an important mediator of LPS interactions with immune cells and the LPS-induced transcription of pro-inflammatory cytokines (27). Because of the documented difficulties in measuring LPS in biological fluids (28), the LBP levels have been proposed and widely used as a reliable marker of endotoxemia (29-31). Increased levels of circulating LBP have been determined in obesity and the metabolic syndrome and associated with increased circulating IL-6 levels, impaired insulin resistance and cardiovascular risk (31-34). In the present study we observed significantly increased plasma LBP in the overweight group compared with the lean group (**Figure 1A**). In addition, plasma levels of CRP, a general inflammatory marker, were significantly higher in the overweight women (**Figure 1B**), as were the cytokine IL-6 and the adipokine leptin (**Figure 1C, D).** In contrast, the levels of adiponectin were significantly lower in the overweight group **(Figure 1E).** In addition, the leptin/adiponectin ratio values were significantly increased in the overweight compared with the lean group (**Figure 1F).** Together these results indicate that overweight women exhibit a significant systemic pro-inflammatory phenotype when compared to matched lean controls.

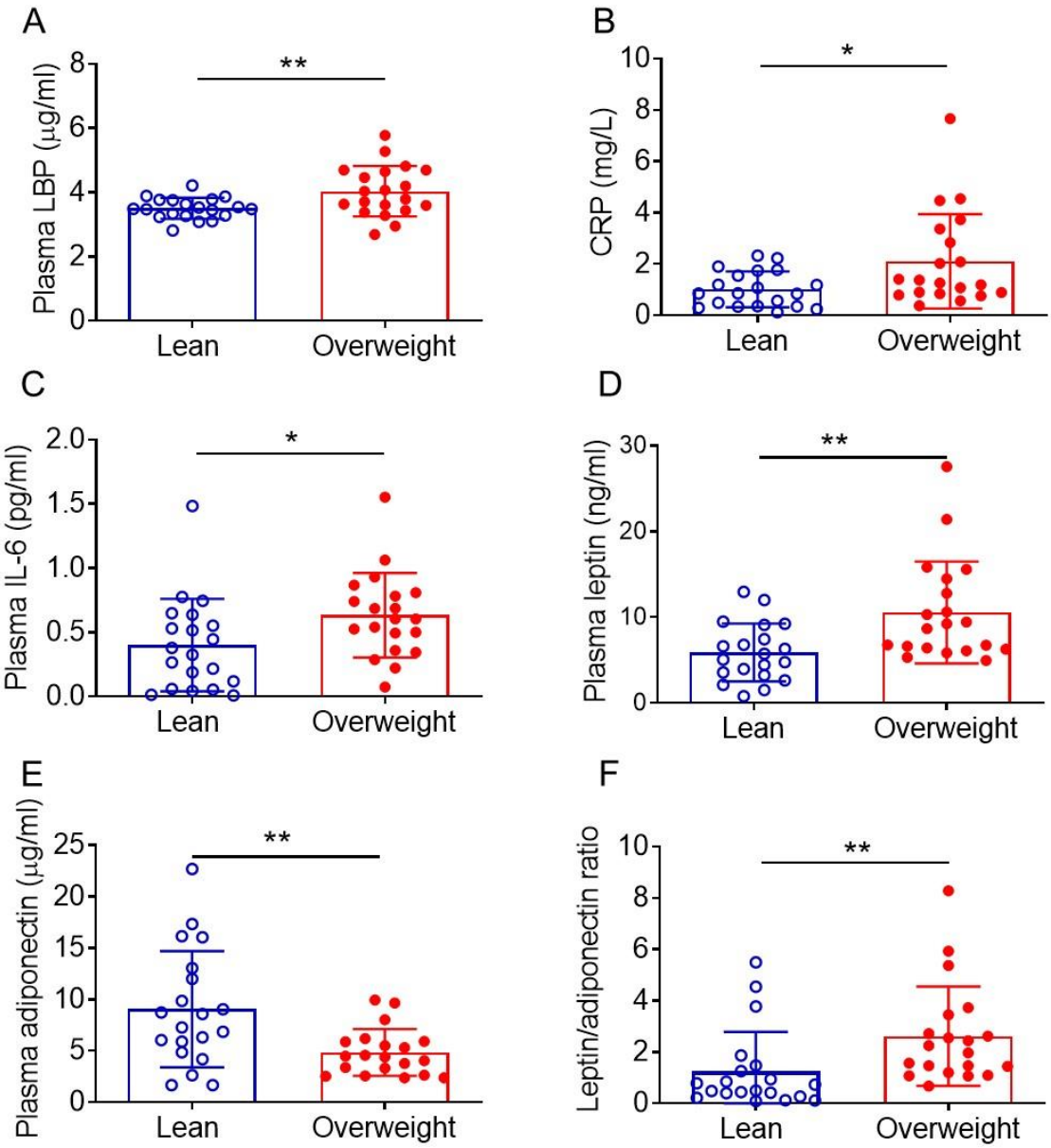

Figure 1. Levels of circulating markers of inflammation are altered in overweight women compared with lean women. Plasma samples of overweight and lean subjects were analyzed for (**A**) LPB, (**B**) CRP, (**C**) IL-6, (**D**) leptin, and (**E**) adiponectin were analyzed as described in Materials and methods, and leptin/adiponectin ratios (**F)** were calculated. (*p<0.05; **p<0.01)

Additional data evaluation revealed that plasma inflammatory marker alterations correlated with BMI of the study subjects (**Figure 2).** A moderate, but significant correlation was observed between plasma LBP and BMI and plasma CRP and BMI (**Figure 2A, B**) while a weak, non-significant correlation was found between plasma IL-6 levels and BMI (**Figure 2C**). A moderate and significant correlation was observed between plasma leptin and BMI (**Figure 2D**) and between plasma adiponectin and BMI (**Figure 2E**).

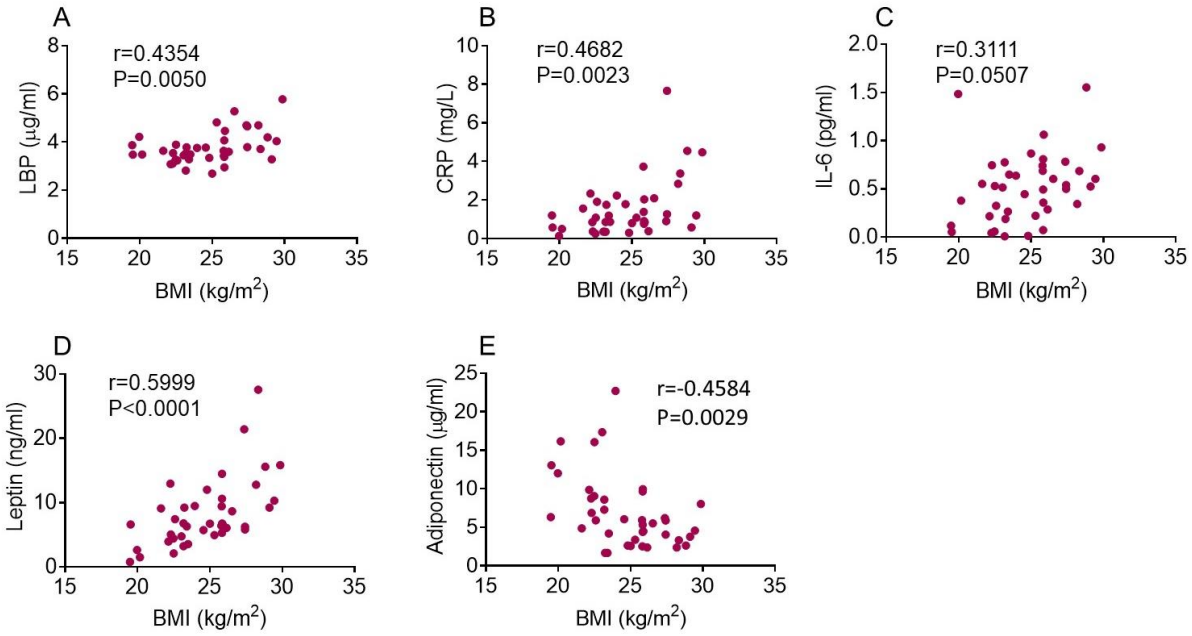

Figure 2. Correlation of plasma inflammatory indices and BMI. Plasma LBP (**A**), CRP (**B**), leptin (**D**), and adiponectin (**E**) levels significantly correlate with BMI as indicated by Pearson correlation coefficients (r) and p values. Plasma IL-6 levels do not correlate with BMI (**C**).

Correlation analysis of the analytes showed that LBP was weakly, but significantly correlated with IL-6 (r=0.34; p=0.03) and nearly significantly with CRP (r=0.3; p=0.06). Additionally, leptin was weakly, but significantly correlated with CRP (r=0.30; p=0.05), and with a trend to LBP (r=0.28;

p=0.08). Of note, no significant correlations were observed between the plasma inflammatory analytes and the age of the study participants **(Supplementary Figure 1).**

Discussion

These data show that in a small cohort of women matched for age and having self-declared hypercholesterolemia and/or hypertension, overweight individuals exhibited higher plasma concentrations of markers for endotoxemia, and a pro-inflammatory state, compared to lean controls. These differences are small, but notable for the existence of a significant correlation with BMI, as well as for weak positive correlations between LBP and the pro-inflammatory cytokines CRP and IL-6. Although almost none of the analytes exceeded the upper limits observed in larger clinical studies evaluating normal lean individuals, the observation that the pro-inflammatory molecules LBP, CRP, and IL-6 covary is consistent with a state of subclinical endotoxemia, rather arising from other factors, e. g., assay variability.

Previously, the results of one large study evaluating 500 apparently healthy lean individuals, a majority being female, has shown that LBP is weakly, but highly significantly, correlated with BMI (35). Like the results of the present study, LBP was also positively correlated with IL-6 and CRP, although to a much stronger degree, presumably due to the considerably larger study population. Further, LBP was also significantly positively correlated with blood pressure, HDL and LDL cholesterol, triglycerides, as well as to leptin. The current results conform to these findings and together indicate that markers of endotoxemia and a pro-inflammatory state vary continuously with the BMI, suggesting that adiposity of any degree, even if considered to be normal, is associated with detectable levels of pro-inflammatory molecules. Whether these have clinical significance will require additional large, prospective clinical studies, but it stands to reason that a sustained low grade inflammatory milieu likely has clinical consequences.

Although there is a lack of data regarding the risk of subclinical endotoxemia in overweight individuals, previous studies in obese individuals have clearly indicated the presence of metabolic endotoxemia (based on LBP levels) and a chronic inflammatory state and their role in promoting further metabolic dysfunction and pathogenesis (3, 7-9, 36-42). Metabolic endotoxemia in obesity has been specifically linked to the pathogenesis of CVD (15, 21) and increased LBP levels have been directly associated with an increased risk of CVD (21). Endotoxemia increases the production of IL-6 and other cytokines and significantly contributes to a pro-inflammatory state. IL-6 and the adipokine leptin are key mediators of inflammation in obesity (3, 43). IL-6 has been characterized as an important link between obesity and coronary heart disease (44). Importantly, in a large prospective study, increased IL-6 levels were associated with a higher risk of CVD, specifically coronary heart disease, as strongly as major established risk factors, such as blood pressure and blood cholesterol levels (45) Leptin is an adipokine with an essential role in energy balance through a variety of functions, some of which are related to cardiovascular health (7, 18, 20). Increased leptin levels in obesity are associated with activation of pro-inflammatory signaling and increased thrombosis and arterial distensibility in obese patients (11, 46, 47). Elevated leptin levels arising from leptin resistance in obesity are associated with insulin resistance and CVD (18). In contrast, adiponectin is an adipokine with anti-inflammatory and antithrombotic properties (7, 48). Decreased plasma adiponectin levels were associated with an increased risk of myocardial infarction (49). Plasma levels of CRP (high-sensitive C-reactive protein), a general marker of chronic subclinical inflammation, have been positively correlated with plasma leptin levels and inversely with plasma adiponectin (50-52). The leptin/adiponectin ratio is indicated as a more reliable marker in CVD assessment compared with individual leptin and adiponectin measures (53-55) and proposed as a better marker of a first cardiovascular event in men than plasma leptin and adiponectin levels alone (56).

Until longitudinal data concerning subclinical endotoxemia are available, it may be prudent to institute proactive measures to monitor and reduce the circulating levels of LBP, as a surrogate biomarker for endotoxemia. As composition of the diet has been shown to be a critical driver of metabolic endotoxemia (reviewed in (57)), with high saturated fat ingestion causing postprandial endotoxemia with increases in IL-6 even in lean subjects (58, 59), dietary intervention would be a reasonable initial step. Other possibilities include pharmacological interventions, including the potential development of anti-LPS peptides which neutralize LPS signaling of immune system activation (57). What about exercise?

In addition to the small number of subjects assessed, there are several limitations of this study. One important one is that the degree of abdominal adiposity (in contrast to subcutaneous fat deposits) has been implicated in the pathogenesis of an systemic inflammatory response and correlates well with the production of pro-inflammatory cytokines (60). Unfortunately, as a single measure the BMI cannot fully capture this variable as it is insensitive to changes in regional body composition. That is, a smaller waist circumference for any given BMI is indicative of subcutaneous fat deposits, in contrast to an abdominal location in an individual possessing a larger waist circumference. A consensus has appeared that including the waist circumference as a measured variable provides information independent of the BMI, and when both are considered together the predictive accuracy of cardiometabolic risk is significantly increased (61). Additional limitations include other potentially contributing factors that were not assessed, including the existence of abnormal glucose tolerance, degree of sedentary behavior, and the confirmation and severity of self-reported hypercholesterolemia and hypertension, among others. Lastly, as dietary factors are a major driver of the appearance of LPS into the circulation, in future studies plasma samples should be obtained under standardized fasting conditions.

Conclusion

Here, we demonstrate that despite the presence of hypertension and/or high cholesterol levels in two groups of women characterized using BMI as overweight versus lean, circulating biomarkers of inflammation, including LBP, CRP, IL-6, and leptin are increased and adiponectin is decreased in the overweight group. Although these differences seem to be of subclinical importance, more extensive alterations of these molecules observed in obese individuals have been linked to an increased cardiometabolic risk. While the number of subjects in this exploratory study was small, the presence of subclinical endotoxemia the degree of which varied continuously with BMI encourage designing and implementing a larger confirmatory study to better characterize individuals who are overweight, but not yet classified as obese. These individuals may benefit from therapy to alleviate chronic, low-grade inflammation as an additional risk factor for the development of CVD and other disorders.

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Author contributions:

CNM and VAP proposed the experimental concept and designed the experiments. XX and PKC performed the analyses. CNM, VAP, XX, PKC, and RA analyzed data. CNM, VAP, and MB wrote the manuscript. PG and KJT provided additional comments to finalize the paper.

Data availability statement:

Data is available at the authors' discretion upon direct request to the corresponding authors.

References

1. Heymsfield SB, Wadden TA. Mechanisms, Pathophysiology, and Management of Obesity. The New England journal of medicine. 2017;376(3):254-66.
2. Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: public-health crisis, common sense cure. The Lancet. 2002;360(9331):473-82.
3. Pavlov VA. The evolving obesity challenge: targeting the vagus nerve and the inflammatory reflex in the response. Pharmacology & therapeutics. 2021;222:107794.
4. Grundy SM. Obesity, Metabolic Syndrome, and Cardiovascular Disease. The Journal of Clinical Endocrinology & Metabolism. 2004;89(6):2595-600.
5. Ritchie SA, Connell JMC. The link between abdominal obesity, metabolic syndrome and cardiovascular disease. Nutrition, Metabolism and Cardiovascular Diseases. 2007;17(4):319-26.
6. Vogel B, Acevedo M, Appelman Y, Bairey Merz CN, Chieffo A, Figtree GA, et al. The Lancet women and cardiovascular disease Commission: reducing the global burden by 2030. The Lancet. 2021;397(10292):2385-438.
7. Pavlov VA, Tracey KJ. The vagus nerve and the inflammatory reflex--linking immunity and metabolism. Nature reviews Endocrinology. 2012;8(12):743-54.
8. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. Gastroenterology. 2007;132(6):2169-80.
9. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Research and Clinical Practice. 2014;105(2):141-50.

10. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annual review of immunology. 2011;29:415-45.
11. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nature reviews Immunology. 2011;11(2):85-97.
12. Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. Current pharmaceutical design. 2009;15(13):1546-58.
13. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761-72.
14. Mohammad S, Thiemermann C. Role of metabolic endotoxemia in systemic inflammation and potential interventions. Frontiers in immunology. 2021;11:594150.
15. Neves AL, Coelho J, Couto L, Leite-Moreira A, Roncon-Albuquerque R. Metabolic endotoxemia: a molecular link between obesity and cardiovascular risk. 2013;51(2):R51.
16. de Gusmao Correia ML, Haynes WG. Leptin, obesity and cardiovascular disease. Current Opinion in Nephrology and Hypertension. 2004;13(2):215-23.
17. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. The Journal of clinical investigation. 2011;121(6):2111-7.
18. Martin SS, Qasim A, Reilly MP. Leptin Resistance. Journal of the American College of Cardiology. 2008;52(15):1201-10.
19. Mathieu P, Lemieux I, Després JP. Obesity, inflammation, and cardiovascular risk. Clinical pharmacology & therapeutics. 2010;87(4):407-16.
20. Nakamura K, Fuster JJ, Walsh K. Adipokines: a link between obesity and cardiovascular disease. Journal of cardiology. 2014;63(4):250-9.
21. Roberts LM, Buford TW. Lipopolysaccharide binding protein is associated with CVD risk in older adults. Aging Clin Exp Res. 2021;33(6):1651-8.
22. Kivimäki M, Kuosma E, Ferrie JE, Luukkonen R, Nyberg ST, Alfredsson L, et al. Overweight, obesity, and risk of cardiometabolic multimorbidity: pooled analysis of individual-level data for 120 813 adults from 16 cohort studies from the USA and Europe. The Lancet Public Health. 2017;2(6):e277-e85.
23. Wilson PW, D'Agostino RB, Sullivan L, Parise H, Kannel WB. Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. Archives of internal medicine. 2002;162(16):1867-72.
24. Garcia M, Mulvagh SL, Merz CNB, Buring JE, Manson JE. Cardiovascular Disease in Women. Circulation Research. 2016;118(8):1273-93.
25. Schenck-Gustafsson K. Risk factors for cardiovascular disease in women. Maturitas. 2009;63(3):186-90.
26. Jousilahti P, Vartiainen E, Tuomilehto J, Puska P. Sex, Age, Cardiovascular Risk Factors, and Coronary Heart Disease. Circulation. 1999;99(9):1165-72.
27. Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, et al. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. The Journal of experimental medicine. 1994;179(1):269-77.
28. Cohen J. The detection and interpretation of endotoxaemia. Intensive care medicine. 2000;26:S051-S6.
29. Albillos A, de la Hera A, González M, Moya JL, Calleja JL, Monserrat J, et al. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. Hepatology (Baltimore, Md). 2003;37(1):208-17.
30. Guerra Ruiz A, Casafont F, Crespo J, Cayón A, Mayorga M, Estebanez A, et al. Lipopolysaccharide-binding protein plasma levels and liver TNF-alpha gene expression in obese patients: evidence for the potential role of endotoxin in the pathogenesis of non-alcoholic steatohepatitis. Obesity surgery. 2007;17:1374-80.

31. Lepper PM, Schumann C, Triantafilou K, Rasche FM, Schuster T, Frank H, et al. Association of lipopolysaccharide-binding protein and coronary artery disease in men. Journal of the American College of Cardiology. 2007;50(1):25-31.
32. Trøseid M, Nestvold TK, Rudi K, Thoresen H, Nielsen EW, Lappegård KT. Plasma lipopolysaccharide is closely associated with glycemic control and abdominal obesity: evidence from bariatric surgery. Diabetes care. 2013;36(11):3627-32.
33. Moreno-Navarrete J, Ortega F, Serino M, Luche E, Waget A, Pardo G, et al. Circulating lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. International journal of obesity. 2012;36(11):1442-9.
34. Gonzalez-Quintela A, Alonso M, Campos J, Vizcaino L, Loidi L, Gude F. Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. PloS one. 2013;8(1):e54600.
35. Sun L, Yu Z, Ye X, Zou S, Li H, Yu D, et al. A marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese. Diabetes Care. 2010;33(9):1925-32.
36. Nathan C. Epidemic inflammation: pondering obesity. Mol Med. 2008;14(7-8):485-92.
37. Chang EH, Chavan SS, Pavlov VA. Cholinergic Control of Inflammation, Metabolic Dysfunction, and Cognitive Impairment in Obesity-Associated Disorders: Mechanisms and Novel Therapeutic Opportunities. Frontiers in neuroscience. 2019;13:263.
38. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. The Journal of clinical investigation. 2017;127(1):1-4.
39. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nature Reviews Immunology. 2011;11(2):98-107.
40. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nature reviews Immunology. 2006;6(10):772-83.
41. Esser N, Paquot N, Scheen AJ. Anti-inflammatory agents to treat or prevent type 2 diabetes, metabolic syndrome and cardiovascular disease. Expert opinion on investigational drugs. 2015;24(3):283-307.
42. Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, et al. Obesity and the risk of heart failure. The New England journal of medicine. 2002;347(5):305-13.
43. Elks CM, Francis J. Central adiposity, systemic inflammation, and the metabolic syndrome. Current hypertension reports. 2010;12(2):99-104.
44. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis. 2000;148(2):209-14.
45. Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. PLoS Med. 2008;5(4):e78.
46. Konstantinides S, Schäfer K, Koschnick S, Loskutoff DJ. Leptin-dependent platelet aggregation and arterial thrombosis suggests a mechanism for atherothrombotic disease in obesity. The Journal of clinical investigation. 2001;108(10):1533-40.
47. Singhal A, Farooqi IS, Cole TJ, O'Rahilly S, Fewtrell M, Kattenhorn M, et al. Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? Circulation. 2002;106(15):1919-24.
48. Katsiki N, Mantzoros C, Mikhailidis DP. Adiponectin, lipids and atherosclerosis. Current opinion in lipidology. 2017;28(4):347-54.
49. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. Jama. 2004;291(14):1730-7.

50. Shetty GK, Economides PA, Horton ES, Mantzoros CS, Veves A. Circulating adiponectin and resistin levels in relation to metabolic factors, inflammatory markers, and vascular reactivity in diabetic patients and subjects at risk for diabetes. Diabetes care. 2004;27(10):2450-7.
51. Ble A, Windham BG, Bandinelli S, Taub DD, Volpato S, Bartali B, et al. Relation of plasma leptin to C-reactive protein in older adults (from the Invecchiare nel Chianti study). The American journal of cardiology. 2005;96(7):991-5.
52. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation. 2003;107(5):671-4.
53. Satoh N, Naruse M, Usui T, Tagami T, Suganami T, Yamada K, et al. Leptin-to-adiponectin ratio as a potential atherogenic index in obese type 2 diabetic patients. Diabetes care. 2004;27(10):2488-90.
54. Kotani K, Sakane N, Saiga K, Kurozawa Y. Leptin: adiponectin ratio as an atherosclerotic index in patients with type 2 diabetes: relationship of the index to carotid intima–media thickness. Diabetologia. 2005;48:2684-6.
55. Norata GD, Raselli S, Grigore L, Garlaschelli K, Dozio E, Magni P, et al. Leptin: adiponectin ratio is an independent predictor of intima media thickness of the common carotid artery. Stroke. 2007;38(10):2844-6.
56. Kappelle PJWH, Dullaart RPF, van Beek AP, Hillege HL, Wolffenbuttel BHR. The plasma leptin/adiponectin ratio predicts first cardiovascular event in men: A prospective nested case–control study. European Journal of Internal Medicine. 2012;23(8):755-9.
57. Mohammad S, Thiemermann C. Role of Metabolic Endotoxemia in Systemic Inflammation and Potential Interventions. Front Immunol. 2020;11:594150.
58. Laugerette F, Vors C, Alligier M, Pineau G, Drai J, Knibbe C, et al. Postprandial Endotoxin Transporters LBP and sCD14 Differ in Obese vs. Overweight and Normal Weight Men during Fat-Rich Meal Digestion. Nutrients. 2020;12(6).
59. Vors C, Pineau G, Drai J, Meugnier E, Pesenti S, Laville M, et al. Postprandial Endotoxemia Linked With Chylomicrons and Lipopolysaccharides Handling in Obese Versus Lean Men: A Lipid Dose-Effect Trial. J Clin Endocrinol Metab. 2015;100(9):3427-35.
60. Rexrode KM, Pradhan A, Manson JE, Buring JE, Ridker PM. Relationship of total and abdominal adiposity with CRP and IL-6 in women. Ann Epidemiol. 2003;13(10):674-82.
61. Ross R, Neeland IJ, Yamashita S, Shai I, Seidell J, Magni P, et al. Waist circumference as a vital sign in clinical practice: a Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. Nat Rev Endocrinol. 2020;16(3):177-89.