International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jms.htm
2014 Vol. 4 (2) May-August, pp. 111-121/Zaman et al.
Research Article

RELATIONSHIP BETWEEN POSTPRANDIAL ENDOTOXEMIA IN PRE- AND POST MENOPAUSAL WOMEN AFTER A FAT OVERLOAD

***Gaffar Sarwar Zaman[1], Amanullah Mohammed[1] and Fawzia Zaman[2]**
[1]Department of Clinical Biochemistry, Government College of Medicine, King Khalid university, Abha, Kingdom of Saudi Arabia
[2]Ekopath Metropolis, Guwahati, Assam, India
**Author for Correspondence*

ABSTRACT

Bacterial endotoxemia has been associated with postprandial lipemia, though this relation is not fully understood. The objective of this study was designed to test the hypothesis that young premenopausal (PrW) and postmenopausal women (PoW) may be independently associated with postprandial endotoxemia and indirectly associated with atherosclerosis. The lipopolysaccharide (LPS) levels and circulating lipopolysaccharide-binding protein (LBP) were determined in serum at fasting, 1 hr, 2 hrs, 3 hrs, and 4 hrs after a fat overload and their levels related with postprandial lipid levels in 70 premenopausal women and 70 post-menopausal women. The postmenopausal women with the highest postprandial hypertriglceridemia showed a significant increase in LPS levels and circulating LBP in serum after the fat overload. Elevated LPS and circulating LBP was associated significantly with PoW, especially after a fat overload. These findings suggested a role of LPS and LBP in atherosclerosis. Prospective studies are needed to confirm these results.

Keywords: *Premenopause, Postmenopause, Endotoxemia, Lipopolysaccharide, Lipopolysaccharide-Binding Protein, Fasting, Postprandial*

INTRODUCTION

The term endotoxin was coined by Richard Friedrich Johannes Pfeiffer, who considered endotoxin to be a toxin kept "within" the bacterial cell and to be released only after destruction of the bacterial cell wall. Today, the term 'endotoxin' is used synonymously with the term lipopolysaccharide (LPS), which is a major constituent of the outer cell membrane of Gram-negative bacteria (GNB). LPS consists of a polysaccharide (sugar) chain and a lipid moiety, known as lipid A, which is responsible for the toxic effects. The polysaccharide chain is highly variable among different bacteria and determines their serotype. In recent years, there has been an increasing recognition of the link between inflammation and atherosclerosis (Ross, 1993; de Boer *et al.,* 1996; Gerszten *et al.,* 2000; Glass *et al.,* 2001; Libby, 2002; Libby *et al.,* 2002; Hansson *et al.,* 2002; Curtiss *et al.,* 2000). One potentially important source of inflammation is endotoxin (LPS), a unique glycolipid that comprises most of the outer leaflet of the outer wall of GNB (Rietschel *et al.,* 1994, Preston *et al.,* 1996, Wilkinson, 1996; Holst *et al.,* 1996; Raetz, 2002). GNB colonize the human gastrointestinal, genitourinary, and respiratory tracts and generate endotoxin not only during overt infections but also in common subclinical or chronic conditions such as periodontitis, sinusitis, bronchitis, or diverticulitis (Li, 2002; De Nardin, 2001, Kuramitsu *et al.,* 2002). In animal studies, weekly injections of endotoxin accelerated the development of atherosclerotic lesions in rabbits on hypercholesterolemic diets (Lehr *et al.,* 2001) and in apolipoprotein E-deficient mice (Ostos *et al.,* 2002). These observations support the hypothesis that chronic exposure to endotoxin may be pathogenically linked to atherosclerosis. Serum lipoproteins, particularly HDL, are believed to play a major role in clearance of circulating endotoxin (Read *et al.,* 1993; Flegel *et al.,* 1989; Feingold *et al.,* 1995; Levine *et al.,* 1995; Parker *et al.,* 1995; Flegel *et al.,* 1993). However, excess dietary fat not only increases systemic exposure to potentially proinflammatory free fatty acids and their derivatives, but its intestinal absorption was recently found to also facilitate the absorption of highly proinflammatory

bacterial LPS from the gut (Cani *et al.*, 2007; Erridge *et al.*, 2007). However, chylomicrons also have high affinity for LPS (Vreugdenhil *et al.*, 2003; Read *et al.*, 1995; Harris *et al.*, 1993) and thus not only transport postprandial fat, but likely also significant amounts of concomitantly absorbed gut LPS. Whereas sequestration of absorbed LPS on chylomicrons would reduce LPS toxicity and enhance its hepatic clearance, it nevertheless is possible that the inflammatory effect of chylomicrons could correlate with their LPS content. However, the cause of these postprandial events that occur in association with the postprandial triglyceride response remains poorly understood. A possible link is bacterial endotoxin (LPS), a component of the Gram-negative bacteria cell wall that is present in large quantities in the human gut (Berg, 1996). Endotoxins circulate in the plasma of healthy human subjects at low concentrations (known as metabolic endotoxemia), and an elevated concentration of circulating LPS has been associated with a higher risk for atherosclerosis (Wiedermann *et al.*, 1991). There is evidence that metabolic plasma LPS levels are modulated by food content: the higher the fat content, the higher the concentration of plasma LPS (Amar *et al.*, 2008). Small amounts of LPS are absorbed from the gut in healthy animals (Ravin *et al.*, 1960), and there is evidence that chylomicrons likely also transport significant amounts of absorbed gut LPS (Ghoshal *et al.*, 2006; Laugerette *et al.*, 2011; Vreugdenhil *et al.*, 2003). Obesity tends to be accompanied by the consumption of a high-fat diet, and interestingly, the proportion of GNB in microflora is higher in obese subjects than in lean subjects (Ley *et al.*, 2008; Turnbaugh *et al.*, 2006). Thus, these conditions would enhance the translocation of endogenous LPS from the gut during fat absorption, which would lead to the low-grade inflammation observed in these patients (Erridge *et al.*, 2007; Cani *et al.*, 2007). However, no studies have yet examined metabolic endotoxemia in obese patients. Little is known about the involvement of endotoxin absorption from the gut during the digestion of lipids. To our knowledge, this is the first study evidencing in healthy humans that, following a mixed meal containing lipids, increased endotoxemia is associated with raised sCD14 and a peak of IL-6. On a repeated basis, this may thus be a triggering cascade for the onset of atherosclerosis. Lipopolysaccharide-binding protein (LBP) is a protein that in humans is encoded by the LBP gene (Gray *et al.*, 1993). LBP is a soluble acute-phase protein that binds to bacterial lipopolysaccharide (or LPS) to elicit immune responses by presenting the LPS to important cell surface pattern recognition receptors called CD14 and TLR4 (Muta *et al.*, 2001). LPS is detoxified in the circulation by incorporation into lipoproteins (reviewed in ref. 1). Physiological levels of lipoproteins protect against endotoxicity in vitro and in vivo (Feingold, 1995; Flegel *et al.*, 1989). Early studies have demonstrated an interaction of LPS with HDL (Ulevitch *et al.*, 1979); albeit later, also VLDL and LDL were found to bind and inactivate LPS (Lenten *et al.*, 1986; Victorov, 1989; Netea, 1998). Consistent with this, LDL, VLDL, chylomicrons, and HDL all have been observed to reduce the lethal effect of endotoxin in mice (Read, 1995; Harris, 1993; Harris *et al.*, 1990). Postprandial lipoprotein metabolism is affected by dietary habits, meal composition (amount and type of fat, carbohydrates, proteins, fiber, and alcohol), lifestyle practices, (physical activity and tobacco use), physiological factors (age, gender, and menopausal status), and pathological conditions (obesity, insulin resistance, diabetes mellitus (DM), etc) (Gaag *et al.*, 2000; López-Miranda *et al.*, 2007; Kabagambe *et al.*, 2009). Abnormalities during the postprandial state contribute to the development of atherosclerosis and cardiovascular risk (Karamanos *et al.*, 2001). The TG-rich lipoproteins are involved in many pathways leading to atherosclerosis. They are carriers of cholesteryl esters to the vessel all (University College London Medical School, 1993) and they are toxic to the endothelial cells (ECs) and induce endothelial dysfunction Funada *et al.*, 2002; Jagla *et al.*, 2001; Gokce *et al.*, 2001). A myriad of seemingly unrelated risk factors may cause EC damage, leading to atherosclerosis. Dyslipidemia has been accorded a crucial role, but our understanding of the contribution of different lipids and lipoproteins continues to evolve (Bae *et al.*, 2001; Libby, 2003). Recent studies have shown that postprandial handling of TG-rich lipoprotein is important for the propensity of endothelial dysfunction and atherosclerosis (Ross, 1995; Patsch, 1994; Dubois *et al.*, 1994). There are very few studies comparing endotoxemia and postprandial lipid metabolism before and after menopause (Lewis *et al.*, 1991; Nabeno *et al.*, 2007; Richal *et al.*, 2004). Very few data exist regarding the response of endotoxemia and postprandial lipid

metabolism in premenopausal (PrW) and postmenopausal women (PoW). Therefore, this study was undertaken with the following objectives:

(1) To find the relationship of postprandial endotoxemia between pre- and post menopausal women after a fat overload.

(2) Whether post menopausal state has got relationship with increased lipids and atherosclerosis.

MATERIALS AND METHODS

Patients Inclusion and Exclusion Criteria

This study was conducted in accordance with the ethical rules of the Helsinki Declaration. The study was approved by the Ethics Committee of the hospital, and all women gave written informed consent. Prior to the study, participants were informed that their confidentiality would be maintained and consent was obtained. 70 premenopausal women and 70 post-menopausal women were selected for the study. For the group of young women, individuals selected had to be healthy, between 18 and 45 years old. Patients were excluded if they had cardiovascular disease, arthritis, acute inflammatory disease, infectious disease, renal disease, were receiving treatment for hyperlipidemia or diabetes or were taking medications that could influence gastric emptying or the absorption time.

Preparation of Patients and Sample Collection

On the morning of the visit, blood pressure, weight, and height were measured and compliance with dinner instructions was verified with a questionnaire. After that, each participant underwent a structured examination, which included an interview. Height, weight, waist circumference (WC) and hip measurements, a fasting venipuncture, and sequential determination of serum lipids were done. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight (kilogram) divided by height (in meter) squared. WC was determined to the nearest 0.1 cm using a measuring tape positioned at the midpoint between the lowest rib and the iliac crest and hips were measured at the largest gluteal circumference. These measurements were used to calculate the waist-to-hip ratio (WHR). Then, blood pressure was measured using a standard mercury sphygmomanometer. Blood samples were obtained from the antecubital vein and placed in vacutainer tubes. Postprandial blood samples were taken 1, 2, 3, and 4 hr after the end of the study meal. Samples were centrifuged; serum was collected and stored at 20 °C until analyzed. Lipid profiles comprising TC, HDL-C, LDL-C, and TG concentrations were measured at fasting and at 1, 2, 3, and 4 h post-load. Calculation of LDL-C concentrations was based on the Friedewald equation (Cohn *et al.*, 1988). The diagnosis of DM was based on WHO criteria (Friedewald *et al.*, 1972), i.e. a fasting plasma glucose level > 7.0 mmol/L or > 126 mg/dL, or a 2-h postprandial plasma glucose level > 11.1 mmol/L or > 200 mg/dL on more than one occasion, with symptoms of diabetes.

Serum LPS concentrations were measured by endotoxin assay, based on a Limulus amebocyte extract with a chromogenic LAL assay (QCL-1000, Lonza Group Ltd.). Samples were diluted in pyrogen-free water and heated at 70ºC for 10 min to inactivate endotoxin-neutralizing agents that inhibit the activity of endotoxin in the LAL assay. Internal control of recovery calculation was included in the assessment. All samples were tested in duplicate. The endotoxin content was expressed as endotoxin units (EU) per mL. Exhaustive care was taken to avoid environmental endotoxin contamination and all material used for sample preparation and the test was pyrogen-free. Plasma LBP levels were determined by a sandwich ELISA Technology. Plasma samples were diluted at least 200 times and assayed according to the manufacturer's instructions. The assay has a sensitivity of 0.2 ng/ml. The intra-assay and interassay coefficients of variation were < 5 and < 10%, respectively.

Statistical Analysis

All data were entered into an Excel spreadsheet, and were analyzed using standard statistical software such as SPSS. Chi-square test was used for categorical variables. All numerical data were presented as mean ± standard deviation. A P value of less than 0.05 was considered statistically significant.

RESULTS

Table 1

	Premenopausal women	Postmenopausal women
Age (years)	18-42	48+
BMI (kg/m^2)	22.0 ± 2.4 kg/m^2	23.0 ± 3.1 kg/m^2
Waist circumference (cm)	71.9 ± 6.1	73 ± 8.2
Systolic blood pressure (mmHg)	110.4 ± 17.3	112.4 ± 13.3
Diastolic blood pressure (mmHg)	72.7 ± 9.8	74.9 ± 6.8
Fasting Plasma Glucose (mg/dL)	94±8	97±7

The mean BMI values were 23.0 ± 3.1 kg/m^2 in PoW and 22.0 ± 2.4 kg/m^2 in PrW (Table 1). The mean waist circumference were 73 ± 8.2 in PoW and 71.9 ± 6.1 in PrW. The mean systolic blood pressure (mmHg) was 112.4 ± 13.3 in PoW and 110.4 ± 17.3 in PrW, whereas the diastolic blood pressure (mmHg) was 74.9 ± 6.8 in PoW and 72.7 ± 9.8 PrW Compared with PrW, PoW were more likely to have higher values for waist circumference, blood pressure, glucose. Fasting plasma glucose and postprandial plasma glucose were in the range of normal in both categoies (94±8 mg/dL in PrW and 97±7 mg/dL in PoW).

The result of the study on the relationship between the serum lipids in PrW and PoW are represented in Figures 1–4. The mean TC in mg/dL was 154, 169, 183, 176, and 159 at fasting, 1, 2, 3, and 4 h in the PrW vs. 164, 177, 201, 178, and 169 in the PoW during the same duration. Cholesterol concentrations showed a significant reduction after 2 h, to reach values similar to the baselineafter 4 h in PrW but not in PoW. The mean HDL-C in mg/dL was 48.74, 44.38, 42.71, 43.47 and 40.1 at fasting, first, second, third and fourth hours after the test meal in the PrW vs. 45.66, 44.7, 43.12, 42.11, and 39.04 in the PoW during the same time interval. This shows that HDL-C concentration was decreased more in PoW compared to PrW but it was not significant. The mean LDL-C in mg/dL was 127.44, 125.77, 116.23, 109.6, and 94.9 at fasting, 1, 2, 3, and 4 h in the PrW vs. 137.92, 141.26, 128.4, 129.8, and117.64 in the PoW during the same amount of time. The mean TC in mg/dL was 136, 143, 157, 148, and 146 at fasting, 1, 2, 3, and 4 h in the PrW *vs*. 159, 173, 194, 182, and 170 in the PoW during the same duration.

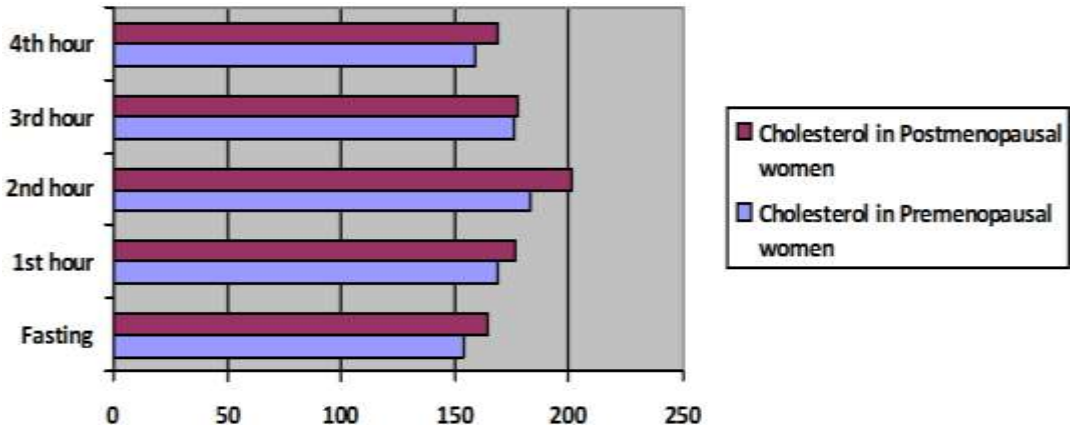

Figure 1: Fasting and Postprandial Cholesterol in premenopausal and postmenopausal women

The mean Plasma endotoxin (LPS) in PrW in EU/mL was 0.34, 0.38, 0.45, 0.44 and 0.42 at fasting, 1, 2, 3, and 4 hr in the PrW vs. 0.36, 0.46, 0.67, 0.61 and 0.60 in the PoW during the same duration. The mean LPS binding protein (LBP) in PrW in µg/ml was 10.2, 11.6, 13.9, 11.8 and 11.6 at fasting, 1, 2, 3, and 4 h in the PrW vs. 10.9, 14.4, 19.8, 13.8 and 13.4 in the PoW during the same duration.

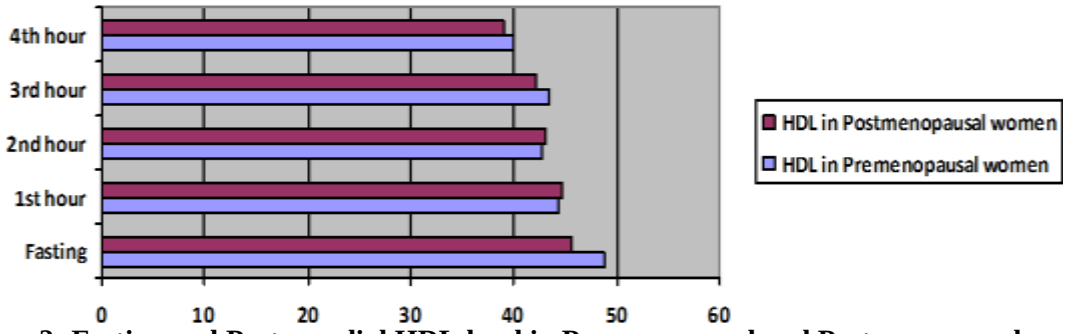

Figure 2: Fasting and Postprandial HDL level in Premenopausal and Postmenopausal women

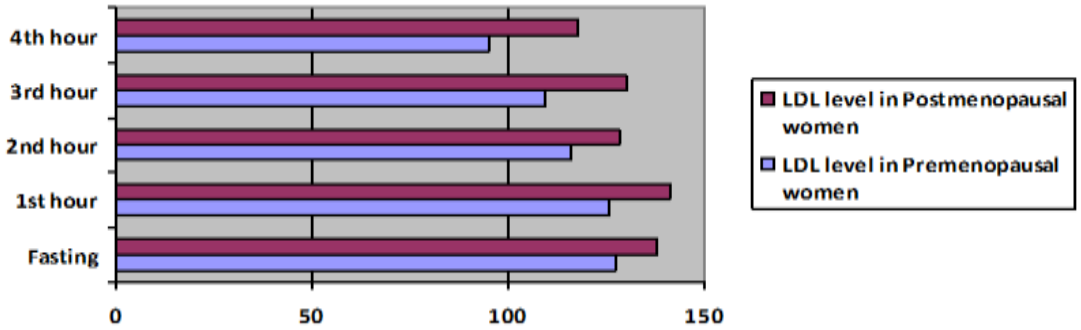

Figure 3: Fasting and Postprandial LDL level in Pre and Postmenopausal women

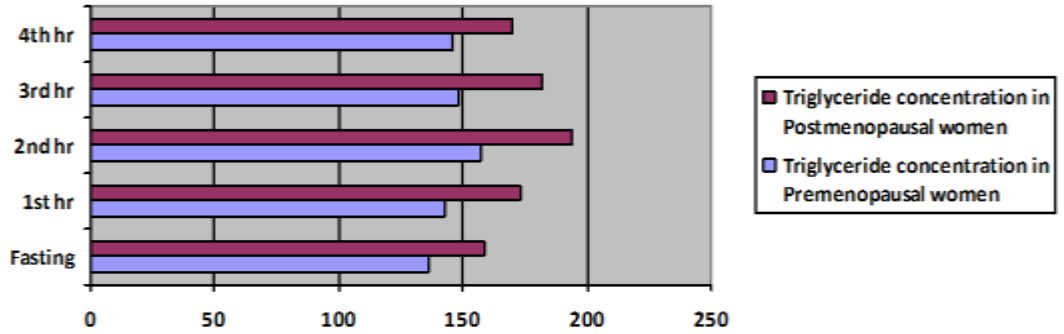

Figure 4: Fasting and Postprandial Triglyceride level in Pre and Postmenopausal women

Serum endotoxin activity had a significant positive correlation with cholesterol, triglyceride and LDL concentration, and a negative correlation with HDL cholesterol concentration. When endotoxin concentrations measured at all time points before meals were compared with all time points after meals, plasma endotoxin was significantly higher after a high-fat meal.

Table 2:

	Fasting	1 hr	2 hr	3 hr	4hr	P value
Plasma endotoxin (LPS) in Premenopausal women(EU/mL)	0.34	0.38	0.45	0.44	0.42	$P < 0.05$
Plasma endotoxin (LPS) in Postmenopausal women (EU/mL)	0.36	0.46	0.67	0.61	0.60	$P < 0.05$
LPS binding protein (LBP) in Premenopausal women (µg/mL)	10.2	11.6	13.9	11.8	11.6	$P < 0.05$
LPS binding protein (LBP) in Postmenopausal women(µg/ml)	10.9	14.4	19.8	13.8	13.4	$P < 0.05$

Discussion

Bacterial endotoxin is increasingly being considered as a potential inflammatory mediator of atherosclerosis (American Diabetes Association, 2004; Stoll *et al.,* 2004; Ostos *et al.,* 2002; Lehr *et al.,* 2001) and has emerged as an independent predictor of atherosclerosis risk (Wiedermann *et al.,* 1999), although the mechanisms for increased endotoxin in the plasma of some healthy individuals remain unknown. Chylomicrons promote intestinal absorption of lipopolysaccharides more than 1 g of LPS can be found in the gut lumen (Berg, 1996). Even small amounts of this highly proinflammatory substance could elicit strong inflammatory responses in the body proper, and it is therefore thought that the gut epithelium acts to effectively block the "translocation" of LPS and other microbial proinflammatory substances. However, it was shown several decades ago that small amounts of LPS are absorbed from the gut in healthy animals (Ravin *et al.,* 1960). Excessive LPS absorption, however, could evidently be harmful and could lead to acute or chronic inflammation. Increased LPS absorption, for example, could exacerbate the risk for several chronic diseases, such as alcoholic liver injury (Adachi *et al.,* 1995) nonalcoholic steatohepatitis, HIV/AIDS, and inflammatory bowel disease Caradonna *et al.,* 2000; Wellmann *et al.,* 1986). In theory, dietary fat could increase LPS absorption in several ways. One way would be through promotion of paracellular uptake of macromolecules as a result of deleterious effects of fatty acids on tight-junction integrity (Wellmann *et al.,* 1986). An alternative mechanism explaining fatty-acid dependent LPS absorption involves internalization of LPS by the enterocyte, followed by association of some of the internalized LPS with chylomicrons and concomitant basolateral secretion of LPS with the chylomicrons or by association of independently transcytosed LPS with newly released chylomicrons. Chylomicrons have been associated with metabolic endotoxemia. Both animal and in vitro studies have demonstrated that chylomicron formation promotes LPS absorption (Kvietys *et al.,* 1991; Ghoshal *et al.,* 2009). A recent study has also shown human chylomicrons can be postprandial carriers of LPS in healthy humans (Laugerette *et al.,* 2011; Cardona *et al.,* 2008). Our study agrees with the idea of chylomicron LPS transport since the patients with higher increases in triglyceride levels over baseline displayed higher levels of chylomicron LPS after the fat overload. Concordantly with the idea that chylomicrons promote LPS absorption, a high fat meal leads to increased endotoxemia in healthy humans. Husam *et al.,* also observed an increase in the plasma concentration of LPS and LBP after the high fat meal intake but not after the AHA meal. Whereas the LPS content of the meal probably contributes substantially to the increase in plasma concentrations of LPS, it is possible that some contribution also comes from LPS in the gastrointestinal tract since LPS is fat soluble. In addition, it was recently shown that fat intake leads to increased intestinal permeability for LPS. However, it was remarkable that the AHA meal did not alter plasma LPS concentrations in spite of having LPS content similar to that of the HFHC meal. In consequence, it has been hypothesized that endogenous LPS levels could be responsible for the low-grade inflammation observed in obese subjects who have a high fat intake. It has been reported that patients with morbid obesity have a greater postprandial response to fat overload, and the postprandial response is associated with a greater increase in oxidative stress and inflammation. In the study by Mariann *et al.,* most diabetic patients with high serum LPS activity had elevated serum triglycerides and low HDL-cholesterol concentrations. Of all the tested clinical variables, the strongest correlation was observed between the LPS/HDL ratio and serum triglyceride concentrations. High fasting concentrations of triglycerides predict postprandial hypertriglyceridemia and the development of insulin resistance. Bacterial endotoxin is increasingly being considered as a potential inflammatory mediator of obesity, diabetes and atherosclerosis (Gubern *et al.,* 2006). In addition, positive correlations between LBP and metabolic traits such as BMI, diastolic blood pressure, fasting glucose, insulin, and triglycerides were observed in 60 men with glucose intolerance by Gubern *et al.,* Moreover, a higher LBP level was associated with increased prevalence of coronary artery disease independent of established cardiovascular risk factors in 247 male patients by Lepper *et al.,* With a relatively large sample size of apparently healthy men and women, our study provides more convincing evidence about the relationship between LBP and metabolic abnormalities. In recent years, the effects of microbiota on health have attracted increasing attention, and low-grade endotoxemia or LPS was found to link to various metabolic consequences.

However, most studies have been performed in mice and few in human populations. Studies in mice demonstrated that two- to threefold increased circulating LPS induced by a high-fat diet or LPS infusion led to increased levels of fasting glucose and insulin and body weight gain (Cani *et al.,* 2008; Cani *et al.,* 2007). PPL is influenced by various parameters such as gastric emptying time, intestinal absorption, and lipoprotein lipase activity. Some studies have shown that the gastric emptying of liquids and solids decreases with age (Greaves *et al.,* 2002), but intestinal motility is not altered with age (Stoll *et al.,* 2004). Pancreatic secretion slightly decreases with age (Evans *et al.,* 1981). However, Kupfer *et al.,* (Kupfer *et al.,* 1985; Fikry, 1968; Arora *et al.,* 1989) studying healthy individuals have reported that fecal excretion, and, consequently, fat absorption changes slightly with age, suggesting that the decrease in pancreatic secretion is not enough to hinder the normal digestive process. One could imagine that because older individuals have a longer gastric emptying time, the absorption of fat would be slowed, justifying a late elevation in triglyceridemia. With age, gastric emptying rate and lipoprotein lipase activity are known to decrease, and a reduction of pancreatic lipase secretion and a delay in the clearance of TG-rich lipoproteins have also been observed. Bibliographical data on postprandial metabolism in PrW and PoW women are scarce and the studies that have been undertaken involve very small numbers of subjects. It is also difficult to compare data due to of the variety of food employed in the different studies. The lower PPL displayed by the PrW in this study was also found in other studies, with levels of TG for PrW and PoW similar to our data (Krasinski *et al.,* 1990; Masding *et al.,* 2003).

REFERENCE

Adachi Y, Moore LE, Bradford BU, Gao W and Thurman RG (1995). Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology* **108** 218–224.

Amar J, Burcelin R, Ruidavets JB, Cani PD, Fauvel J, Alessi MA, Chamontin B and Ferriéres J (2008). Energy intake is associated with endotoxemia in apparently healthy men. *The American Journal of Clinical Nutrition* **87**(5) 1219-23.

American Diabetes Association (2004). Diagnosis and classification of Diabetes Mellitus. *Diabetes Care* **27** S5-10.

Arora S, Kassarjian Z, Krasinski SD, Croffey B, Kaplan MM and Russell RM (1989). Effect of age on tests of intestinal and hepatic function in healthy humans. *Gastroenterology* **96** 1560-5.

Bae JH, Bassenge E, Kim KB, Kim YN, Kim KS and Lee HJ (2001). Postprandial hypertriglyceridemia impairs endothelial function by enhance oxidant stress. *Atherosclerosis* **155** 517-23.

Berg RD (1996). The indigenous gastrointestinal microflora. *Trends in Microbiology* **4**(11) 430-5.

Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delme´e E, Cousin B, Sulpice T, Chamontin B, Ferrie` res J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC and Burcelin R (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56** 1761–1772.

Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM and Burcelin R (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* **57** 1470–1481.

Caradonna L, Amati L, Magrone T, Pellegrino NM, Jirillo E and Caccavo D (2000). Enteric bacteria, lipopolysaccharides and related cytokines in inflammatory bowel disease: biological and clinical significance. *Journal of Endotoxin Research* **6** 205–214.

Cardona F, Tunez I, Tasset I, Montilla P, Collantes E and Tinahones FJ (2008). Fat overload aggravates oxidative stress in patients with the metabolic syndrome. *European Journal of Clinical Investigation* **38**(7) 510-5.

Cohn JS, McNamara JR, Cohn SD, Ordovas JM and Schaefer EJ (1988). Postprandial plasma lipoprotein changes in human subjects of different ages. *The Journal of Lipid Research* **29** 469-79.

Curtiss LK, Kubo N, Schiller NK and Boisvert WA (2000). Participation of innate and acquired immunity in atherosclerosis. *Immunologic Research* **21** 167–176.

De Boer OJ, van der Wal AC and Becker AE (2000). Atherosclerosis, inflammation, and infection. *The Journal of Pathology* **190** 237–243.
De Nardin E (2001). The role of inflammatory and immunological mediators in periodontitis and cardiovascular disease. *Annals of Periodontology* **6** 30–40.
Dubois C, Armand M, Azais-Braesco V, Portugal H, Pauli AM and Bernard PM (1994). Effects of moderate amounts of emulsified dietary fat on postprandial lipemia and lipoproteins in normolipidemic adults. *The American Journal of Clinical Nutrition* **60** 374-82.
Erridge C, Attina T, Spickett CM and Webb DJ (2007). A high-fat meal induces lowgrade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *The American Journal of Clinical Nutrition* **86**(5) 1286-92.
Evans MA, Triggs EJ, Cheung M, Broe GA and Creasey H (1981). Gastric emptying rate in elderly: Implications for drug therapy. *Journal of the American Geriatrics Society* **29** 201-5.
Feingold KR, Funk JL, Moser AH, Shigenaga JK, Rapp JH and Grunfeld C (1995). Role for circulating lipoproteins in protection from endotoxin toxicity. *Infection and Immunity* **63** 2041–2046.
Fikry M (1968). Exocrine pancreatic functions in the aged. *Journal of the American Geriatrics Society* **16** 463-7.
Flegel WA, Baumstark MW, Weinstock C, Berg A and Northoff H (1993). Prevention of endotoxin-induced monokine release by human low- and high-density lipoproteins and by apolipoprotein A-I. *Infection and Immunity* **61** 5140–5146.
Flegel WA, Wölpl A, Männel DN and Northoff H (1989). Inhibition of endotoxin-induced activation of human monocytes by human lipoproteins. *Infection and Immunity* **57** 2237–2245.
Friedewald WT, Levy RI and Frederickson DS (1972). Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18** 499-502.
Funada J, Sekiya M, Hamada M and Hiwada K (2002). Postprandial elevation of remnant lipoprotein leads to endothelial dysfunction. *Circulation Journal* **66** 127-32.
Gerszten RE, Mach F, Sauty A, Rosenzweig A and Luster AD (2000). Chemokines, leukocytes, and atherosclerosis. *Journal of Laboratory and Clinical Medicine* **136** 87–92.
Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Real JMF and Dandona P (2009). Increase in Plasma Endotoxin. *Diabetes Care* **32**(12) 2281-2287.
Ghoshal S, Witta J, Zhong J, de Villiersa W and Eckhardt E (2009). Chylomicrons promote intestinal absorption of lipopolysaccharides. *The Journal of Lipid Research* **50**(1) 90-7.
Glass CK and Witztum JL (2001). Atherosclerosis: the road ahead. *Cell* **104** 503–516.
Gokce N, Duffy SJ, Hunter LM, Keaney JF and Vita JA (2001). Acute hypertriglyceridemia is associated with peripheral vasodilatation and increased basal flow in healthy young adults. *American Journal of Cardiology* **88** 153-9.
Gray PW, Corcorran AE, Eddy RL Jr, Byers MG and Shows TB (March 1993). The genes for the lipopolysaccharide binding protein (LBP) and the bactericidal permeability increasing protein (BPI) are encoded in the same region of human chromosome 20. *Genomics* **15**(1) 188–90. doi:10.1006/geno.1993.1030. PMID 8432532.
Greaves DR and Channon KM (2002). Inflammation and immune responses in atherosclerosis. *Trends in Immunology* **23**(11) 535-41.
Gubern C, Lo´pez-Bermejo A, Biarne´s J, Vendrell J, Ricart W and Ferna´ndez-Real JM (2006). Natural antibiotics and insulin sensitivity: the role of bactericidal/permeability- increasing protein. *Diabetes* **55** 216–224.
Hansson GK, Libby P, Schonbeck U and Yan ZQ (2002). Innate and adaptive immunity in the pathogenesis of atherosclerosis. *Circulation Research* **91** 281–291.
Harris HW (1993). Chylomicrons alter the fate of endotxin, decreasing tumor necrosis factor release and preventing death. *Journal of Clinical Investigation* **91** 1028–1034.

Harris HW, Grunfeld C, Feingold KR and Rapp JH (1990). Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *Journal of Clinical Investigation* **86** 696–702.
Harris HW, Grunfeld C, Feingold KR, Read TE, Kane JP, Jones AL, Eichbaum EB, Bland GF and Rapp JH (1993). Chylomicrons alter the fate of endotoxin, decreasing tumor necrosis factor release and preventing death. *Journal of Clinical Investigation* **91** 1028–1034.
Holst O, Ulmer AJ, Brade H, Flad H-D and Rietschel ET (1996). Biochemistry and cell biology of bacterial endotoxins. *FEMS Immunology and Medical Microbiology* **16** 83–104.
Jagla A and Schrezenmeir J (2001). Postprandial triglycerides and endothelial function. *Experimental and Clinical Endocrinology & Diabetes* **109** S533-47.
Kabagambe EK, Ordovas JM, Tsai MY, Borecki IB, Hopkins PN and Glasser SP (2009). Smoking, inflammatory patterns and postprandial hypertriglyceridemia. *Atherosclerosis* **203** 633-9.
Karamanos BG, Thanopoulou AC and Roussi-Penesi DP (2001). Maximal postprandial triglyceride increase reflects post-prandial hypertriglyceridaemia and is associated with the insulin resistance syndrome. *Diabetic Medicine* **18** 32-9.
Krasinski SD, Cohn JS, Schaefer EJ and Russell RM (1990). Postprandial plasma retinyl ester response is greater in older subjects compared with younger subjects. *Journal of Clinical Investigation* **85** 883-92.
Kupfer RM, Heppell M, Haggith JW and Bateman DN (1985). Gastric emptying and small-bowel transit rate in the elderly. *Journal of the American Geriatrics Society* **33** 340-3.
Kuramitsu HK, Kang IC and Qi M (2003). Interaction of Porphyromonas gingivalis with host cells: implications for cardiovascular diseases. *Journal of Periodontology* **74** 85–89.
Kvietys PR, Specian RD and Grisham MB (1991). Jejunal mucosal injury and restitution: role of hydrolytic products of food digestion. *American Journal of Physiology - Gastrointestinal and Liver Physiology* **261** G384–G391.
Laugerette F, Vors C, Géloën A, Chauvin MA, Soulage C, Lambert-Porcheron S, Peretti N, Alligier M, Burcelin R, Laville M, Vidal H and Michalski MC (2011). Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. *The Journal of Nutritional Biochemistry* **22**(1) 53-9.
Lehr HA, Sagban TA, Ihling C, Zahringer U, Hungerer K-D, Blumrich M, Reifenberg K and Bhakdi S (2001). Immunopathogenesis of atherosclerosis: endotoxin accelerates atherosclerosis in rabbits on hypercholesterolemic diet. *Circulation* **104** 914–920.
Lepper PM, Schumann C, Triantafilou K, Rasche FM, Schuster T, Frank H, Schneider EM, Triantafilou M and von Eynatten M (2007). Association of lipopolysaccharide-binding protein and coronary artery disease in men. *Journal of the American College of Cardiology* **50** 25–31.
Levine DM, Parker TS, Donnelly TM, Walsh A and Rubin AL (1993). In vivo protection against endotoxin by plasma high density lipoprotein. *Proceedings of the National Academy of Sciences* **90** 12040–12044.
Lewis GF, O'Meara NM, Soltys PA, Blackman JD, Iverius PH and Pugh WL (1991). Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. *The Journal of Clinical Endocrinology and Metabolism* **72** 934-44.
Ley RE, Turnbaugh PJ, Klein S and Gordon JI (2006). Microbial ecology-Human gut microbes associated with obesity. *Nature* **444** 1022–3.
Li L, Messas E, Batista EL Jr, Levine RA and Amar S (2002). Porphyromonas gingivalis infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. *Circulation* **105** 861–867.
Libby P (2002). Inflammation in atherosclerosis. *Nature* **420** 868–874.
Libby P (2003). Vascular biology of atherosclerosis: Overview and state of the art. *American Journal of Cardiology* **91** 3A-6A.

Libby P, Ridker PM and Maseri A (2002). Inflammation and atherosclerosis. *Circulation* **105** 1135–1143.
López-Miranda J, Williams C and Lairon D (2007). Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. *British Journal of Nutrition* **98** 458-73.
Mariann I, Lassenius MI, Pietiläinen KH, Kaartinen K, Pirkko JP, Syrjänen J, Forsblom C, Pörsti I, Rissanen A, Kaprio J, Mustonen J, Groop PH and Lehto M (August 2011). Bacterial Endotoxin Activity in Human Insulin Resistance, Obesity, and Chronic Inflammation Is Associated With Dyslipidemia. *Diabetes Care* **34** 1809-1815.
Masding MG, Stears AJ, Burdeg GC, Wootton SA and Sandeman DD (2003). Premenopausal advantages in postprandial lipid metabolism are lost in women with type 2 diabetes. *Diabetes Care* **26** 3243-9.
Muta T and Takeshige K (2001). Essential roles of CD14 and lipopolysaccharide-binding protein for activation of toll-like receptor (TLR)2 as well as TLR4 Reconstitution of TLR2- and TLR4-activation by distinguishable ligands in LPS preparations. *European Journal of Biochemistry* **268**(16) 4580–9. doi:10.1046/j.1432-1327.2001.02385.x. PMID 11502220.
Nabeno Y, Fukuchi Y, Matsutani Y and Naito M (2007). Influence of aging and menopause on postprandial lipoprotein responses in healthy adult women. *Journal of Atherosclerosis and Thrombosis* **14** 142-50.
Netea MG (1998). Bacterial lipopolysaccharide binds and stimulates cytokine producing cells before neutralization by endogenous lipoproteins can occur. *Cytokine* **10** 766–772.
Ostos MA, Recalde D, Zakin MM and Scott-Algara D (2002). Implication of natural killer T cells in atherosclerosis development during a LPS induced chronic inflammation. *FEBS Letters* **519** 23–29.
Parker TS, Levine DM, Chang JCC, Laxer J, Coffin CC and Rubin AL (1995). Reconstituted high-density lipoprotein neutralizes Gram-negative bacterial lipopolysaccharides in human whole blood. *Infection and Immunity* **63** 253–258.
Patsch JR (1994). Triglyceride-rich lipoproteins and atherosclerosis. *Atherosclerosis* **110** S23-6.
Preston A, Mandrell RE, Gibson BW and Apicella MA (1996). The lipooligosaccharides of pathogenic Gram-negative bacteria. *Critical Reviews in Microbiology* **22** 139–180.
Raetz CR and Whitfield C (2002). Lipopolysaccharide endotoxins. *Annual Review of Biochemistry* **71** 635–700.
Ravin HA, Rowley D, Jenkins C and Fine J (1960). On the absorption of bacterial endotoxin from the gastro-intestinal tract of the normal and shocked animal. *The Journal of Experimental Medicine* **112** 783-92.
Read TE (1995). Triglyceride-rich lipoproteins improve survival when given after endotoxin in rats. *Surgery* **117** 62–67.
Read TE, Grunfeld C, Kumwenda ZL, Calhoun MC, Kane JP, Feingold KR and Rapp JH (1995). Triglyceride-rich lipoproteins prevent septic death in rats. *The Journal of Experimental Medicine* **182** 267–272.
Read TE, Harris HW, Grunfeld C, Feingold KR, Kane JP and Rapp JH (1993). The protective effect of serum lipoproteins against bacterial lipopolysaccharide. *European Heart Journal* **14**(Suppl. K) 125–129.
Richter V, Rassoul F, Hentschel B, Kothe K, Krobara M and Unger R (2004). Age dependence of lipid parameters in the general population and vegetarians. *Zeitschrift für Gerontologie und Geriatrie* **37** 207-13.
Rietschel ET, Kirikae T, Schade FU, Mamet U, Schmidt G, Loppnow H, Ulmer AJ, Zahringer U, Seydel U, Di Padova F, Schreier M and Brade H (1994). Bacterial endotoxin: molecular relationships of structure to activity and function. *The FASEB Journal* **94**(8) 217–225.
Ross R (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* **362** 801-809.
Ross R (1995). Cell biology of atherosclerosis. *Annual Review of Physiology* **57** 791-804.

Stoll LL, Denning GM and Weintraub NL (2004). Potential role of endotoxin as a proinflammatory mediator of atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* **24** 2227–36.
Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER and Gordon JI (2007). An obesity associated gut microbiome with increased capacity for energy harvest. *Nature* **444** 1027–31.
Ulevitch RJ, Johnston AR and Weinstein DB (1979). New function for high density lipoproteins. *Journal of Clinical Investigation* **64** 1516–1524.
University College London Medical School (1993). Hypertriglyceridaemia and vascular risk. Report of a meeting of physicians and scientists. *The Lancet* **342** 781-7.
Van der Gaag MS, Sierksma A, Schaafsma G, Van Tol A, Geelhoed-Mieras T and Bakker M (2000). Moderate alcohol consumption and changes in postprandial lipoproteins of premenopausal and postmenopausal women: A diet- controlled, randomized intervention study. *Journal of Women's Health and Gender-Based Medicine* **9** 607-16.
Van Lenten BJ, Fogelman AM, Haberland ME and Edwards PA (1986). The role of lipoproteins and receptor-mediated endocytosis in the transport of bacterial lipopolysaccharide. *PNAS, Proceedings of the National Academy of Sciences* **83** 2704–2708.
Victorov AV (1989). Composition and structure of lipopolysaccharide- human plasma low density lipoprotein complex. *Biochimica et Biophysica Acta* **984** 119–127.
Vreugdenhil AC, Rousseau CH, Hartung T, Greve JW and Buurman WA (2003). Lipopolysaccharide (LPS)-binding protein mediates LPS detoxification by chylomicrons. *The Journal of Immunology* **170** 1399–1405.
Wellmann W, Fink PC, Benner F and Schmidt FW (1986). Endotoxaemia in active Crohn's disease. Treatment with whole gut irrigation and 5-aminosalicylic acid. *Gut* **27** 814–820.
Wiedermann CI, Kiechl S, Dunzendorfer S, Schratzberger P, Egger G, Oberhollenzer F and Willeit J (1999). Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. *Journal of the American College of Cardiology* **34** 1975–81.
Wilkinson SG (1996). Bacterial lipopolysaccharides–themes and variations. *Progress in Lipid Research* **35** 283–343.