Accumulation of Stimulants of Toll-Like Receptor (TLR)-2 and TLR4 in Meat Products Stored at 5 °C

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Abstract: Recent evidence suggests that exposure to stimulants of the innate immune receptors Toll-like receptor (TLR)-2 and TLR4 may contribute to the development of atherosclerosis and insulin resistance. We showed recently that common foods can contain TLR-stimulants, and that the greatest concentrations were present in meat-based products. Using a recently developed quantitative bioassay, we here examined the kinetics of accumulation of TLR2- and TLR4-stimulants in a variety of meat products held at 5 °C in air or under a modified atmosphere for up to 8 d. Meat content of TLR-stimulants increased with time in each meat examined and was paralleled by growth of pseudomonads and Enterobacteriaceae, suggesting that bacterial lipopeptides and lipopolysaccharides are the likely sources of TLR2- and TLR4-stimulants, respectively. TLR-stimulants reached the highest levels (approximately 80 μg lipopeptide-equivalents per gramme and approximately 7 μg lipopolysaccharide-equivalents per gram) in meat that was minced rather than intact, and when stored in air rather than under a modified atmosphere. TLR2- and TLR4-stimulants in meat products cooked for 1 h retained approximately 20% and approximately 40% of their bioactivity, respectively. In summary, storage conditions and microbial flora critically regulate the kinetics of TLR2- and TLR4-stimulant accumulation in meat products and these may retain biological activity after cooking.

Keywords: bacteria, inflammation, lipopeptide, lipopolysaccharide, meat, Toll-like receptors

Practical Application: The novel assays presented in this work could be used to predict the potential of foods to promote inflammatory signaling in human subjects, which may be deleterious to health. These assays may also be used to monitor the historical microbial flora in food products after cooking or other forms of food processing may have rendered the original microflora nonviable.

Introduction

Chronic inflammatory processes underpin the development of insulin resistance and atherosclerosis (Ross 1999; Xu and others 2003). In particular, activation of the innate immune system via stimulation of Toll-like receptor (TLR)-2 and TLR4 has been shown to play a central role in the progression of these diseases in animal models (Michelson and others 2004; Mullick and others 2005; Tsukumo and others 2007). Experimental administration of the established ligands of TLR2 or TLR4, namely, bacterial lipopeptide (BLP) or lipopolysaccharide (LPS, endotoxin), was shown to result in a marked increase in disease burden in mouse models of atherosclerosis (Michelson and others 2005; Westerterp and others 2007; Madan and Amar 2008), and administration of LPS to mice or human subjects led to a marked reduction in insulin sensitivity (Cani and others 2007; Mehta and others 2010). Accordingly, mice genetically deficient in TLR2 or TLR4 are resistant to the development of diet-induced insulin resistance (Tsukumo and others 2007; Himes and Smith 2009; Ebnes and others 2010) and atherosclerosis (Michelson and others 2004; Mullick and others 2005; Madan and Amar 2008).

It has been proposed that dietary saturated fatty acids (SFAs) are the agents most likely to be responsible for stimulating TLR2- and TLR4-dependent signaling in these models (Lee and others 2001). However, we showed recently that SFAs are unable to directly stimulate TLR-signaling (Erridge and Samani 2009). Instead, we have proposed that systemic exposure to the established ligands of TLR2 and TLR4, namely, BLP and LPS, may be more likely to promote these diseases (Erridge 2009). In support of this notion, it has been shown that circulating levels of LPS are associated with both type 2 diabetes (Creely and others 2007; Al-Attas and others 2009; Dasu and others 2010) and atherosclerosis (Pussinen and others 2007; Wiedermann and others 1999) in human subjects. We and others also showed recently that fatty meals promote the translocation of endotoxin from the intestine into the circulation in human volunteers (Erridge and others 2007; Laugerette and others 2011), and oral administration of LPS was reported to result in systemic cytokine release and inflammation in mice (Youngher 1972; Yoshino and others 1999; Yoshino and others 2005).

We previously assumed that the majority of circulating TLR2- and TLR4-stimulants were likely to derive from the microflora of the large intestine (Erridge and others 2010). However, since the small intestine has recently been shown to be the principle site of intestinal absorption of endotoxins into the circulation (Ghoshal and others 2009), and since the small intestine contains only a limited Gram-negative bacterial flora (Berg 1996), we recently investigated the possibility that foods may contain TLR2- and
TLR4-stimulants. These studies revealed that meat products contained the highest levels of TLR2- and TLR4-stimulants among a wide variety of common foodstuffs (Erridge 2010).

However, as meat content of TLR-stimulants was quite variable in the previous study, we here aimed to examine the factors that may lead to the accumulation of TLR2- and TLR4-stimulants in stored meats. In particular, we sought to define the kinetics of accumulation of TLR-stimulants in meat products held at 5 °C under a variety of storage conditions that may occur under typical domestic circumstances, and to investigate the potential role of microbial growth in these processes.

Materials and Methods
Meats and storage conditions
Fresh beef, pork, and turkey minced meats and intact steaks or breast, all packaged under a modified atmosphere, were purchased from local supermarkets 6 d before the advertised “use by” dates. Samples were stored promptly in a refrigerator at 5 °C. For measurement of TLR-stimulant accumulation under a typical commercial modified atmosphere, individual packs of each meat were opened from their original packaging at 2-d intervals up to 8 d. Alternatively, 25 g portions of each mince, steak or breast meat were removed from their protective packaging on the day of purchase, and transferred to individual sterile plastic bags, open on one end to permit access to the air and stored at 5 °C.

Preparation of meat extracts
A total of 25 g of each meat product was homogenized in 10-volumes of phosphate-buffered saline (PBS) using a domestic blender (HR2000, Phillips, Eindhoven, The Netherlands) until homogeneity was achieved (approximately 1 min). An aliquot of each homogenate was set aside immediately for microbiological analysis, and the remainder was centrifuged (400 g for 5 min) to pellet insoluble material. Clarified supernatants of meat homogenates were then filter-sterilized (0.22 μm, Acrodisc, Pall, Portsmouth, U.K.) to yield a sterile extract of each meat product that was stored at ~20 °C prior to batch analysis for quantification of soluble TLR-stimulants.

Microbiological analyses
Total aerobic mesophiles (aerobic plate counts, APC), pseudomonads, and Enterobacteriaceae were quantified in meat extracts according to previously described protocols (Fallowfield and Patterson 1985; Lambropoulou and others 1996). Briefly, serial 10-fold dilutions of each meat homogenate were prepared in sterile PBS and plated onto plate count agar (APC, for determination of total aerobic mesophile plate count), violet red bile glucose agar (VRBGA, for enumeration of Enterobacteriaceae), or pseudomonas agar with cetrimide-fucidin-cephaloridine supplement (Oxoid), selective for pseudomonads. Plates were incubated at 30 °C for 24 h and colony forming units (CFU)/g were calculated for each meat extract.

Quantification of TLR-stimulants in meat extracts
The relative biological activities of TLR-stimulants present in meat extracts were measured using a recently developed quantitative bioassay (Erridge 2010; Erridge and others 2010), which employs the measurement of NF-κB-dependent reporter activation in TLR-deficient HEK-293 cells transfected with either TLR2 or TLR4/MD2. Briefly, HEK-293 cells maintained in Dulbecco’s modified eagle medium (DMEM, Sigma) supplemented with 10% foetal calf serum (FCS) and 2.5 μg/mL Plasmocin (Invivogen, Toulouse, France), were plated in 96-well plates at 2 × 10^4 cells per well and transfected after 24 h using Genejuice (Novagen, San Diego, Calif., U.S.A.). Amounts of construct per well were 30 ng of human TLR2, TLR4 (co-expressing MD-2) (Invivogen), 30 ng of CD14, and 10 ng of firefly luciferase-reporter construct driven by the NF-κB-dependent E-selectin promoter (pELAM). Three days after transfection, cells were challenged in triplicate with appropriate dilutions of sterile-filtered supernatants of each meat extract (typically 1:100 in DMEM/1%
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FCS). On the same plate, an 8-point standard curve (from 100 ng/mL to 0.03 ng/mL) was prepared using either the synthetic BLP Pam3CSK4, or Escherichia coli R1 LPS, as calibrating standards for determination of the relative biological activity of TLR2- and TLR4-stimulants in each test sample, respectively.

After 18 h, NF-κB-dependent reporter expression was measured using Dual-Glo reagent (Promega, Madison, Wis., U.S.A.) and fold-induction was calculated relative to cells cultured in medium alone. Log-transformed concentrations of TLR-stimulant standards were then plotted against fold-induction of NF-κB reporter to generate a standard curve that was used to estimate the relative biological activities of TLR2- and TLR4-stimulants present in each food sample relative to Pam3CSK4 or LPS standards, as described previously (Erridge 2010; Erridge and others 2010). The abundance of TLR-stimulants present in each extract is therefore presented as a relative biological activity with respect to Pam3CSK4 or LPS. For example, 200 ng/g BLP-equivalent means that each gramme of product contains TLR2-stimulants with a capacity to stimulate TLR2-signaling equal to that of 200 ng Pam3CSK4. TLR-stimulant standards did not induce signaling in cells expressing heterologous TLRs, or in cells transfected with CD14 alone (Erridge and others 2008; and data not shown). The mean intraassay coefficient of variance was 18% for TLR2-stimulant measurements, and 23% for TLR4-stimulant measurements in this study.

Measurement of TLR-stimulants in cultured bacterial isolates

Bacterial cultures were grown from 8 colonies picked at random from VRBGA and pseudomonas-selective agar plates (4 from each) on which diluted extracts of minced meats held at 5 °C for 8 d had been grown. The panel of 8 isolates were grown in luria broth (LB) at 5 °C for 10 d or at 37 °C for 18 h. Cultures were then centrifuged and bacterial cell pellets were washed twice in PBS before resuspension to an absorbance of 1.0 at 600 nm (equivalent to approximately 10^9 bacteria/mL). Resuspended bacteria were then heat-killed by treatment at 100 °C for 10 min. HEK-293 cells transfected with TLR2 or TLR4/MD2 and NF-κB reporter construct as described above were then challenged in triplicate with each heat-killed isolate (10^6 bacteria/mL in tissue culture medium) heat-killed E. coli R1 (NCTC 13114), 10 ng/mL E. coli R1 LPS or 10 ng/mL Pam3CSK4. After 18 h, fold-induction of NF-κB reporter was calculated relative to cells cultured in medium alone as described above.

Meat cooking protocol

To determine if TLR2- or TLR4-stimulants in meat products retain biological activity after cooking, 400 g of turkey mince held at 5 °C under a modified atmosphere for 8 d was browned in a hot pan with 10-mL sunflower oil and then simmered over a medium heat for up to 1 h, with small amounts of pyrogen-free water added as necessary to prevent overdrying. A total of six 25 g samples were taken at 0, 5, 10, 20, 30 and 60 min after cooking for determination of TLR2- and TLR4-stimulant biological activity as described above. Alternatively, 100 g patties were prepared without binder and cooked in a domestic clam-shell style grill for 8 min, and 25 g was then assayed for remaining biological activity of TLR2- and TLR4-stimulants.

Statistical analysis

Differences between atmosphere and air-treated, or minced and unminced, meat content of plate counts (log_{10}-transformed), or stimulants of TLR2/TLR4 were compared using the Student’s t-test. The capacities of cultured bacterial isolates grown at 5 and 37 °C to stimulate TLR2- and TLR4-dependent signaling were compared by analysis of variance (ANOVA) using Tukey’s posttest. Recovery of TLR-stimulant biological activity from cooked and uncooked meat was compared by ANOVA using Dunnett’s posttest.

Results

Accumulation of TLR-stimulants in intact meat products stored at 5 °C

Levels of TLR2- or TLR4-stimulants were below 100 ng/g in beef, pork, or turkey steak or breast, each bought packaged under a modified atmosphere, on the day of purchase (that is, day 0). Exposure to air led to a time-dependent accumulation of TLR2- and
TLR4-stimulants in each of the meats. By day 8 (that is, 2 d after
the advertised “use-by” date), the mean peak of TLR2-stimulants
among the 3 types of meat reached 7450 ng/g BLP-equivalents,
and mean TLR4-stimulants reached 1290 ng/g LPS-equivalents
(Figure 1A and B). By contrast, mean TLR2- and TLR4-stimulant
content in meat steaks held under a modified atmosphere for 8 d
reached 330 ng/g BLP-equivalents and 198 ng/g LPS-equivalents,
respectively (Figure 1C and D). Exposure to air thus led to an
approximately 22-fold increase in accumulation of TLR2-stimulants
and an approximately 6.5-fold increase in TLR4-stimulants, rela-
tive to meat steaks held under a modified atmosphere for the same
amount of time (P = 0.024 and P = 0.094, respectively).

Mean APC in intact meats were low at purchase (4.5 log
CFU/g), but increased to 7.1 log CFU/g after 8 d storage under
a modified atmosphere, while pseudomonad and Enterobacteriaceae counts also rose to 6.5 and 6.8 log CFU/g, respectively
(Figure 2). Mean APC reached 10.2 log CFU/g in these meats
exposed to air for 8 d, at which time mean pseudomonad and
enterobacterial counts were 10.7 and 10.9 log CFU/g, respectively.
As expected, storage under a modified atmosphere was therefore
associated with a mean reduction in pseudomonad growth of
approximately 4.2 logs, enterobacterial growth of approximately 4.1
logs, and aerobic mesophile growth of approximately 3.1 logs (all
P < 0.001).

Accumulation of TLR-stimulants in minced meat products
stored at 5°C

We next aimed to determine if the kinetics of TLR-stimulant
accumulation may be different in minced meat products. In each
of the minced meats held under a modified atmosphere, TLR2
and TLR4-stimulant concentrations generally increased with time
to reach a mean peak of 1700 ng/g BLP-equivalents and 420 ng/g
LPS-equivalents by day 8 (Figure 3).

In minced meats exposed to air, TLR2- and TLR4-stimulant
concentrations also increased with time, reaching a mean of 78500
ng/g BLP-equivalents, and 8360 ng/g LPS-equivalents by day 8
(Figure 3). Exposure to air thus led to an approximately 46-fold
increase in mean BLP-equivalents and an approximately 20-fold
increase in LPS-equivalents, relative to minced meats held under
a modified atmosphere for 8 d (P = 0.012 and P < 0.001, respectiv-
ely). Levels of TLR2-stimulants were therefore approximately
5-fold higher in minced meats stored under a modified atmo-
sphere for 8 d than those found in intact meats held under the
same conditions for the same length of time (P = 0.023). TLR4-
stimulants were approximately 2-fold more abundant in minced
meats than in intact meats held under a modified atmosphere for
8 d, although this difference did not reach significance (P = 0.129).

Microbiological analysis revealed that mean APC were higher
in the minced meats (6.3 log CFU/g) than the steaks at purchase
(P < 0.01). In minced meats stored under a modified atmosphere,
mean plate counts increased in each product, but plateaued to reach
a mean of 7.2 log CFU/g for pseudomonads, 7.4 log CFU/g for
Enterobacteriaceae, and 8.4 log CFU/g for APC (Figure 4). Mean
bacterial counts in minced meats exposed to air were significantly
higher than those stored under a modified atmosphere by day 8,
reaching 11.2 log CFU/g for pseudomonads, 10.3 log CFU/g for
Enterobacteriaceae, and 11.3 log CFU/g for APC (all P < 0.05).

Correlation of TLR-stimulant accumulation with microbial
growth

We next examined whether meat content of TLR-stimulants
correlated with microbial growth. When data points for all
measurements made for meat products were compared, log-
transformed TLR2-stimulants correlated with log-transformed
APC (r = 0.897), pseudomonads (r = 0.885), and Enterobacteriaceae (r = 0.864), all P < 0.0001. Log-transformed
TLR4-stimulants also correlated well with log-transformed APC
(r = 0.877), pseudomonads (r = 0.878), and Enterobacteriaceae
(r = 0.862), all P < 0.0001.

TLR-stimulating capacity of cultured meat-derived bacteria

As it was shown recently that several species of Gram-
negative bacteria can express LPS with either a TLR4-stimulating
or TLR4-antagonist type lipid-A in a manner dependent on
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culture conditions or growth temperature (Hajjar and others 2003; Robinson and others 2008), we next examined the effect of growth temperature on the capacity of bacterial isolates derived from meat cultures to stimulate human TLR2 or TLR4. Four enterobacterial strains and 4 pseudomonads cultured from minced meats held under a modified atmosphere for 8 d were cultured in LB at 5 or 37 °C. Growth temperature had no significant effect on stimulation of TLR2 by each bacterial isolate (Figure 5A). Growth temperature also had little impact on stimulation of TLR4 by most bacterial isolates, although isolates E1 and P4 demonstrated significantly reduced capacity to stimulate TLR4-signaling when grown at 5 °C compared to the same isolate grown at 37 °C (both P < 0.001, Figure 5B).

Effect of cooking on biological activity of TLR-stimulants in minced meat

Turkey mince, which had been stored for 8 d under a modified atmosphere, was cooked over a medium heat with oil in a pan, or as a burger in a clam-shell grill, in a manner intended to reflect that likely to occur in a typical domestic setting. Cooking for between 5 min (after which time the meat was completely browned) and 60 min led to an approximately 80% reduction in TLR2-stimulant biological activity, which did not diminish further with time (P < 0.01). Likewise, cooking for between 5 and 60 min led to an approximately 60% reduction in recoverable biological activity of TLR4-stimulants in the minced meat (P < 0.01).

Discussion

Murine models of diet-induced insulin resistance and atherosclerosis have revealed that the stimulation of innate-immune signaling via TLR2 and TLR4 plays a key role in the development of these diseases (Michelson and others 2004; Mullick and others 2005; Tsukumo and others 2007; Madan and Amar 2008; Himes and Smith 2009; Ehres and others 2010). However, the source of the agents responsible for stimulating TLR-signaling in these conditions remains to be identified. We showed recently that a number of foodstuffs common to the Western diet can contain stimulants of TLR2 and TLR4, and that the highest levels of these stimulants were present in meat products (Erridge 2010). As TLR-stimulant content was found to vary between similar meat products in the previous study, the aim of the present study was to investigate the factors that may lead to the accumulation of TLR2- and TLR4-stimulants in meat products.

Soluble stimulants of TLR2 and TLR4 were found to accumulate in a time-dependent manner in all meat products examined when stored at 5 °C (Figure 1 and 3). The accumulation of TLR2- and TLR4-stimulants was minimized by storing meats in the intact, rather than in the minced form, and by storage under a modified atmosphere, rather than exposed to air. Stimulants of both TLRs correlated well with bacterial counts, which were very similar to those reported in previous studies of microbial growth in minced meat products stored in air or under a modified atmosphere (Fallowfield and Patterson 1985; Lambropoulou and others 1996; Jay and others 2003). This information, taken together with the results from our previous studies that showed that meat-derived TLR-stimulants are not likely to be SFAs (Erridge and Samani 2009) and are inhibited by both polymyxin-B and oxidized palmitoyl-arachidonyl-phosphatidyl choline (Erridge 2010), strongly suggest that the TLR2- and TLR4-stimulants identified in meats are of bacterial origin, being presumably BLPs and LPS, respectively. It is well established that pseudomonads and Enterobacteriaceae are among the major contributors to the microflora of spoiling meat (Borch and others 1996; Lambropoulou and others 1996; Gram and others 2002). Since both enterobacterial and pseudomonas strains isolated from meats in the present study stimulated TLR4-dependent signaling when grown at typical refrigeration temperatures (Figure 5), it is likely that both pseudomonads and Enterobacteriaceae contribute to the accumulation of TLR2- and TLR4-stimulants in stored meats.

An alternative method that has been widely used for the quantification of LPS in foodstuffs is the limulus amoebocyte lysate (LAL) assay (Jay and others 1979; Fallowfield and Patterson 1985). As discussed previously, however (Erridge 2010), the LAL assay was found to be not suitable for quantifying the biological activities of TLR-stimulants in foodstuffs for a number of reasons. First, endotoxins, which are antagonists of human TLR4, or which are

Figure 4–Microbial profile of minced meats stored at 5 °C. Total aerobic mesophiles (A), pseudomonads (B), and Enterobacteriaceae (C) were enumerated in beef, pork, and turkey minced meat stored under modified atmosphere packaging (MAP), or exposed to air, for up to 8 d at 5 °C. Results are expressed as log10 CFU/g.
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otherwise of low biological activity with respect to human TLR-4, can be potent stimulants of the LAL assay (Rossignol and others 2006), thereby potentially leading to the overestimation of the inflammatory potential of foodstuffs. Second, common food constituents such as glucans can promote a false-positive reaction in the assay (Elin and Wolff 1973). Finally, the limulus system is insensitive to BLPs, and so cannot be used for their quantification (Erridge and Samani 2009). Despite these limitations, however, the LAL assay has been used previously to demonstrate that the endotoxin content of spoiling meat correlates well with microbial load, a finding supported by the present study (Fallowfield and Patterson 1985; Jay and others 1979). The LAL assay has also been used to estimate the amount of endotoxin present in minced beef stored at refrigeration temperature for 6 d to range from approximately 32 μg/g to approximately 2.5 mg/g (Jay and others 1979; Fallowfield and Patterson 1985). The present findings suggest that TLR4-stimulants (presumably LPS) in minced meats reached a maximum of approximately 860 ng/g under a modified atmosphere and 7 μg/g when exposed to air for 8 d. For the reasons outlined above, it is likely that previous reports may have significantly overestimated the level of TLR4-stimulating LPS present in meat products.

The finding that typical cooking times and temperatures reduced the recoverable biological activity of TLR2- and TLR4-stimulants in minced meat by approximately 80% and approximately 60%, respectively (Figure 6), was surprising, as this

Figure 5–TLR-stimulating capacity of meat-derived bacterial isolates. Four enterobacterial species and 4 pseudomonas species were isolated from minced meats held at 5 °C for 8 d and cultured subsequently in LB at 4 °C or 37 °C. The capacity of each heat-killed isolate to stimulate TLR2- or TLR4-dependent signalling was measured in HEK-293 cells transfected with human TLR2 or TLR4 with CD14 and NF-κB-sensitive reporter (pELAM). Also measured were responses to heat-killed E. coli, 10 ng/mL E. coli LPS, or 10 ng/mL Pam3CSK4 (Pam3). Results are presented as mean fold-induction of reporter relative to cells cultured in medium alone (Ctrl) ±SD of triplicate cultures and are representative of 3 similar experiments.

Figure 6–Effect of cooking on recovery of TLR-stimulants from minced meat. 400 g of turkey mince stored at 5 °C under a modified atmosphere for 8 d was browned in a pan with 10-mL hot sunflower oil and then simmered over medium heat for up to 1 h. A total of six 25 g samples were taken at 0, 5, 10, 20, 30, and 60 min after cooking for determination of TLR2- and TLR4-stimulant biological activity. Alternatively, 100 g patties were prepared and cooked in a domestic clam-shell style grill for 8 min, and 25 g was then assayed for remaining biological activity of TLR2- and TLR4-stimulants. Results are expressed as mean biological activities relative to Pam3CSK4 (TLR2) or E. coli LPS (TLR4) ±SD of triplicate cultures and are representative of 3 similar experiments. ** P < 0.01 compared with uncooked meat.
contrasts with previous findings that boiling LPS or lipopeptide in saline alone for up to 1 h resulted in a limited or no reduction in the biological activity of these agents (Erridge 2010). In light of these observations, and the fact that no further reduction in biological activity was observed between 5 min and 1 h of cooking, it seems likely that the cooking process results in reduced efficiency of recovery of TLR2- and TLR4-stimulants during the extraction process, rather than significantly degrading the biological activity of the molecules themselves.

Further studies using in vivo models will be required to determine if the presence of TLR2- and TLR4-stimulants in foodstuffs is of any relevance to the progression of TLR-dependent diseases such as atherosclerosis and insulin resistance. Supportive of this notion are the demonstrations that the ingestion of LPS via the oral route results in systemic cytokine release and inflammation in mice (Youngner 1972; Yoshino and others 1999; Yoshino and others 2005), and that a proportion of orally ingested LPS translocates into the systemic circulation in a biologically active form (Yoshino and others 1999). Current evidence suggests that only a very small fraction of orally ingested endotoxin is absorbed into the systemic circulation. In mice, for example, only approximately 0.2% of orally ingested radiolabeled endotoxin translocates into the circulation by 90 min postgavage (Ghoshal and others 2009). This is consistent with the finding that the oral dose of LPS required to promote systemic inflammation is approximately 1000-fold higher than that required by intraperitoneal injection of the same reagent (Youngner 1972; Yoshino and others 1999; Yoshino and others 2005). In human subjects, bolus intravenous injection of as little as 7 ng LPS (0.1 μg/kg) results in significant increases in circulating inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α (Krogh-Madsen and others 2008), while the present results suggest that an oral challenge of as much as 100 μg LPS could feasibly occur, at least occasionally, among subjects following the Western dietary pattern.

The effects of chronic ingestion of meat products rich in LPS and lipopeptides on human health may also merit further investigation. Interestingly, it was shown recently that the consumption of meats, induce the expression of cyclooxygenase-2 mediated through Toll-like receptor 4. Metabolism Endocr 22:53–9.

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