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 hypertriglyceridemia 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

© Copyright 2014 | Centre for Info Bio Technology (CIBTech)
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 (Baehrens 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 h 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 70°C 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

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Premenopausal women</th>
<th>Postmenopausal women</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>22.0 ± 2.4 kg/m²</td>
<td>23.0 ± 3.1 kg/m²</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>71.9 ± 6.1</td>
<td>73 ± 8.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>110.4 ± 17.3</td>
<td>112.4 ± 13.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72.7 ± 9.8</td>
<td>74.9 ± 6.8</td>
</tr>
<tr>
<td>Fasting Plasma Glucose (mg/dL)</td>
<td>94±8</td>
<td>97±7</td>
</tr>
</tbody>
</table>

The mean BMI values were 23.0 ± 3.1 kg/m² in PoW and 22.0 ± 2.4 kg/m² 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 categories (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 baseline after 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, and 117.64 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](image)

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.
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:**

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma endotoxin (LPS) in</td>
<td>0.34</td>
<td>0.38</td>
<td>0.45</td>
<td>0.44</td>
<td>0.42</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Premenopausal women (EU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma endotoxin (LPS) in</td>
<td>0.36</td>
<td>0.46</td>
<td>0.67</td>
<td>0.61</td>
<td>0.60</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Postmenopausal women (EU/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS binding protein (LBP) in</td>
<td>10.2</td>
<td>11.6</td>
<td>13.9</td>
<td>11.8</td>
<td>11.6</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Premenopausal women (μg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPS binding protein (LBP) in</td>
<td>10.9</td>
<td>14.4</td>
<td>19.8</td>
<td>13.8</td>
<td>13.4</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Postmenopausal women (μg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Research Article

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 (Kviety 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.
Research Article

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, Kupper et al., (Kupper 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


© Copyright 2014 | Centre for Info Bio Technology (CIBTech)
Research Article


Research Article


© Copyright 2014 | Centre for Info Bio Technology (CIBTech)
Research Article


