Energy intake is associated with endotoxemia in apparently healthy men\textsuperscript{1–4}

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ABSTRACT

Background: The bridge between food intake and weight is not fully understood. Recently, the role of gut microbiota and bacterial lipopolysaccharides (LPS) in weight has been noted.

Objective: The objective was to evaluate the relation between plasma LPS concentration and food intake.

Design: A dietary survey was conducted in 1015 subjects randomly recruited in France. The participants were given oral and written instructions on how to keep a consecutive 3-d food record. Plasma LPS was measured in a subsample of 201 men. To assess, under controlled conditions, the differential impact of various high-energy diets, plasma LPS concentrations were measured in mice fed a high-fat or a high-carbohydrate diet over a 4-wk period.

Results: In humans, no significant relation was observed between cardiovascular disease risk factors, carbohydrate and protein intakes, and plasma LPS concentration. Conversely, positive correlations were observed with fat and energy intakes. In a multivariate analysis, endotoxemia was independently associated with energy intake. Compared with the control mice, mice fed a high-energy diet showed an increase in plasma LPS. However, in mice fed a high-carbohydrate diet, the increase in plasma LPS was blunted compared with mice fed a high-fat diet.

Conclusions: In this large sample of healthy men from a population-based sample, we found a link between food intake and plasma LPS. Experimental data suggest that fat was more efficient in transporting bacterial LPS from the gut lumen into the bloodstream. The results of this study add to the knowledge of mechanisms responsible for relations between food intake and metabolic diseases. \textit{Am J Clin Nutr} 2008;87:1219–23.

INTRODUCTION

Obesity has reached epidemic proportions globally. More than 1 billion adults are overweight, at least 300 million of whom are clinically obese. Obesity and overweight pose a major risk of chronic diseases, including type 2 diabetes, cardiovascular disease, hypertension, and stroke. The key causes are an increased consumption of energy-dense foods high in saturated fats and sugars (1). However, the bridge between food intake and weight is not fully understood. The hypothesis that gut microbiota influences weight gain was posited recently. An original observation reported that young adult mice have 40\% more total body fat than their germ-free counterparts fed the same diet (2, 3). Similarly, lean axenic mice colonized with microbiota from genetically obese mice gained weight. Moreover, axenic mice fed a high-fat obesitogenic diet did not gain weight, which suggests that, indeed, a bacterially related factor is responsible for obesity induced by a high-fat diet (4). The authors suggested that gut microbiota from obese mice allows energy to be salvaged from otherwise indigestible dietary polysaccharides (5). Recently, we reported in mice the role of bacterial lipopolysaccharide (LPS) from the gram-negative intestine (6). We first showed that metabolic concentrations of plasma LPS are modulated by food content: the higher the fat food content, the higher the concentration of plasma LPS. Second, we found that the metabolic concentration of plasma LPS is a sufficient molecular mechanism for triggering the high-fat diet–induced metabolic diseases such as obesity and diabetes. Whether these experimental results are relevant in humans deserves to be studied. We sought to evaluate in healthy men the relation between plasma LPS concentration and food intake.

SUBJECTS AND METHODS

Population

Subjects (n = 1015) randomly selected from polling lists were recruited between 1995 and 1997 by the Toulouse MONICA Center in Haute Garonne, a region of southwestern France. Authorization from the appropriate ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Lille) was obtained, and each subject signed an informed consent form (7). The examination was performed in the morning, and a blood sample was drawn after the subjects fasted overnight. With the help of medical staff, each subject filled in a questionnaire about his or her medical history, drug intake, smoking habits, and alcohol consumption. The dietary survey

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was carried out only in the subsample of the men aged 45–64 y. Plasma LPS was measured in half of the population. Finally, the correlative analysis of plasma LPS concentration and the dietary survey were conducted in a subgroup of men (n = 201) in whom both plasma LPS concentration was measured and the dietary survey was conducted.

Experimental data

Because we observed a positive correlation between energy intake and plasma LPS in humans, we tested whether high-energy, high-fat or high-energy, high-carbohydrate diets resulted in changes in plasma LPS. Male 12-wk-old C57Bl6/J mice (Charles River, Lyon, France) were housed in a controlled environment (inverted 12-h daylight cycle, lights off at 1000) and had free access to food and water. All of the following animal experimental procedures were validated by the local ethical committee of the Rangueil Hospital. Mice were fed for 4 wk a control diet (A04; Usine d’Alimentation Rationnelle, Villemoisson sur Orge, France) or a high-energy diet: either a high-fat, carbohydrate-free diet (HF; 72% fat from corn oil and lard, 28% of energy as protein, and <1% carbohydrate as energy) or a high-fat, high-carbohydrate diet (HC; 35% fat from corn oil and lard, 28% of energy as protein, and 37% of carbohydrate as energy). Blood was collected by retroorbital bleeding and kept frozen (−80 °C) until LPS measurement.

Dietary survey

Food and alcohol intakes were assessed by using a food-record method (8). Participants were given oral and written instructions by the medical staff on how to keep a consecutive 3-d food record. They recorded in a food diary all the food and beverages (types and amounts) consumed throughout 3 consecutive days in the week after the exam (2 weekdays and 1 weekend day). Two to four days after the food record was completed, each participant was interviewed at home by a certified dietitian in the presence of the person who prepared the meals. The contents of household measures were evaluated by the dietitian with a measuring glass. Food estimates were facilitated by the use of photographs showing portion sizes and their respective weight. The recorded data were carefully checked by the dietitian, who, to avoid forgotten or misreported data, submitted a list of various food categories to the participants to check the reliability of the data. For meals that were not eaten at home (eg, meals eaten in restaurants and cantines), the cook was contacted and the composition of the dishes and their respective portion size were recorded.

Biochemical analysis

Plasma total cholesterol and triglycerides were measured by enzymatic methods (Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was measured in the supernatant after sodium phosphotungstate–magnesium chloride precipitation (Boehringer Mannheim). LDL cholesterol was determined by the Friedewald formula. Interleukin-6 (IL-6) concentrations were determined with an immunoenzymatic method (Immunochem, Marseille, France). Fibrinogen was measured in plasma with the Clauss method (9). To take into account the dilution induced by the anticoagulant factor, the fibrinogen measurements obtained were corrected according to the hematocrit value.

Plasma LPS concentrations were measured in humans and mice in 2 distinct laboratories using 2 different methods. In both laboratories, endotoxin assay, based on a Limulus amebocyte extract with Kinetic-QCL test (Bio Whittaker, Cambrex BioScience, Walkersville, MD), was used. It is a quantitative, kinetic assay for the detection of gram-negative bacterial endotoxin. In humans, sera were diluted 1/2000 with 0.5% pyrosperse to minimize interferences in the reaction (inhibition or enhancement). In such a condition, the lower limit of detection of human plasma LPS concentration was 9 U/mL. In mice, sera were diluted 1/40 to 1/80 and heated for 10 min at 70 °C. The lower limit of detection of LPS was 1 U/L.

Statistical analysis

The population-based sample was divided into 3 groups according to plasma LPS concentration: subjects with plasma LPS concentrations below the limit of detection (9 U/mL) and subjects with detectable plasma LPS concentrations, who were divided into 2 groups according to the median value of LPS (39 U/mL). A P value <0.05 indicated statistical significance. In a bivariate analysis, the chi-square test was used to compare the distribution of qualitative variables between levels of endotoxemia. Mean values of continuous variables were compared by one-factor analysis of variance. Shapiro-Wilks and Levene’s tests were used to test the normality of the distribution of residuals and the homogeneity of variances, respectively. When basic assumptions of analysis of variance were not satisfied, a logarithmic transformation of the variables was done or a Kruskal-Wallis test was performed. Energy variables were adjusted for total energy intake by using the residual method for energy adjustment (10). Multivariate linear regression analysis was used to assess the relation between plasma LPS concentration and food intake. We estimated the β regression coefficients and their SEs for each endotoxemia level. These coefficients represent the absolute difference in energy or fat intake between each of the endotoxemia levels and the reference group (endotoxin concentration <9 U/mL). These analyses were performed by using SAS statistical software, release 9.2 (SAS institute Inc, Cary, NC).

Mice data are presented as means ± SEs. The statistical significance of the differences was analyzed by one-factor analysis of variance followed by a post hoc Bonferroni multiple comparison test using GraphPad Prism software (version 4.00 for WINDOWS; GraphPad Software, San Diego, CA).

RESULTS

Population-based sample

Characteristics of the study population and macronutrient intakes according to endotoxemia levels are shown in Table 1. No significant differences were observed in body mass index, age, cardiovascular disease risk factors, lifestyle habits, income, duration of schooling, residence, and standard of medical coverage between the 3 LPS groups. Also, we found no differences in IL-6 plasma concentrations by LPS group. In addition, no significant correlation was observed with carbohydrate and protein intakes. Conversely, positive and significant relations were observed with fat and energy intakes. All fat types ingested, saturated, monounsaturated, and polyunsaturated (P = 0.06, 0.02, and 0.05, respectively), showed a similar statistical trend (data not shown). In the multivariate analysis (Table 2), endotoxemia was independently associated with energy intake but not fat with intake.
FOOD INTAKE AND METABOLIC ENDOTOXEMIA

TABLE 1
Characteristics of the study population and macronutrient intakes according to endotoxin concentration

| Endotoxin concentration | <9 U/mL (n = 130) | 9–39 U/mL (n = 44) | >39 U/mL (n = 27) | P for trend
|-------------------------|-------------------|-------------------|------------------|-----------------
| Age (y)                 | 54.8 ± 6.1        | 54.3 ± 5.9        | 52.8 ± 5.2       | 0.11
| Weight (kg)             | 77.0 ± 11.5       | 76.6 ± 13.5       | 77.8 ± 7.1       | 0.74
| BMI (kg/m²)             | 26.1 ± 3.2        | 26.2 ± 3.7        | 26.0 ± 2.2       | 0.82
| Waist circumference (cm)| 93.8 ± 9.3        | 93.0 ± 11.1       | 93.1 ± 7.9       | 0.74
| Systolic blood pressure (mm Hg) | 137.3 ± 16.2 | 140.1 ± 17.7 | 135.9 ± 16.2 | 0.71
| Total cholesterol (mmol/L) | 6.09 ± 0.86 | 5.85 ± 0.88 | 5.91 ± 1.24 | 0.37
| HDL cholesterol (mmol/L) | 1.30 ± 0.34 | 1.24 ± 0.39 | 1.24 ± 0.30 | 0.42
| Triglycerides (mmol/L)   | 1.41 ± 0.84 | 1.74 ± 1.27 | 1.20 ± 0.49 | 0.29
| Glucose (mmol/L)         | 5.87 ± 0.92 | 5.74 ± 0.71 | 5.95 ± 1.16 | 0.70
| Interleukin-6 (µg/L)     | 12.1 ± 7.8 | 13.2 ± 6.4 | 11.5 ± 7.1 | 0.71
| Fibrinogen (g/L)         | 3.56 ± 0.71 | 3.49 ± 0.71 | 3.60 ± 0.73 | 0.75
| Smoking (packs/y)        | 4.3 ± 4.0 | 3.4 ± 2.2 | 4.2 ± 3.4 | 0.85
| Alcohol (g/d)            | 18.6 ± 22.9 | 21.1 ± 19.4 | 27.2 ± 31.4 | 0.09
| Drug use for hypertension [n (%)] | 20 (15.4) | 12 (27.3) | 6 (13.7) | 0.58
| Drug use for dyslipidemia [n (%)] | 29 (22.3) | 4 (9.1) | 3 (11.1) | 0.06
| Drugs use for diabetes [n (%)] | 6 (4.6) | 1 (2.3) | 1 (3.7) | 0.65

Macronutrient intake

| Total energy (kcal/d) | 2307.1 ± 567.1 | 2517.9 ± 689.9 | 2616.9 ± 713.1 | 0.02
| Carbohydrate (g/d)   | 233.4 ± 69.4 | 251.1 ± 77.7 | 262.8 ± 85.3 | 0.07
| Protein (g/d)        | 95.6 ± 24.4 | 101.1 ± 29.2 | 102.0 ± 24.2 | 0.24
| Fat (g/d)            | 91.7 ± 27.8 | 102.7 ± 39.5 | 110.7 ± 46.5 | 0.009
| Alcohol (g/d)        | 25.6 ± 21.7 | 28.2 ± 22.3 | 24.6 ± 19.6 | 0.82

1 ANOVA did detect potential differences between the 3 groups of endotoxin concentrations.
2 ± SD (all such values).

Changes in plasma LPS in response to various high-energy diets in mice

Compared with control mice, mice fed a high-energy diet (either the high-fat diet or the high-carbohydrate diet) showed an increase in plasma LPS. In response to the high-fat diet, endotoxemia increased 2- to 3-fold above that in mice fed a normal chow diet. Importantly, this increase was still present but blunted when the percentage of energy as fat was reduced and replaced with carbohydrate (Figure 1).

DISCUSSION

This study showed for the first time, to the best of our knowledge, that endotoxemia correlates with energy intake in healthy men. The epidemic of high-fat diets in Western countries is becoming a problem of the utmost importance. Indeed, changes in eating habits to increase energy intake are involved in the growing occurrence of metabolic diseases, such as obesity and diabetes. In addition, it was recently determined that abdominal obesity is associated with a low-grade chronic systemic inflammation (11). In models of diet-induced and genetic obesity, the adipose tissue contains an increased expression and content of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) (12, 13), IL-1 (12, 13), and IL-6 (13). The production of cytokines adversely affects muscle insulin action. For example, TNF-α has been shown to cause insulin resistance by increasing serine phosphorylation on insulin receptor substrate-1 (IRS-1) (14), which leads to its inactivation. The consequent insulin resistance favors hyperinsulinemia and excessive hepatic and adipose tissue lipid storage. Consistently, epidemiologic data in humans have repeatedly established correlations between inflammatory proteins, such as C-reactive protein, and metabolic syndrome (15–

TABLE 2
Relations between energy and fat intakes and endotoxemia (linear regression of energy or fat on endotoxemia)

<table>
<thead>
<tr>
<th>Endotoxin concentration</th>
<th>Total energy</th>
<th>Energy without alcohol</th>
<th>Fat</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>β   SE  P</td>
<td>β   SE  P</td>
<td>β   SE  P</td>
<td>β   SE  P</td>
<td></td>
</tr>
<tr>
<td>&lt;9 U/mL (n = 130)</td>
<td>0  51 0.22</td>
<td>0  46 0.22</td>
<td>1.2 3.1 0.71</td>
<td>0.9 1.8 0.61</td>
</tr>
<tr>
<td>9–39 U/mL (n = 44)</td>
<td>62.9 62.7 0.04</td>
<td>57.9 46.9 0.22</td>
<td>4.6 3.8 0.23</td>
<td>2.5 2.1 0.26</td>
</tr>
<tr>
<td>&gt;39 U/mL (n = 27)</td>
<td>132.2 62.7 0.04</td>
<td>121.8 57.7 0.04</td>
<td>4.6 3.8 0.23</td>
<td>2.5 2.1 0.26</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.03 — —</td>
<td>0.03 — —</td>
<td>0.25 — —</td>
<td>0.25 — —</td>
</tr>
</tbody>
</table>

1 Adjusted for age, physical activity, BMI, and residuals from linear regression of energy on protein, carbohydrate, fat, and alcohol.
2 Linear regression of fat-adjusted energy on endotoxemia.
3 Adjusted for age, physical activity, BMI, and residuals from linear regression of energy on protein, carbohydrate, and alcohol.
In this respect, chronic subclinical inflammation has been proposed as part of the insulin resistance syndrome (18).

However, the triggering factor linking inflammation to high-fat diet–induced metabolic syndrome remains to be determined. Recently, we found in mice that the metabolic concentration of plasma LPS is modulated by food content: the higher the fat food content, the higher the concentration of plasma LPS (6). Second, we found that the metabolic concentration of plasma LPS is a sufficient molecular mechanism for triggering high-fat diet–induced metabolic diseases such as obesity and diabetes via inflammation mediated by the CD14 receptor, which is the main receptor of LPS. Thus, we suggested that LPS would be a newly identified inflammatory factor from microbiota that, on binding to CD14, serves as vector for the triggering of obesity and insulin resistance induced by high-fat feeding. However, the clinical relevance of these data derived from animal models remains to be established. Significant correlations were observed between the CD14 pathway and insulin resistance in humans. Importantly, the soluble CD14 concentration and a polymorphism of the CD14 gene—a C-to-T transition at bp −159 that plays a significant role in regulating the serum sCD14 concentration, has been shown to correlate with waist diameter and insulin sensitivity in cross-sectional and longitudinal studies (19, 20). Furthermore, low-grade endotoxemia has been shown to increase adipose TNF and IL-6 concentrations and the homeostasis model of assessment of insulin resistance in healthy volunteers (21). By linking energy intake and endotoxemia in a large sample of healthy men from population-based sample, the present study adds important information to this body of evidence. Taken together, these results suggest that diet-induced changes in endotoxemia may bridge the gap between food intake and metabolic diseases in humans. Second, we showed for the first time to our knowledge that the confounding factor of the relation between fat food intake and endotoxemia is likely to be energy intake. This latter result has important pathophysiological implication. Indeed, this finding suggests that changes in diet-induced endotoxemia are associated with general digestion rather than with fat digestion. Most importantly, this finding suggests that various high-energy diets, independently of fat content, may result in changes in metabolic endotoxemia in humans. This hypothesis is supported by experimental data: indeed, we found a significant increase in plasma LPS in mice fed various high-energy diets (either high in fat or high in carbohydrate) over 4 wk. However, the increase in plasma LPS is more pronounced in mice fed a high-fat diet, which suggests that fat is more efficient in transporting bacterial LPS from the gut lumen into the bloodstream.

In this cross-sectional study, we failed to find any relation between plasma LPS and weight, body mass index, insulin, glycemia, or IL-6. This finding is in contrast with results obtained in mice infused with LPS for 4 wk (4). However, it should be noted that the data from animal models also suggest that LPS is a catalyst for inflammation and weight gain with a high-fat diet (4). Once the process is underway, other mechanisms are likely to be involved in weight gain and insulin resistance. One obvious responsible factor is lipotoxicity, which is associated with a high-fat diet. Insulin resistance and impaired insulin secretion may be direct consequences of lipotoxicity because of the metabolic mechanisms involved with excessive lipid storage and oxidation (22–24). Regarding these hypotheses, the study design did not allow any firm conclusion to be drawn. A prospective study is required to assess the influence of changes in plasma LPS associated with a high-energy diet on body weight and metabolic disease. Also, it is likely that increased intestinal permeability precedes increased endotoxemia; indeed, no effect of a single intravenous dose of endotoxin was observed on markers of large bowel permeability, metabolism, or inflammation in healthy male subjects (25). Furthermore, it has been shown that a loss-of-function mutation in Toll-like receptor 4 (TLR4), which plays a key role in the LPS-sensing machinery, prevents diet-induced obesity and insulin resistance (26). Indeed, TLR4 is a major component of the LPS-sensing machinery consisting primarily of LPS-binding protein (LBPs), CD14, a glycosylphosphatidylinositol (GPI)-anchored monocyte differentiation antigen, and TLR4—a signal-transducing integral membrane protein (27). In this respect, an increased intestinal permeability, a higher circulating concentration of endotoxins, and an increased expression of membrane CD14 mRNA concentrations (the main receptor of LPS) were found in genetically obese (leptin-deficient) mice (28). Therefore, in light of these data, it could be relevant to determine whether manipulation of gut microbial communities may result in changes in weight and body mass index (29).

**Study limitations**

There is no consensus on how to measure plasma LPS concentrations. Distinct methods were used in humans and in mice. The assay system applied (Limulus amebocyte lysate) in human sera detects a broad spectrum of bacterial endotoxin and showed adequate performance in previous studies (30, 31). It bears the disadvantage of undetermined interlaboratory variability and is not approved for use in clinical practice. Also, in the present study, the lower limit of detection of plasma LPS concentration was 9 U/mL. Therefore, the analyses were performed in a semiquantitative way. However, inaccuracies in assessing plasma LPS in humans tend to weaken true relations rather than create spurious ones.

This study adds human data to the body of evidence from animal studies supporting the role of gut microbiota and plasma LPS in metabolic diseases. In this large sample of men from a population based-sample, we found a link between energy food intake and plasma LPS. Taken together, these data suggest that a reduction in plasma LPS concentration might be a potent strategy for the control of metabolic diseases in humans.

The authors’ responsibilities were as follows—JA, RB, JBR, and JF: contributed to the study conception, study design, analysis and interpretation of the data, and the draft of the manuscript and gave final approval of the manuscript; PDC, JF, MCA, and BC: contributed to the interpretation of data, revision of the manuscript critically for important intellectual content, and final approval of the manuscript. None of the authors had a conflict of interest.
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