Diet influences levels of plasma lipopolysaccharide (LPS) and its soluble receptor (sCD14) in Saudis

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Abstract
Objective: This study aimed to investigate the association between serum levels of LPS, sCD14 and hs-CRP, and markers of obesity, and dietary composition of healthy adults residing in Jeddah, Saudi Arabia.
Methods: Apparently healthy adults, aged 18-55 years, were recruited from Jeddah population in a cross-sectional design. Anthropometric measurements, and vital signs were taken using standardized techniques. Serum glucose, cholesterol (TC), triglycerides (TG), high-density lipoprotein- cholesterol (HDL-C), hs-CPR, LPS and sCD14 were assayed, and LDL- cholesterol (LDL-C), and atherogenic index of plasma (AIP) were calculated. Means of estimated variables were compared using t-test or Mann–Whitney U-test for two groups and ANOVA for multiple groups. Chi-square, and Pearson’s correlation coefficient were used to identify association and correlations between studied variables.
Results: Means of TG, LDL-C, and hs-CRP increased significantly in both genders with increasing BMI (p= 0.019, and 0.040 for TG, 0.049, and 0.002 for LDL-C in males and females respectively, and <0.001 for hs-CRP for both). Mean hs-CRP was significantly higher in subjects with abdominal obesity (p=0.025 for men, and 0.001 for women), identified to have metabolic syndrome (p<0.001). Mean sCD14 was significantly elevated in females consuming high quantity of bread (p= 0.033) or drinking tea (p = 0.018). LPS correlated positively with sCD14 in men (p=0.049).
Conclusion: An association between dietary composition and development of bacterial endotoxaemia was found. However, no association between measures of endotoxaemia and increased adiposity and inflammation was found.
Keywords: Metabolic syndrome; sCD14; Endotoxaemia; obesity, hs-CPR

Introduction
The relationship between more than normal weight, metabolic dysregulation, inflammation, dietary composition and the composition of gut microbiota is still unclear, and the question remains whether health complications is more related to excess weight or to type of microbiota or to dietary practices.

Various studies have reported a causal relationship between the composition of intestinal microbiota and the development of obesity and its complications due partly to its involvement in energy harvesting from nondigestible foodstuffs, and hence in the storage of fat by adipocytes. Furthermore, the detection of elevated levels of circulating lipopolysaccharide (LPS) molecules, the glycolipids in the outer membrane of gram-negative bacteria, in chronic diseases associated with obesity suggested that it might have an important role in the inflammatory process and onset of these metabolic diseases. However, other studies reported that dietary composition is more relevant to the composition of intestinal microbiota, and hence endotoxaemia and metabolic dysfunction, rather than the presence of obesity as such.

Indeed, studies on mice indicated that ingestion of high fat diets caused an increase in the ratio of gram-negative to gram-positive bacteria leading to disruption of the gastrointestinal barrier function and the development of metabolic endotoxaemia, causing dysregulation of the inflammatory tone and triggering body weight gain. This would result in low-grade inflammation, and is associated with components and presence of metabolic syndrome (MetS), as well as diabetes, and the risk of incident cardiovascular disease (CVD) events. In contrast, another study reported that lipopolysaccharide from microbiota is not essential for impaired glucose or insulin tolerance in mice. The circulating soluble form receptor for LPS in plasma, sCD14, has been suggested as clinical marker that reflect plasma exposure to endotoxins.

Therefore, we aimed in this study to investigate the relationship of body mass index (BMI), abdominal obesity, and dietary composition in apparently healthy adults, with the serum levels of highly sensitive C-reactive protein (hs-CRP), LPS and sCD14, to find out the relationship between
weight gain, inflammation, and bacterial endotoxaemia.

Subjects and methods

This cross-sectional study was conducted between September 2015 and March 2016 on apparently healthy adults age 18-55 years, both genders, and diverse body mass index (BMI), recruited from the student population and staff at King Abdulaziz University Medical campus, as well as members of their families and friends. The target sample size was calculated, taking into account similar previous studies, to detect the association between markers of endotoxaemia, inflammation, metabolic dysfunction, and obesity, with 95% confidence interval and 0.05 precision. Thus, the actual calculated sample size using "Open epi program" was 88 but a total of 98 was aimed to ensure adequate gender representation, and to cover for missing data. Exclusion criteria included history of colon cancer, inflammatory bowel disease, acute or chronic diarrhoea in the previous 8 weeks and treatment with antibiotics in the previous 2 months, as well as intake of medication or nutritional supplements. The study was approved by the Ethics Committee of Human Research at King Abdulaziz University under agreement number 014-CEGMR-2-ETH-P. All participants were asked to sign a written informed consent after being briefly informed about the purpose of the study and ensured confidentiality of data. They were then requested to fill a pre-designed questionnaire covering their socio-demographic information, medical history, lifestyle and dietary practices. Anthropometric measurements were taken using standardised techniques. Height was measured to the closest 0.5 cm while barefooted using a stationary stadiometer, and weight was measured while the participant was dressed in light clothing to the closest 0.5 kg using a portable calibrated scale (Omron BF511). Waist circumference (WC) was measured between the lowest rib and iliac crest at the level of the umbilicus, to the closest 0.5 cm. Weight and Height were used to calculate Body Mass Index (BMI= kg/m²), which was then used to classify subjects as being underweight (BMI < 18.5), normal (BMI = 18.5–25), overweight (BMI = 25–<30), or obese (BMI ≥ 30). Using WC measurements to indicate abdominal obesity the cut-off value for increased risk was defined as >94cm for men, >80 cm for women.9 Blood pressure (BP) was measured following the recommendations of the 8th Joint National Committee using a standard mercury sphygmomanometer with the cuff on the right upper arm. A systolic BP greater than 140 mmHg, or diastolic BP greater than 90 mmHg was considered elevated.10 Fasting blood samples were obtained for conducting various biochemical measurements. Serum glucose, cholesterol, triglycerides, and high-density lipoprotein(HDL-C) were assayed in the accredited Clinical Chemistry laboratories of the "National Guard Hospital" in Jeddah on ABBOTT, Architect c8000 using spectrophotometric method. On the same the immunoturbidimetric latex immunoassay method was used to measure hs-CRP test. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation.11 The atherogenic index of plasma (AIP) was calculated using Dobiasova and Frohlich equation.12 The consensus definition was used to diagnose metabolic syndrome (MS).13 The Bacterial endotoxin (lipopolysaccharide -LPS), and the human soluble cluster of differentiation 14 (sCD14) were both measured using ELISA methods according to manufacturer's instructions in the "Food, Nutrition, and Lifestyle Research Unit" at King Fahd Research Center- King Abdulaziz University. Limulus Amebocyte Lysate (LAL) chromogenic endpoint assay from Hycult Biotech (Plymouth Meeting, PA, USA) was used for bacterial endotoxin detection, and a quantitative sandwich enzyme immunoassay kit from CUSABIO (Houston, USA) was used to measure sCD14.

Statistical Analysis: Data were entered and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 21 for Windows software (Chicago, IL, USA). Descriptive statistics were presented in the form of mean ±standard deviation (SD) for parametric data. Qualitative variables were presented as relative frequencies (percentages). Comparison between two means was performed using independent samples t-test for normally distributed parameters. When normality was not confirmed, the Mann–Whitney U-test was used. One way repeated measure analysis of variance (ANOVA) test was performed to examine differences among the groups for different variables. Chi-square test was used to identify the association between hs-CRP, LPS, and sCD14 with various variables. Correlation between variables was examined using Pearson's correlation coefficient. A P value < 0.05 was accepted to be statistically significant.

Results

A total of 48 men and 50 women were included in the study, divided according to BMI categories into 1) underweight: 11 men, and 10 women, 2) Normal weight: 14 men, and 16 women, 3) overweight: 12 men, and 13 women, 4) obese: 11 men, and 11 women. This distribution was not significantly different between men and women (P= 0.653). Characteristics of the study group are presented in Table 1.

Even though almost half of the studied group were overweight or obese, and more than 30% showed abdominal obesity, only one man had elevated DBP. However, the means of triglycerides, and LDL- cholesterol increased significantly with increasing BMI in men and
women. In contrast, the means of total cholesterol and AIP showed an increase, and that of HDL-C showed a decrease in women only.

Using the consensus definition for metabolic syndrome, 13 individuals were identified. The means and standard deviations (SD) of estimated serum hs-CRP, LPS, and sCD14 in different categories of BMI, and in the presence and absence of abdominal obesity, as well as in the presence and absence of metabolic syndrome are presented in Table 3.

An increase in waist circumference was associated with an increase in the level of hs-CRP in a linear manner ($r=0.363$, $p=0.013$ in men, and $r=0.638$, $p<0.001$ in women). Furthermore, hs-CRP level also positively correlated with triglycerides but negatively with HDL-C in men only. In addition, LPS correlated positively with sCD14 in men only.

There was a difference in reported dietary intake patterns between men and women, with men eating larger meals later in the evening than women, and showing different food preferences.

Table 2.

The mean hs-CRP increased significantly with increasing BMI in both genders, being highest in the obese subjects. Furthermore, the group of males and females with abdominal obesity had significantly higher mean hs-CRP than the corresponding group with normal waist circumference ($p=0.025$ in males, and $0.001$ in females). Moreover, a significantly higher mean hs-CRP ($p<0.001$) was found in subjects with metabolic syndrome. However, neither the mean of LPS nor that of sCD14 were affected by general or abdominal obesity, nor by the presence of metabolic syndrome.

Correlation between levels of hs-CRP, LPS, and sCD14 with each other, and with components of the metabolic syndrome are presented in Table 3.

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Studying the relationship between the quantity of intake of different types of food and serum hs-CRP, no effect of quantity of red meat, fruits and vegetables, fresh fruit juice, fried foods, dairy products, flavoured drinks, and tea (black or green), were noted on mean value. However, the mean hs-CRP of males reporting moderate intake of Turkish coffee was significantly lower than the mean of those reporting low or no intake (0.87±0.54 vs. 1.71±1.67, $p=0.016$). On the other hand, this was not noted among females ($p=0.362$). Similarly, a higher mean was noted among males reporting high consumption of bread, but without reaching statistical significance (2.69±2.28 vs 1.26±0.87, $p=0.077$). Alternatively, the mean of females reporting moderate intake of Cappuccino was lower, but not significantly so, than those reporting low intake (0.77±0.55 vs 1.26±1.27, $p=0.088$).

The effect of intakes of different types of food on the level of serum LPS and sCD14 were studied similarly; however, only statistically significant or near significant results are presented.

Non significantly lower mean LPS was noted in groups of females ingesting moderate intake of green tea (0.84±0.27 vs 1.12±0.52 EU/ml, $p=0.083$), and Turkish coffee compared

### Table 1: Anthropometric, demographics, and biochemical characteristics of studied subjects.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>p-value</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometric and Demographics</strong></td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>27.81 ± 8.15</td>
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<td>25.22 ± 9.2</td>
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<td>Height (m)</td>
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<td>1.68 ± 0.05</td>
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<td>Weight (kg)</td>
<td>68.95 ± 16.22</td>
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<td>62.02 ± 14.86</td>
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<td><strong>Biological characteristics</strong></td>
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<tr>
<td>SBP (mmHg)</td>
<td>120.4 ± 11.02</td>
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<td>110.56 ± 12.3</td>
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<tr>
<td>DBP (mmHg)</td>
<td>83.18 ± 7.69</td>
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<td>81.57 ± 8.38</td>
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<tr>
<td>WC (inch)</td>
<td>35.1 ± 5.83</td>
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<td>30.37 ± 5.48</td>
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<td>WC:HC</td>
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<td>0.75 ± 0.079</td>
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<tr>
<td><strong>Clinical characteristics</strong></td>
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<td>TG (mg/dl)</td>
<td>82.06 ± 7.99</td>
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<td>79.75 ± 9.39</td>
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<tr>
<td>FBG (mg/dl)</td>
<td>80.95 ± 8.98</td>
<td>0.238</td>
<td>79.99 ± 16.22</td>
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<tr>
<td>TC (mg/dl)</td>
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<td>83.87 ± 21.36</td>
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<tr>
<td>HDL-C (mg/dl)</td>
<td>22.33 ± 13.2</td>
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<td>18.23 ± 6.75</td>
<td>0.000</td>
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<tr>
<td>LDL-C (mg/dl)</td>
<td>20.08 ± 5.01</td>
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<td>15.23 ± 3.43</td>
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<tr>
<td>AIP</td>
<td>-0.33 ± 0.18</td>
<td>---</td>
<td>-0.27 ± 0.13</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*p*-value of independent t-test between males and females of studied population

*p*-value of ANOVA test within each gender, FBG: fasting blood Glucose, TC: Total cholesterol, TG: Triglyceride, AIP: atherogenic index of plasma

Women in contrast, the means of total cholesterol and AIP showed an increase, and that of HDL-C showed a decrease in women only.
The intake of bread and some beverages had some effect of statistical significance on the mean serum level of sCD14, which was also gender specific. Females consuming a high quantity of bread had higher mean sCD14 than those with moderate or low intake (10.8±2.6, 8.2±2.9, and 7.7±4.6 ng/ml for high, moderate, and low intake respectively, \( p = 0.033 \)). Similarly, the group of females drinking high or moderate amounts of tea had a higher mean than those with low intake (9.5±2.7, 10.3±3.0, and 7.6±3.1 ng/ml for high, moderate, and low intake respectively, \( p = 0.018 \)). However, females reporting moderate intake of American Coffee had a lower mean than the group reporting low or no intake (6.62±2.91, and 9.42±3.14 ng/ml for moderate, and low intake respectively, \( p = 0.019 \)).

### Discussion

Obesity, and specially abdominal obesity, is reported to increase both inflammation and oxidative stress, both of which cause metabolic dysregulation. This explains the link between obesity as well as abdominal obesity and disturbance in normal metabolism or metabolic dysregulation. Various studies have suggested that endotoxins (or lipopolysaccharides, LPS) produced by gram-negative microbiota contribute to the onset and maintenance of inflammation during obesity. However, another study disagreed with these suggestions. Most studies were carried out on murine models fed controlled diets, and none were carried out on Saudis. Therefore, we aimed to investigate the relationship between different measures of obesity, as well as dietary composition in apparently healthy adults, with the serum levels of hs-CRP, LPS and the circulating form of its receptor (sCD14), in the hope of clarifying the situation.

In view of the well documented gender differences in response to known risk factors of various diseases, as well as in dietary and lifestyle practices, data for men and women were presented separately.

It was not surprising to find an increasing mean of hs-CRP with the increase in BMI, and in abdominally obese individuals, as well as in those with metabolic syndrome.
This finding is in agreement with previously published studies. The association between CRP and diet has also been reported in another study. This study did not take into account overall dietary patterns for analysis. However, moderate intake of Turkish coffee in men and Cappuccino in women were associated with lower mean hs-CRP, substantiating earlier reports of the beneficial effects of moderate consumption of coffee including reduction of cardiovascular disease, incident cancer, and metabolic, and liver conditions.

However, the mean level of LPS was not affected by BMI, nor by the presence of abdominal obesity, contrary to previous reports linking increased levels to obesity, metabolic syndrome, and cardiometabolic risk. The difference in results could be attributed to inclusion of mainly healthy and obese individuals, as compared to morbidly obese subjects in the Norwegian study. Similarly compared to the large Finnish cohort, with a high percentage suffering from metabolic disorders, only seven subjects in our study had metabolic syndrome, and not all of them were overweight or obese. Furthermore, the concentrations of plasma LPS were reported to be modulated by food content in healthy adult males, with higher energy and fat content increasing its concentration. Unfortunately, our study did not allow us to evaluate the total dietary energy or fat content of the participants. However, non-significantly lower mean LPS was noted in females ingesting moderate intake of green tea, and Turkish coffee compared to the group with low intake, supporting the earlier studies suggesting that diet rather than obesity per se controls the composition of the gut microbiome, hence the level of LPS in the blood. In addition, this association was only found in lean, healthy people, not those who were obese, or had metabolic disorders.

The circulating form of the LPS membrane-bound receptor (sCD14), has been suggested as clinical markers that reflect plasma exposure to LPS more accurately. Moreover, it was reported to have a blunting effect on LPS activity by driving detoxification. However, in an earlier study, no significant correlation was found between plasma sCD14 levels and LPS concentrations in non-obese subjects, while a positive association was found in the obese. The latter was in agreement with our results for males. In the same study fasting triglycerides was reported to contribute independently to circulating sCD14 variance, which might be a cause for the lack of association between the parameters in the females since the mean triglycerides level was higher in males, and we did not adjust for this factor.

In our study, the mean sCD14 level was not affected by general or abdominal obesity, nor by the presence of metabolic syndrome. An earlier study reported a positive association between circulating sCD14 level and waist circumference in non-obese healthy subjects, but not in obese subjects, and morbidly obese subjects had on average higher sCD14 levels compared to the non-obese. Moreover, fasting blood samples were collected for the measurement of all parameters, and sCD14 has a longer half-life compared with that of LPS, which is known to increase following food intake, then decrease gradually. Therefore, this could be another cause for the lack of association noted in females, who reported earlier dinner time, and smaller meals compared to males.

In another cohort study, healthy men were overfed to induce weight gain, and the ratio of lipopolysaccharide binding protein to sCD14 (LBP/sCD14) was found to be increased significantly after overfeeding (OF), but LPS, LBP, and sCD14 did not vary significantly. Researchers concluded that the setup of low-grade inflammation during the initial stage of weight gain is linked to the relative variations of LBP and sCD14. This could also explain the lack of difference in mean sCD14 (and LPS) between the different BMI categories since the timing of weight gain was not recorded in our study. LBP was not estimated either, hence the ratio of LBP/sCD14 could not be calculated. Moreover, it was reported that even though overweight and obese subjects had increased circulating hs-CRP (similar to our findings), the level of sCD14 was not, and instead was dependent on the dietary pattern of the studied group.

Even though dietary patterns were not investigated in our study, the intake of some types of food and beverages had an effect on the mean serum level of sCD14. Females reporting a high quantity of bread, and those drinking higher amounts of black tea had higher mean sCD14 than those with moderate or low intake. However, females reporting moderate intake of American coffee had a lower mean than those reporting low or no intake, supporting further the beneficial effects of coffee. The increased mean sCD14 associated with high bread intake could be due to an increase in overall energy intake, which is reported to increase LPS level, and hence sCD14 to modulate its effect. However, it was surprising to find an increase in mean sCD14 associated with high intake of black tea which is rich in polyphenols that are reported to decrease plasma LPS, and hence sCD14. However, the addition of sugar to black tea, as commonly practised, could increase the total energy intake, and hence LPS, as well as sCD14 levels.

In conclusion, our study supported the proposed association between diet and the development of bacterial endotoxaemia. However, it did not prove the association...
between serum LPS level, or sCD14 and increased adiposity and inflammation as suggested in earlier reports.

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References