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Reduced dietary intake of pro-inflammatory Toll-like receptor stimulants favourably modifies markers of cardiometabolic risk in healthy men

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Abstract *Background and aims:* Because pro-inflammatory stimulants of Toll-like receptor-2 and TLR4 (pathogen-associated molecular patterns, PAMPs), are abundant in some processed foods, we explored the effects of diets enriched or depleted in these molecules on markers of cardiometabolic risk in man.
Methods and results: Adherence to a low PAMP diet for 7 days reduced LDL-cholesterol (−0.69 mM, $P = 0.024$) and abdominal circumference (−1.6 cm, $P = 0.001$) in 11 habitual consumers of high PAMP foodstuffs, and these markers, together with leukocyte counts (+14%, $P = 0.017$) increased significantly after 4 days consuming predominantly high PAMP foods. Change in LDL-cholesterol and leukocyte counts correlated well with change in frequency of intake of high PAMP foodstuffs per individual ($r = 0.540$, $P = 0.0095$ and $r = 0.6551$, $P = 0.0009$, respectively). In an independent group of 13 healthy men, leukocyte counts and expression of the activation marker CD11b on granulocytes and monocytes were significantly reduced after a fresh onion meal ($P < 0.05$), but these effects were reversed by a high PAMP equivalent meal.
Conclusions: A low PAMP diet is associated with reduced levels of several cardiometabolic risk factors, while a high PAMP diet reverses these effects. These findings suggest a novel potential mechanistic explanation for the observed association between processed food consumption and risk of cardiometabolic diseases. The study is registered at clinicaltrials.org (reference NCT02430064).

Introduction

Evidence is accumulating to suggest that chronic metabolic diseases, including type II diabetes and atherosclerosis, may be promoted by low-grade inflammation induced by specific dietary patterns [1–3]. For example, observational studies have reported that the consumption of processed meats, relative to unprocessed meats, is associated with elevated C-reactive protein (CRP) levels [4], risk of type II diabetes [4,5] and risk of cardiovascular disease [6,7]. However, the specific components of these

Abbreviations: BLP, bacterial lipoprotein; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model of insulin resistance; hsCRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; LPS, lipopolysaccharide (endotoxin); PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood mononuclear cells; TLR, Toll-like receptor; WBC, white blood cell.

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diets which are responsible for promoting inflammatory signalling, or metabolic risk, remain to be clearly defined.

Recent evidence suggests that conserved molecules of microbial origin termed pathogen-associated molecular patterns (PAMPs) may play a key role in this relationship [8,9]. In particular, parenteral administration of PAMPs which stimulate the innate immune receptors Toll-like receptor (TLR)-2 or TLR4 (bacterial lipopeptides, BLP, and lipopolysaccharides, LPS, respectively), promotes inflammation, insulin resistance, impaired reverse cholesterol transport and atherosclerosis in murine models [8–10]. Circulating endotoxin levels also correlate positively with risk of atherosclerosis and type II diabetes in man [11].

Because the human large intestine contains a very large number of bacteria (~100 trillion), and a high PAMP content [12], it has been widely assumed that food-borne TLR-stimulants are unlikely to be significant contributors to systemic inflammatory tone. However, we found recently that of the four major phyla that dominate the human intestinal microbiota (Bacteroidetes, Actinobacteria, Firmicutes and Proteobacteria), only key marker species of the numerically minor (~0.1%) Proteobacteria group secreted large quantities of soluble stimulants of TLR2 and TLR4 [12]. Accordingly the soluble PAMP content of the human faecal microbiota was much lower than expected [12].

By comparison, we found that a variety of commonly consumed foodstuffs associated with the Western diet, particularly processed foods containing minced meats or chopped onion stored at refrigeration temperature, frequently contained bacterial TLR-stimulants at concentrations that were several orders of magnitude higher than those measured in the healthy murine small intestine, which normally has a very low PAMP content but is the major site of endotoxin absorption [12–14]. The present study was therefore conducted to explore the hypothesis that dietary TLR-stimulants may modify systemic markers of inflammation and cardiometabolic risk in human volunteers *in vivo*.

Methods

Recruitment and subjects

Two studies were conducted, one chronic sequential dietary intervention sampling at three timepoints over 12 days and one acute, single blinded, cross-over study with 2 interventions each sampled on three timepoints over 24 h (see Supplemental Fig. 1 for a graphical depiction of the study protocols). For the chronic study, 15 healthy male volunteers (age 37.5 ± 10.0 years) were recruited, of whom 4 reported onset of mild infections during the trial and were excluded from further study. For the acute study, 13 healthy male volunteers (age 27.8 ± 10.7 years) were recruited and all completed the study. Baseline physical characteristics of each cohort are summarised in Supplemental Table 1. For both studies, inclusion criteria were healthy men between the ages of 18 and 65. Exclusion criteria included evidence of any current inflammatory condition, infection or vaccination within two weeks prior to the study and use of medications. All subjects gave informed consent, and ethical approval for the study (reference NCT02430064 at clinicaltrials.org) was granted by the University of Leicester College of Medicine Ethics Committee.

Study design and dietary interventions

For the chronic study, volunteers were asked to avoid specific types of food we found in recent studies to be at relatively high risk of containing high levels of PAMPs [14,15], and to consume any quantity of fresh produce, including any form of meat, fish or vegetables that had not been minced or processed unless immediately before consumption, for a run-in period of 7 days. There were no restrictions on salt, sugar or non-alcoholic beverages. Fasting blood samples were collected on days 0 and 7 of this run-in period. Then, over the next 4 days, subjects consumed a set lunch and evening meal provided to them, each chosen on the basis of high PAMP content from prior screens (Fig. S2, Table S2). Subjects were asked to maintain a quantitative diet diary and to avoid excessive alcohol consumption for the duration of the study. A more detailed description of dietary advice, nutrient and PAMP content of provided food items, methods for assessing dietary intake and power calculations is provided in the Supplementary material.

The acute study was of single-blinded, crossover design. 13 healthy male volunteers fasted overnight before giving blood then ingesting either a low PAMP (control) or high PAMP onion-based breakfast on separate occasions with at least two weeks washout between visits. Subjects provided a postprandial blood sample at 3 h and a second fasted sample at 24 h. The low and high PAMP meals differed only in the PAMP content of the onion used to prepare each meal, and were otherwise nutritionally identical. Detailed methods for test meal preparation and composition, biochemical measurements and nutritional analyses are provided in the Supplementary material section.

Statistics

For the chronic study, responses were compared using linear mixed models with Sidak's post-test. For the acute study, linear mixed models were used with meal and time as within subject factors. Associations between cytokine production and TLR-stimulant contents of foods were tested by Spearman correlation. Data were analysed using Graphpad Prism 6 and IBM SPSS 22, and are presented as mean ± SE unless otherwise indicated. Statistical significance was assumed at $P < 0.05$.

Results

PAMP content of test meals and diets

To identify foods at high risk of inducing inflammatory signalling for use in the study, a panel of 23 potential test