Metabolic endotoxaemia: is it more than just a gut feeling?

Milan K. Piya[a,b], *Alison L. Harte*[a], *and Philip G. McTernan*[a]

Purpose of review
This article reviews the evidence linking gut bacteria, endotoxin, and its circulating levels with inflammatory induced obesity and metabolic disease (metabolic endotoxaemia).

Recent findings
Gut flora analyses have allowed gut microbiota signatures (GMS) to be observed in animal studies of obesity/metabolic disease. In these studies, specific GMS result in a change in obesity and metabolic disease state whereas in humans, analysis remains unclear. Serum studies, examining metabolic endotoxaemia as a biomarker, appear to link long-term cardiovascular disease and type 2 diabetes mellitus (T2DM) through activation of inflammatory pathways. More recent studies note the importance of diet, which shows the dramatic rise in endotoxin following acute or long-term high-fat diet, with the effects exacerbated in T2DM.

Summary
Gut flora appears to act as an important determinant in the pathogenesis of inflammatory induced obesity/T2DM. Endotoxin may act as the systemic insult, impacted by a high-fat diet, which may regulate this effect, combined with an altered GMS. As such, clinical and dietary intervention to affect this process – on the gut flora, the 'leaky' mucosal membrane and endotoxin coupled lipid absorption or removal of circulating endotoxin – could reduce the progression of inflammatory induced metabolic disease.

Keywords
endotoxin, inflammation, insulin resistance, lipotoxicity, metabolic syndrome

INTRODUCTION

Lipopolysaccharide (LPS), often referred to as endotoxin, represents the fragments from the cell wall of gram-negative bacteria that, in a human context, may normally reside in the gut. Endotoxin has a strong affinity for chylomicrons [lipoproteins that transport dietary lipids including long-chain saturated fatty acids (SFAs) through the gut wall] and, as such, can cross the gastrointestinal mucosa coupled with damaging lipoproteins. These bacteria appear to mediate systemic inflammation linked to metabolic disease risk, a term referred to as metabolic endotoxaemia. In recent years, a 'specific' animal gut flora has increasingly been linked to metabolic risk; this risk being associated with the future development of metabolic dysfunction. Although there are long-established risk factors that contribute to metabolic dysfunction, such as insulin resistance, obesity, raised triglycerides, reduced HDL cholesterol, elevated blood pressure and increased fasting plasma glucose, and other 'primary inflammatory insults' may also be relevant. Within this context, chronic low-grade inflammation has been considered as another factor, intertwined with obesity, gut microbiota and a raised immune response [1,2]. This raised inflammatory response appears, in part, to be mediated by adipose tissue, which responds to the 'inflammatory insults' by secreting proinflammatory cytokines (adipocytokines), a response that is exacerbated by weight gain leading to further metabolic dysfunction [1]. Besides the functional response from adipose tissue to 'inflammatory insults', gut microbiota has also been shown to impact on inflammatory conditions,

[a]Division of Metabolic and Vascular Health, Warwick Medical School, University of Warwick, Clinical Sciences Research Laboratories and [b]Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism, University Hospitals Coventry and Warwickshire NHS Trust, Walsgrave, Coventry, UK

Correspondence to Philip G. McTernan, Associate Professor, Division of Metabolic and Vascular Health, Warwick Medical School, University of Warwick, Clinical Sciences Research Laboratories, University Hospital Coventry and Warwickshire, Coventry, CV2 2DX, UK. Tel: +024 769 68655; e-mail: p.g.mcternan@warwick.ac.uk

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KEY POINTS

- Endotoxin, -the major constituent of the outer cell membrane of gram negative bacteria- crosses the gut mucosal membrane to enter the circulation and directly stimulates inflammatory pathways.
- The gut microbiota is highly variable and changes with age, diet and obesity. Modulation of the gut microbiota with antibiotics, probiotics and prebiotics has been shown to affect metabolic status in animal models, but not in most current human studies.
- Metabolic endotoxaemia has been shown to be associated with a host of conditions including obesity, metabolic syndrome, T2DM, atherosclerosis, cardiovascular disease and nonalcoholic fatty liver disease.
- High-fat meals result in an increase in endotoxin levels in animal and human studies, with a greater increase in metabolic disease states, especially T2DM.

altered by dietary patterns and weight gain in humans [2–4]. As such, gut flora changes and low-grade systemic inflammation may, in part, be linked through gut-derived circulating endotoxin, which has the capacity to act as a stimulator of inflammation and therefore, may represent a natural biomarker of metabolic risk.

ENDOTOXIN AND THE LIPOPOLYSACCHARIDE MOLECULE

The circulating gut-derived bacterial LPS molecule (endotoxin) consists of an active lipid A component (responsible for the endotoxic activity), an oligosaccharide core and a distal O antigen [5]. Endotoxin has been shown to stimulate the innate immunity pathway in human adipose tissue and adipocytes by stimulating Toll-like receptors (TLRs), mainly TLR4 [6,7], although the nature of the TLR homo-dimerization and hetero-dimerization coupled with their regulation is a review in its own right. In simple terms, stimulation of TLR4, through a series of interactions with several proteins, including the LPS binding protein (LBP), CD14 and myeloid differential protein 2 (MD-2), leads to intracellular activation of nuclear factor kappa B (NF-κB), a key transcription factor in the inflammatory cascade that regulates the transcription of numerous pro-inflammatory adipocytokines [6,8], as shown in Figure 1 [9].

GUT BACTERIA, OBESITY AND METABOLIC DISEASE

The human gut microbiota forms the major component of the approximately 1000 species of bacteria, mostly anaerobic, with approximately 10^{12} bacterial cells per gram of faeces [10]. In spite of individual variation of human microbiota, distinct gut microbiota signatures (GMS) have been linked with inflammatory, obese and metabolic conditions [3,11–13]. These distinct GMS have also been noted in rodent studies – in C57/Bl6 male mice with and without diet-induced obesity – despite the same genetic profile and intake of the same high-fat diet [14]. Furthermore, rodent studies have shown that adiposity, *per se*, as well as diet composition impacts on the GMS [15], which again appear to occur in humans.

A strong link between long-term diet and the GMS has been recently demonstrated in a study that performed detailed studies of the faecal samples of

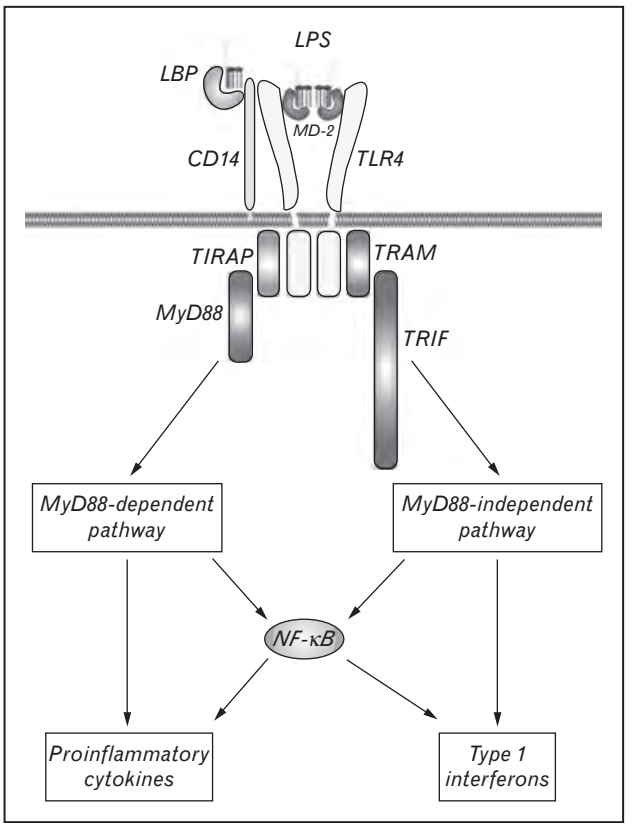

FIGURE 1. Overview of lipopolysaccharide (LPS)/Toll-like receptor 4 (TLR4) signalling pathway. Endotoxin or LPS induces inflammation via the TLR4 pathway by interacting with the LPS binding protein (LBP), myeloid differentiation 2 (MD-2) protein as well as the CD14 protein. Adapter molecules Toll-interleukin 1 receptor adapter protein (TIRAP)), TRIF (and TRIF related adapter molecule (TRAM) are involved in the intracellular activation of nuclear factor kappa B (NF-κB), a key transcription factor in the inflammatory cascade. MyD88, Myeloid Differentiation 88. Reproduced with permission from Lu *et al.* [9].

98 individuals [16▪], whereas Fava *et al.* [17] demonstrated that dietary components were important in GMS, as both high-carbohydrate as well as high-fat diets altered the gut flora. However, despite the apparent importance of obesity to alter GMS, further studies have shown that, in BMI and age-matched individuals with and without insulin resistance, the gut flora – as assessed from their appendix – still appears to be distinct [18]. These findings are affirmed by a Finnish study, which examine women with obesity and metabolic disorders. This study also compared gut flora from lean women and the aforementioned groups, which suggested that the metabolic disorder was the primary key factor for the GMS rather than the obesity [19].

Although previous literature supports the concept that within the first few weeks of neonatal life our gut flora is defined and maintained, leaving to one side the role of infection and antibiotics, adulthood clearly influences GMS composition. The studies examining Egyptian adults and children with and without obesity [20], as well as Swedish preschool children with and without obesity, noted distinct GMS [21]. Therefore at an early age, depending on our metabolic state, our own GMS may be altered. Although our body may try to revert to the GMS defined in early neonatal life, diet or an insulin-resistant state may prevent this. This notion would appear to be supported by studies conducted by Claesson *et al.* [4], who demonstrated a clear difference in the gut flora of community dwelling individuals compared with those in long-term residential care, identifying a link between GMS, age, diet and health status in the elderly. Understanding the rationale for distinct changes in gut microbiota, particularly due to obesity and metabolic disease, has led researchers to propose different mechanisms. These include increased intestinal permeability – resulting in increased circulating endotoxin, the conversion of dietary fibre to short-chain fatty acids – providing extra energy, altered gut hormone production as well as activation of the endocannabinoid system, to name just a few [11,22].

EFFECTS OF ALTERATION OF GUT BACTERIA

However, there is growing evidence in rodent models that alteration of the gut microbiota can affect obesity, adiposity and metabolic disease. To examine this further, Blaut and Klaus [22] transplanted faeces from an obese mouse to nonobese germ-free mice, this resulted in the recipient mice developing features of the metabolic syndrome similar to the donor mice. This study provides further support for the impact of an altered gut microbiota as a key determinant in the development of obesity and metabolic disease, independent of feeding in the germ-free mouse model. In addition, surgical intervention on the gut may also impact on gut flora with health benefits, again without weight change. Studies in rats given a Roux-en-Y gastric bypass (RYGB) showed profound changes in gut microbiota compared with Sham-operated rats, which paralleled with a sudden improvement in insulin resistance following surgery, prior to weight loss [23]. This sudden benefit could suggest that gut microbiota has the capacity to improve insulin sensitivity. However such an improvement could be independent of the gut microbiota, and due to postoperative changes in the physical stresses and/or absorption of nutrients, as well as hormone secretion/regulation changes, coupled with previous data. Although surgical intervention appears to improve metabolic function, more subtle measures affecting the GMS may also be important. Studies in diet-induced obesity in mice and pigs given Lactobacillus supplements appeared to alter GMS in both animals [24,25]. Additional investigation into the systemic effect of Lactobacillus casei strain Shirota supplement, in obese mice, noted a reduction in circulating endotoxin with concomitant improvements in glucose homeostasis, yet intra-abdominal adipose tissue remained unchanged [26]. This, as such, highlights that adipose tissue may merely act in response to the primary inflammatory insult, a response being aggravated by excess weight gain. Therefore, a method to reduce circulating endotoxin may lower the obesity-induced inflammatory response and attenuate both T2DM and cardiovascular disease (CVD) risk. To explore this concept, Carvalho *et al.* [27] tried to reduce circulating endotoxin levels in high-fat-fed mice with the use of antibiotics; unsurprisingly, this led to an altered GMS – concurrently resulting in both a reduction in circulating endotoxin levels and inflammatory signalling. Further treatment of mice with different antibiotics, such as Vancomycin or Bacteriocin-producing Lactobacillus, demonstrated an altered gut microbiota in both treatments but only the Vancomycin-treated group showed improvements in metabolic factors related to obesity [28]. Further use of antibiotic treatment in high-fat-fed, as well as *ob/ob,* mice also noted a GMS change coupled with reduction in endotoxin faecal content, as well as a reduction in glucose intolerance, obesity and inflammation, over time [29]. Although the use of antibiotics is not a treatment option for the general population, for several reasons, it does suggest the importance of specific GMS to improve metabolic function and reduce systemic inflammation. This highlights, in a rudimentary manner, that changes

in the GMS in at risk groups can improve metabolic function, independent of surgical intervention, which may continue to promote the importance of the gut microbiota in disease risk groups. However, subtle dietary changes via either a prebiotic-enriched diet or use of a photochemical tetrahydro iso-α acid derived from hops also appear to improve metabolic function in *ob/ob* and *db/db* mice, including a reduction in metabolic endotoxaemia [30,31]. However the GMS derived in mice may be far easier to control and manipulate than in humans.

Although the human gut microbiota may be more challenging to configure for beneficial effects, the importance of a distinct human GMS in obesity and metabolic disease is evident. The capacity to alter the GMS, as defined in conditions of obesity and metabolic disease, however, still remains unclear. A recent randomized placebo-control trial, conducted in adults with visceral obesity given a probiotic, Lactobacillus gasseri, for 12 weeks, revealed a reduction in abdominal adiposity and body weight [32]. In contrast, individuals with metabolic disease given a probiotic Lactobacillus casei Shirota for 12 weeks were not observed to change their inflammatory status or gut permeability [33▪]. These findings were further affirmed by an obese adolescent randomized double blind trial, in which individuals were also treated for 12 weeks with another probiotic, Lactobacillus salivarius, and no observed changes were noted in their inflammatory status, glucose or lipid profiles [34▪]. Although this may suggest the Lactobacillus supplements have an impact in mice but not man, the subtle variations in doses, clinical background of patients utilized, as well as bacterial sub-species used as the probiotic, may have a significant impact on the human microbiota. As such, improved techniques, such as next generation sequencing, have made the analysis of gut microbiota much quicker and cheaper, and there is now a need for larger scale clinical trials examining GMS to a greater degree and for longer periods of time.

METABOLIC ENDOTOXAEMIA, OBESITY AND METABOLIC DISEASE

Metabolic endotoxaemia has been associated with conditions of obesity, T2DM, CVD [6,35,36] and atherosclerosis [37]. Figure 2 depicts an overview of the impact of systemic endotoxin derived from the gut. Most of these studies from individuals with insulin resistance have consistently shown circulating endotoxin levels to positively correlate with waist circumference, waist–hip ratio, insulin levels, inflammatory cytokines and lipids, including total cholesterol, triglycerides, LDL cholesterol and negatively correlate with HDL cholesterol. As an altered GMS in metabolic disease appears aligned with an insulin-resistance status, independent of diet, or an obese state in some contexts, other insulin-resistance states should also affect GMS, circulating endotoxin levels and inflammation. Although not all studies have examined all three elements, increasing evidence appears to support the systemic effects of gut-derived endotoxin and its impact in an insulin-resistance state. Specifically, a recent study in pregnancy, which itself leads to a physiological state of increased insulin resistance, has shown that there was a doubling of circulating endotoxin levels in obese women compared with lean women, which was concurrent with increased systemic and adipose tissue inflammation in the former [38]. In nonalcoholic fatty liver disease (NAFLD), which represents another insulin-resistance state, high-circulating endotoxin levels were observed even at early stage, biopsy established, liver fibrosis, which was similar across the cohort irrespective of T2DM status [39]. In individuals with chronic alcoholic liver disease (ALD), elevated circulating endotoxin was noted to correlate with alterations in the colonic microbiome, with systemic endotoxaemia considered as the secondary insult on the liver function, postalcohol abuse [40]. Therefore, a compromized liver in individuals with NAFLD may also be impacted by metabolic endotoxaemia, as demonstrated in mouse models [41]. Under normal physiological conditions the liver is essential to the removal of endotoxin so a compromized liver would appear to exacerbate metabolic endotoxaemia. As such, these findings help to explain the higher incidence of nonalcoholic, and possibly alcoholic steatohepatitis, in obesity and metabolic disease states.

Studies have shown that dietary energy intake, especially fat intake, and surgical intervention may influence the GMS in mice, whereas the downstream effects of endotoxin on inflammation may also be observed in a human context [41,42]. Surgical intervention in subjects undergoing RYGB surgery were noted to reduce circulating endotoxin levels by 20%, 6 months postsurgery, accompanied by a reduction in insulin resistance, inflammation and weight loss. Again the role of endotoxin on the reduction of inflammation may correlate with pro-inflammatory markers but this does not necessarily define the primary cause for the noted effect on human metabolism, although collated evidence to date would certainly make a strong case. However, a recent study has tried to directly examine the cause and effect relationship between endotoxin and inflammation. In this study, Shah *et al.* [43] infused a bolus of 3 ng/kg body weight endotoxin into

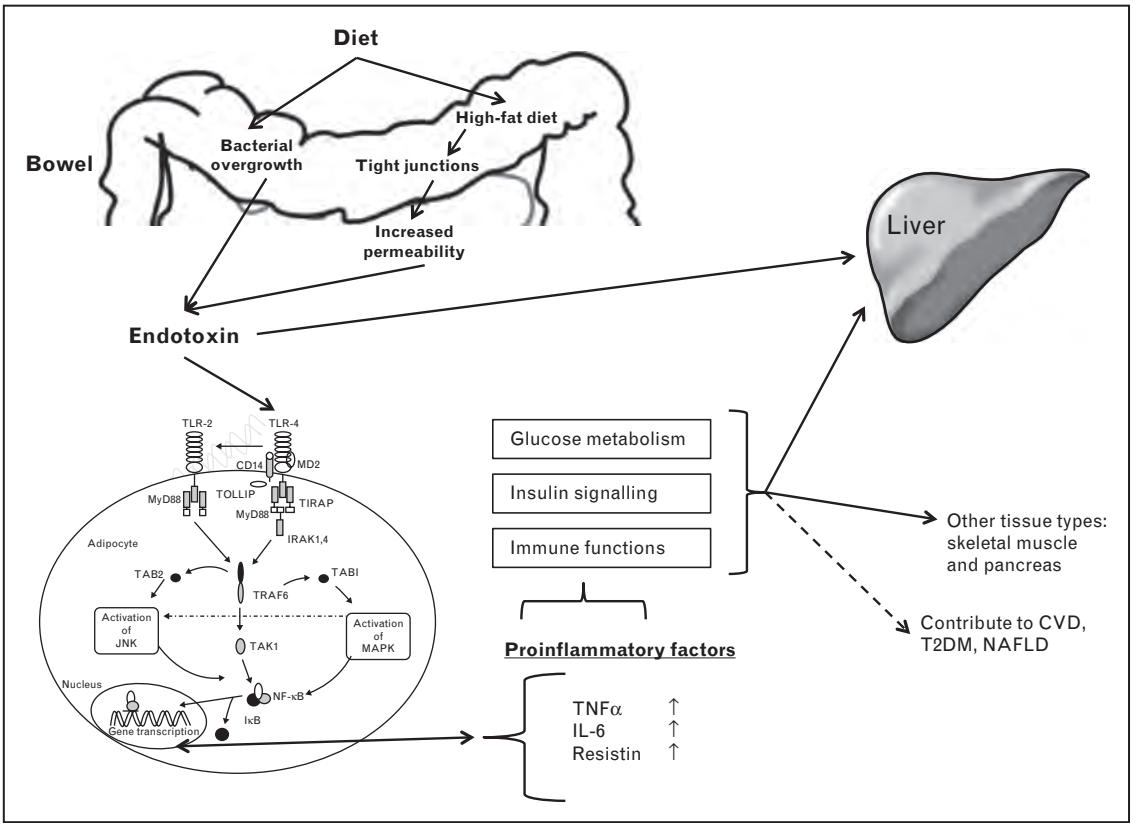

FIGURE 2. An overview of the potential impact of systemic endotoxin derived from the gut. Endotoxin enters the systemic circulation from the gut and activates the Toll-like receptor 4 (TLR4) pathway in adipocytes, as described in Figure 1, which results in the release of proinflammatory cytokines. These cytokines affect glucose metabolism, insulin signalling and immune function which, in turn, affect the liver, as well as other tissue types including skeletal muscle and the pancreas. These effects potentially increase the risk of cardiovascular disease (CVD), type 2 diabetes (T2DM) and nonalcoholic fatty liver disease (NAFLD). Therefore, endotoxin indirectly produces adverse effects within the body whilst simultaneously systemic endotoxin acts directly on the liver. CD14, cluster of differentiation 14; IkB, inhibitor of kappa B; IRAK, interleukin-1 receptor-associated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinases; MD2, extracellular adaptor protein; MYD88, myeloid differentiation primary response gene (88); NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TAB, TAK1-binding proteins; TAK, TGF-β activated kinase; TIRAP, toll-interleukin 1 receptor domain containing adaptor protein; TOLLIP, Toll interacting protein; TRAF6, TNF receptor associated factor 6.

healthy young individuals and biopsied the adipose tissue response over time, examining gene changes. In-vivo analysis noted a modest inflammatory response to endotoxin treatment, whereas ex-vivo adipose tissue analysis noted that endotoxin led to the downregulation of genes involved in both cell development and differentiation, which closely matched the genes suppressed in human obesity. Subsequent in-vitro studies examined which cell type may be more influential, the adipocyte or macrophage (M1, M2). This led the researchers to conclude that the majority of the downregulated genes suppressed by endotoxin (100 ng/ml) were related to the cultured adipocytes, suggesting that adipocytes were mediating the observed *in vivo* changes. As such, this study highlights endotoxin as a potential cause of metabolic change, which indicates a pathogenic role for endotoxin in the prediabetic phase [43]. These studies continue to support the concept that endotoxin may link an altered GMS, as noted in metabolic disease, to a pro-inflammatory state.

POSTPRANDIAL METABOLIC ENDOTOXAEMIA

Endotoxin appears to circulate at low levels, which would impact on metabolic risk over a long-period of time as previous studies suggest. However, the majority of studies have evaluated endotoxin in the fasted state but, as humans, we perhaps spend a significant proportion of our time in the nonfasted state. Therefore, examining endotoxin fluctuations postprandially may highlight how this low-level,

primary, inflammatory insult, observed in the fasted state, may rise substantially. Furthermore such postprandial changes may also highlight the influence of different metabolic states, as well as emphasize the impact of different diets on both our GMS and circulating endotoxin levels.

As noted, endotoxin is transported across the gut mucosa attached to chylomicrons, which are lipoproteins that transport dietary lipids including SFAs across the intestinal wall [41,44]; consequently, a high-fat meal should increase circulating endotoxin. In accordance with this, studies examining the impact of a high-fat meal on morbidly obese patients showed it induced metabolic endotoxaemia, which correlated with baseline and postprandial hypertriglyceridemia, although endotoxin in this specific cohort did not correlate with glucose, insulin resistance status or C-reactive protein level [45▪▪]. Significantly, animal studies have also shown that a high-fat diet in high-fat-fed and *ob/ob* mice increases intestinal permeability along with reduced gene expression of tight junction proteins, increasing the capability of endotoxin to traverse the gut mucosa [29]. As such, prolonged exposure to a high-fat diet, over 4 weeks, resulted in the mice exhibiting 'metabolic endotoxaemia', which produced comparable effects to the administration of chronic subcutaneous LPS infusion – resulting in inflammation and metabolic disturbances [13]. These findings were substantiated by recent studies exploring the inflammatory effects of different types of oils, including milk fat, palm oil, rapeseed oil and sunflower oil, on metabolic risk [46▪]. C57/Bl6 mice were fed high-fat diets for 8 weeks, varying in the aforementioned oil composition, with the greatest inflammatory response induced by palm oil and the lowest with rapeseed oil [46▪]. As a result of these animal studies, several human studies utilizing high-fat meals have been conducted. As such, a single high-fat meal has been shown to increase metabolic endotoxaemia by around 50% in healthy individuals [47]. Furthermore, Ghanim *et al.* [48] have demonstrated increasing circulating endotoxin following a high fat, high carbohydrate meal; whereas, the increase in circulating endotoxin was abrogated by simultaneously drinking orange juice. Separately, the same team also showed metabolic endotoxaemia was induced following ingestion of cream (SFAs) but not glucose or orange juice [49]. These human postprandial studies further support the link between high-fat meals and metabolic endotoxaemia, rather than the impact of total calorie content or high-glucose intake.

Recent, long-term, diet studies examining the impact of a Western diet on metabolic endotoxaemia have shown that, during a 4-week period of the diet, the healthy individuals raised their circulating endotoxin levels on average by 71%, while, in contrast, a 'prudent' low-fat diet led to a 31% reduction in circulating endotoxin levels over the 4 weeks [50▪▪]. These results are interesting given that it was a crossover design and, although the diet composition was different, the total calories were matched, indicating that the type of diet may be more important that the calorific content to induce metabolic endotoxaemia.

In studies by Harte *et al.* [51▪▪], the impact of a single, high, SFA meal given to individuals with T2DM, impaired glucose tolerance (IGT), obesity as well as healthy nonobese controls (NOC) was explored. Prior to the meal, baseline-circulating endotoxin was already higher in the T2DM and IGT group compared with the NOC group, whereas postprandially, there was a significant rise in endotoxin levels in all groups apart from the NOC group. The rise in circulating endotoxin levels was approximately 20% more in the IGT and obese groups compared with the NOC group, but was much higher (125% higher than NOC even at 4 h postmeal) in the T2DM group. The raised circulating endotoxin levels correlated with triglyceride levels at baseline, with the correlation strengthening at each hour following the meal. Over the 4-h period, overall exposure to circulating endotoxin was cumulatively 336% more in the T2DM group compared with the NOC group, and was 167 and 198.5% more in the obese and IGT groups, respectively [51▪▪]. These data highlight that there is a substantial increase in metabolic endotoxaemia in insulin-resistance states, especially in individuals with T2DM, which may explain why T2DM subjects are at a higher CVD risk. Interestingly, the circulating endotoxin levels appeared to be still rising at 4 h postmeal, which may suggest that repeated meals, high in SFAs, would lead to a repeated insult. As such, continual feeding over a day may lead to a substantial rise in circulating endotoxin prior to sleep, therefore highlighting how much fasted studies may omit on the impact of circulating endotoxin on a daily basis.

CONCLUSION

Endotoxin has a direct effect on inflammatory pathways and gut microbiota is altered in obesity and metabolic disease. Animal models have been able to demonstrate an improvement in metabolic markers by altering the GMS, although studies in humans remain unclear but require urgent attention. Metabolic endotoxaemia has been shown to be associated with a host of conditions including obesity,

metabolic syndrome, T2DM, atherosclerosis, CVD and NAFLD. Long-term high-fat diets, as well as high-fat meals, have been shown to cause a dramatic rise in circulating endotoxin, especially in those with metabolic disease such as T2DM. As such, there appears to be little doubt that endotoxin represents a new biomarker for metabolic risk. Furthermore, current data suggest that endotoxin may act as at least one of the 'primary insults' to mediate inflammatory risk in metabolic disease. To address why this 'primary insult' on systemic metabolism occurs will require an in-depth analysis of the human gut microbiota over time. This may herald the next frontier of medical and dietary intervention analysis rather than often targeting the downstream systemic and cellular events leading to metabolic disease.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 93).

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