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Review

Metabolic endotoxemia and diabetes mellitus: A systematic review

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ABSTRACT

In this systematic review we analyzed studies that assessed serum concentrations of lipopolysaccharide (LPS) and/or lipopolysacharide-binding protein (LBP) in diabetic patients compared with healthy people. Articles were selected using PubMed and Scopus. Search terms used were endotoxemia, endotoxins, LPS, LBP, diabetes mellitus (DM), type 1 (T1DM), type 2 (T2DM), insulin resistance, humans, epidemiologic studies, population-based, survey, representative, cross-sectional, case–control studies, observational, and clinical trials. Two authors independently extracted articles using predefined data fields, including study quality indicators. There was a great variability in the estimates of metabolic endotoxemia among the studies. Most of the studies observed higher LPS or LBP concentrations in diabetic subjects than in healthy controls. T1DM and T2DM subjects presented higher mean fasting LPS of 235.7% and 66.4% compared with non-diabetic subjects, respectively. Advanced complications (e.g. macroalbuminuria) and disease onset exacerbate endotoxemia. Antidiabetic medications decrease fasting LPS concentrations. Among these medications, rosiglitazone and insulin present higher and lower effects, respectively, compared with other treatments. T1DM and T2DM seem to increase metabolic endotoxemia. However, some confounders such as diet, age, medication, smoking and obesity influence both diabetes and endotoxemia manifestation. A better understanding of the interaction of these factors is still needed.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Type 1 diabetes (T1DM) results from beta-cell destruction, usually leading to absolute insulin deficiency [1]. Type 2 diabetes (T2DM) occurs due to the progressive loss of insulin secretion and/or insulin action, usually with a contribution from insulin resistance (IR) [1]. The prevalence and incidence of DM have increased during recent decades, especially in Western countries [1]. Short and long-term complications due to uncontrolled glycemia lead to high human, social, and economic burdens [1]. Therefore, understanding the features involved in the

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pathophysiology of DM is of considerable value to treat DM and prevent its progression.

Increased intestinal permeability may contribute to low-grade inflammation, leading to insulin resistance, and DM [2]. The intestinal epithelial monolayer is an important barrier between the organism and the external environment [3]. A healthy intestinal barrier allows the passage of water, nutrients and bioactive compounds, and avoids the passage of harmful substances such as microbial and dietary antigens [3]. Evidence, largely from animal studies, indicates that DM favors endotoxin (especially lipopolysaccharide (LPS)) translocation across the intestinal barrier, leading to its mild increase in concentration in the bloodstream [4]. LPS is the major component of the outer membrane of the Gram-negative bacteria. This endotoxin is composed of three modules: a highly variable O-antigen constituted of repeating oligosaccharide units, a core oligosaccharide and lipid A [5]. Lipid A component is responsible for much of LPS toxicity. Toll-like receptors (TLR) of the innate immune system recognize lipid A and then trigger immune and inflammatory responses [5].

Integrity breakdown and increased intestinal permeability favor LPS translocation from the intestinal lumen to the bloodstream, causing metabolic endotoxemia [2,4]. LPS has a short half-life, so LPS-binding protein (LBP) has been used as a metabolic endotoxemia marker [6,7]. LBP is an acute-phase protein synthesized in the liver [6,7]. The binding of LBP–LPS complex to cluster of differentiation 14 (CD14), which is mainly expressed by macrophages and neutrophils, mediates signal transduction, including nuclear factor kappa B (NF-κ B) activation via TLR4, leading to the activation of innate and adaptive inflammatory responses [6,7]. Considering that LBP represents the innate immune response triggered by LPS, assessing LBP concentrations is an indirect way to evaluate active LPS. Consequently, LBP is a good marker of metabolic endotoxemia [6,7].

Animal and human studies indicate LPS as an antigen that activates the immune system, playing an important role in the pathogenesis of metabolic chronic diseases related to subclinical inflammation, such as obesity, IR, T2DM, and dyslipidemia [2,8,9]. However, the influence of LPS concentrations on glucose homeostasis in humans is not well understood. In this context, new links between endotoxemia and DM should be highlighted to better treat and prevent DM complications. Therefore, in this systematic review we examined the studies that assessed serum concentrations of LPS and/or LBP in diabetic patients compared with healthy controls. We also discuss existing evidence for the proposal of possible mechanisms linking metabolic endotoxemia and DM.

2. Methods

2.1. Protocol and Registration

This systematic review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [10] (S1 Appendix-Checklist) and was registered in PROSPERO (registration number: CRD42015020532).

2.2. Literature Search

Two authors (JMGG and JAC) independently searched for original articles on endotoxemia status in diabetes mellitus type 1 (DM1), DM2 or impaired glucose tolerant (IGT) patients in the following electronic databases: PubMed (www.pubmed.com) and Scopus (www.scopus.com). Keywords were chosen from the Medical Subject Headings terms using the following search strategy: (Endotoxemia OR Endotoxins OR Lipopolysaccharides or Lipopolysaccharide-binding protein) AND (Diabetes Mellitus, Type 2 OR Diabetes Mellitus, Type 1 OR Insulin Resistance) AND humans AND (epidemiologic studies OR population-based OR survey OR representative OR cross-sectional OR case–control studies OR observational OR clinical trials) NOT (reviews).

The search strategies had no date restrictions and included articles published in English, Portuguese, and Spanish. The date last searched was October 30, 2016. References from the extracted articles were also consulted to complete the data bank.

2.3. Studies Selection

We included all published randomized controlled trials (RCTs), cross-sectional and cohort studies comparing fasting plasma LPS or LBP concentrations in diabetic human patients versus healthy non-diabetic controls (at baseline). Studies were included in the present review if they met the following criteria defined a priori: (1) Population: T1DM, T2DM or IR subjects; (2) Control group: non-diabetic healthy subjects; (3) Exposure: presence of T1DM, T2DM or IR; (4) Main outcomes: report of mean or median plasma LPS or LBP concentrations; (5) Study design: cross-sectional comparison of endotoxemia; (6) Measurement of circulating LPS concentrations by chromogenic kinetic limulus amebocyte assay (LAL assay) or LBP concentrations by enzyme-linked immunosorbent assays (ELISA).

We excluded reviews, case reports, letters, commentaries, abstracts, and unpublished articles. We excluded studies that did not have a control group (healthy non-diabetic subjects), did not include diabetic patients or RI or those that did not discriminate diabetic subjects compared with controls, animal studies, studies that did not indicate LPS values or in which LPS values were not adequately described (e.g. only in graphs, only correlation data), studies with LPS infusion, in vitro assays, and other systemic diseases other than diabetes and obesity (e.g. metabolic syndrome, hypertension, periodontitis and AIDS).

2.4. Data Extraction

All studies were independently screened and evaluated for selection by two authors (JMGG and JAC). After all abstracts were reviewed, data comparisons between investigators were conducted to ensure completeness and reliability. We did not contact authors of the original articles in the case of missing data. The inclusion criteria were independently applied to all identified studies. Differing decisions were resolved by consensus. For each included article, we extracted information of the title, authors, publication year, name of the study,

type of study, study aim, sample size, number of diabetics and control subjects, study design, proportion of men, mean age, mean body mass index (BMI, calculated as body weight in kilograms divided by height in meters squared), mean HbA1c or fasting glucose, mean/median LPS or LBP concentrations, and assay used for measuring LPS or LBP concentrations.

2.5. Assessment of Reporting Quality and Risk of Bias

We assessed study quality using data reported in each study on representativeness, validity, and reliability. A study was considered representative if (1) this feature of the study was explicitly addressed in the corresponding full-text article or (2) any statement made by the authors suggested that the actual sample reflected the target population (e.g., sample size calculation, description of inclusion and exclusion criteria, etc.). A study was classified as non-representative if the article's corresponding full-text contained information about an existing selection bias. Assessment validity was evaluated using information about LPS or LBP measure (e.g. detailed information about the method of assessment under nonpyrogenic conditions or citation of a study in which that was previously detailed). Finally, a study was classified as reliable if the intra and inter-assay coefficients of variation (CV) were below 10 and 15%, respectively. In instances where details about representativeness, validity or reliability were not provided, we created a separate category ('unknown') for each quality criterion.

Two authors (JMGG and JAC) assessed risk of bias for each study using predefined criteria described by the Agency for Healthcare Research and Quality "Methods Guide for Effectiveness and Comparative Effectiveness Reviews" [11], using questions specified in the RTI Item Bank [12] and the Cochrane Risk of Bias tool [13]. We selected items based on relevance to the topic and anticipated sources of bias. We assessed the potential for selection bias, performance bias, attrition bias, detection bias, and reporting bias. The tool presents design-specific criteria to assess risk of bias, with 12 questions for RCT, 13 for cohort, and 9 for cross-sectional studies [11]. Studies were classified as having a low risk of bias when >80% questions were answered as "yes (low risk)", a moderate risk of bias when 50 to 79% of the questions were answered as "yes (low risk)" and a high risk of bias when <50% questions were answered as "yes (low risk)". Discrepant opinions between authors were resolved by consensus.

2.6. Data Analyses

A statistical meta-analysis was not justified because of the marked heterogeneity of the included studies. We followed guidance from the Cochrane handbook, which supports the use of a systematic, narrative approach when a meta-analysis is inappropriate [13].

We summarized all studies reviewed in this paper in tables according to main characteristics and results from single studies, geographic distribution, quality assessment, risk of bias, and correlation between LPS or LBP concentrations and other variables. The studies were arranged chronologically by year of publication, beginning with the last published study. The primary outcome measure was the difference in LPS or fasting plasma mean (or medians) comparing diabetic subjects to non-diabetics controls. We also calculated the percentage change in LPS or LBP concentrations comparing cases and controls. We reported the variables that significantly correlated with LPS or LBP according to the information provided by the authors, highlighting the positive and negative correlations, and we described the type of statistical analyses used by the authors.

3. Results

3.1. Study Selection

We identified a total of 867 studies after searching the two databases. A total of 331 duplicates were removed resulting in 536 unique records after which 506 studies were excluded based on their titles and abstracts because they were considered irrelevant to the topic of interest. After reading the full text of the remaining 30 studies, 14 met all the criteria for the systematic review. The most common reasons for exclusion were animal, duplicate, in vitro or LPS infusion studies, language (Chinese, Russian, and Ukrainian), lack of a comparison group (healthy control), lack of diabetics or insulin resistant patients, presence of participants with multiple diseases, and absence of LPS or LBP as outcome (Fig. 1).

3.2. Description of Included Studies

The fourteen studies included in the present review (Table 1) contained data on a total of 9773 subjects (7253 non-diabetic controls, 72 IGT patients, 2424 diabetics patients (1183 T1DM and 1241 T2DM subjects), and 198 overweight or obese non-diabetic subjects) [6,14–26]. These studies had sample sizes ranging from 30 [24] to 6632 [19] participants with a median of 94 (interquartile range 75.7–340). Ten studies (71.4%) included T2DM subjects [6,14,15,19–21,23–26], and four studies (28.6%) included T1DM subjects [16–18,22]. Two studies (14.3%) also evaluated impaired glucose tolerant subjects [6,20], and four (28.6%) discriminated non-diabetic overweight subjects [17,20,21,25]. While the majority of studies contained data on males and females [14–20,22–24], three studies (21.4%) contained data on only females [21,25,26], and another one (7.1%) was restricted to males [6]. The overall proportion of males was 42%. The mean age was 37.3 ± 4.9 years for T1DM subjects (vs. 37.7 ± 5.9 years for healthy controls), and 51 ± 4.8 years for T2DM subjects (vs. 42.1 ± 8.4 years for healthy controls). In terms of geographic distribution, most studies were conducted in Europe and Asia (England, n = 1; France, n = 1; Finland, n = 3; India, n = 1; Russia, n = 1; Saudi Arabia, n = 3; Spain, n = 1; United Kingdom, n = 1) [6,14,15,17–20,22–26]. One study was conducted in Africa [21] and another in the United States [16] (Table 2). The studies included nine cross-sectional studies [6,15–18,21–24], three clinical trials [20,25,26] and two cohort studies [14,19] that compared fasting serum LPS or LBP concentrations of diabetic subjects vs. healthy controls. Only baseline data from the clinical trials and cohorts were used (Table 2).

3.3. Quality Assessment and Risk of Bias

In terms of study quality, ten studies (71.4%) were classified as representative of the target population [6,15–17,19,20,22,24–26].

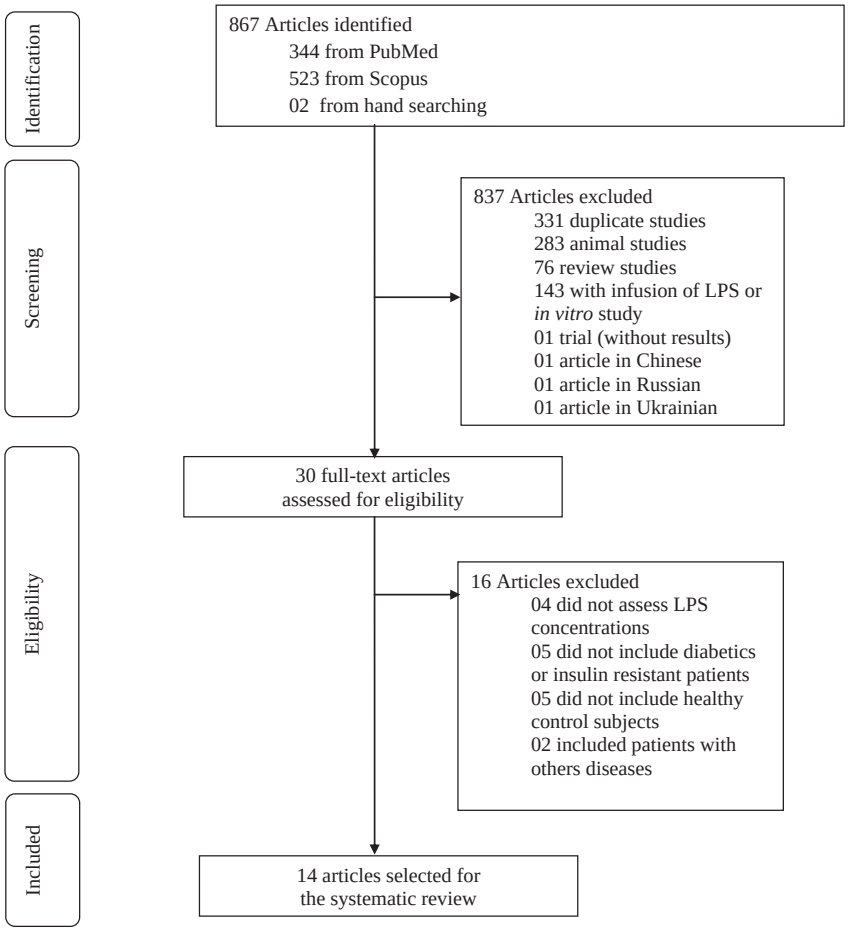

Fig. 1 – Flowchart of study-selection process. PubMed, www.ncbi.nlm.nih.gov/pubmed/; Scopus, www.scopus.com LPS: lipopolysaccharide. Hand searching was conducted on the references lists of papers included in the systematic review.

One study (7.1%) was qualified as non-representative according to the criteria defined previously [21]. Evidence of representativeness could not be established in three (21.4%) studies due to missing information [14,18,23]. Assay validity was reported in nine studies (63.4%) [6,15,19–22,24–26]. Information on assay reliability was provided in seven studies (50%) [6,14,17,20,23,25,26], and all of them were classified as providing reliable LPS or LBP measurements (Table 2).

Three (21.4%) studies were considered as having a high risk of bias [18,21,26], eight (57.1%) were classified as having a moderate risk of bias [14–17,19,22–25] and three (21.4%) were considered as having a low risk of bias [6,17,20]. The major limitations were in the selection bias (inclusion and exclusion criteria not clearly defined or uniformly reported to all comparison groups (nine studies, 64.3%)), and lack of control or adjustment for confounding variables (seven studies, 50%)).

3.4. *Results of Individual Studies*

There was a great variability in the estimates of metabolic endotoxemia among the studies. The mean and median values of LPS in non-diabetic subjects ranged from 0.37 [26] to 61.06 EU/ml [10], and from 3.89 [21] to 66 EU/ml [22], respectively (considering only studies that used the unit EU/ml). Among diabetic subjects, mean and median LPS concentrations ranged from 0.39 (T2DM subjects) [26] to 77.03 EU/ml (T2DM subjects) [19] and from 5.19 (obese T2DM subjects) [21] to 67 EU/ml (T1DM subjects with macroalbuminuria) [17], respectively (considering only studies that used the unit EU/ml).

Higher LPS or LBP concentrations in diabetic subjects compared with healthy controls (Table 1) were observed in most studies. T1DM and T2DM subjects presented higher mean fasting LPS of 235.7% and 66.4% compared with non-diabetic subjects, respectively. Significant differences in LPS concentrations between T2DM subjects compared with healthy controls were not detected in only two studies [24,26]. LPS concentrations in T1DM subjects with microalbuminuria was not different when compared to control subjects instead of T1DM subjects with macroalbuminuria [17]. Considering the studies in which LPS concentrations were statistically different between diabetic and control subjects, the lowest difference was observed in T1DM patients with advanced kidney disease (LPS concentrations 9.8% higher) [17] and a greater difference in T1DM subjects at the disease onset (LPS concentrations 882% higher) [18]. Among the T2DM subjects, the lowest difference compared with control subjects was observed in the study of Jayashree et al. [23] (LPS

Table 1 – Characteristics and main results from single studies on LPS or LBP levels in diabetic subjects and healthy controls.

Author (year)	Study aim	Sample size	Males (%)	Age (years)	BMI (kg/m^2)	HbA1c (%) or glycemia (mmol/L) [2]	Fasting endotoxin (EU/mL) or LBP (μg/ml) [1] levels at baseline	% change in LPS or LBP levels vs. ND
Gubern et al. [6] [1]	To associate bactericidal/ permeability-increasing protein to insulin sensitivity.	114 ND	100	46.2 ± 11.9[a]	27.01 ± 3.6[a]	4.78 ± 0.34[a]	27.82 (9.8, 50.1)[a]	
		60 IGT		53.2 ± 11.2[bc]	29.5 ± 3.9[b]	5.0 ± 0.58[b]	17.26 (9.59, 44.93)[ab]	↓ 37.9%
		170 T2DM		57.2 ± 11.8[c]	32.3 ± 7.0[ab]	7.3 ± 1.7[b]	65.03 (57.9, 72.08)[b]	↑ 133.7%
Creely et al. [14] [2,3]	To examine circulating LPS in T2DM subjects.	25 ND	80	48.1 ± 19.2[a]	29.5 ± 4.3[a]	5.6 ± 0.9[a]	3.1 (1.7)[a]	
		25 T2DM		52.2 ± 11.7[a]	31.8 ± 4.5 [a]	8.6. ± 2.5[b]	5.5 (1.6)[b]	↑ 77.4%
Attas et al. [15] [2,4]	To investigate the relationship between endotoxin and various metabolic parameters of diabetic patients.	67 ND	49	44.1 ± 9.9[a]	30.0 ± 5.2[a]	5.5 ± 1.5[a]	4.2 (3.1–5.1)[a]	
		346 T2DM treated with:						
		36 diet-controlled		48.3 ± 9.[b]	29.6 ± 5.8[a]	7.1 ± 2.8[b]	7.9 (5.7–10.0)[b]	↑ 88%
		141 met		53.0 ± 10.5[cd]	32.0 ± 5.8[a]	9.6 ± 3.4[cd]	7.5 (4.6–8.7)[c]	↑ 78.6%
		22 RSG		52.3 ± 9.5[bd]	29.6 ± 5.8[a]	8.4 ± 1.9[bd]	5.6 (4.2–6.1)[c]	↑ 33.3%
		100 met/RSG		52.5 ± 9.0[cd]	31.0 ± 5.3[a]	9.4 ± 3.9[cd]	7.4 (4.8–9.6)[b]	↑ 76.2%
		47 insulin		55.6 ± 11.4[d]	29.0 ± 6.2[a]	9.5 ± 3.8[d]	9.2 (6.6–10.7)[b]	↑ 119%
Devaraj et al. [16] [5]	To examine circulating levels of TLR2 and TLR4 ligands in T1DM compared with matched healthy controls.	37 ND	51.4	34 ± 11 [a]	24 ± 4[a]	5.4 ± 0.4[a]	2.53 ± 0.67 [a]	
		34 T1DM	44.1	32 ± 11[a]	25 ± 4[a]	7.9 ± 1.4[b]	3.32 ± 0.82[b]	↑ 31.2%
Lassenius et al. [17]	To investigate whether serum LPS is associated with the components of the MetS in T1DM patients.	345 ND:	51.3	33 ± 10	24.3 ± 3.6	NA	61 (44, 79)	Only T1DM patients with macroalbuminuria showed higher LPS levels than ND subjects (↑9.8%).
		219 lean		33 ± 10[a]	22.2 ± 1.7[a]		60 (44, 80)[a]	
		126 overweight		33 ± 9[a]	28.2 ± 2.8 [b]		62 (49, 82)[b]	
		904 T1DM:	47.8					
		587 normal AER		44 (36, 53)[a]	25.6 ± 4.2[a]	7.7 ± 1.3[a]	57 (50, 69)[a]	
		144 with microalbuminuria		46 (37, 55)[ab]	26.4 ± 4.2[b]	7.8 ± 1.8[a]	56 (47, 72)$_a$	
		173 with macroalbuminuria		48 (40, 56)[b]	27.0 ± 4.9[b]	7.8 ± 2.0[a]	67 (52, 96)[b]	
Okorokov et al. [18]	To determine the possible role of the excess of LPS on T1DM onset.	50 ND	58	11.14 ± 0.57	NA	4.56 ± 0.04	0.4 ± 0.03[a]	
		45 T1DM:	53.3					
		15 T1DM (onset)		6.6 ± 1.12		8.1 ± 0.59	3.93 ± 0.79[b]	↑ 882%
		30 T1DM (>2 y of DM)		12.3 ± 0.71		8.73 ± 0.33	2.37 ± 0.27[c]	↑ 492%
Pussinen et al. [19] [4]	To investigate whether endotoxemia and incident diabetes are associated.	6170 ND	50.5	53.2 ± 11.0[a]	26.7 ± 4.1[a]	NA	61.06 ± 36.11[a]	
		462 jlaT2DM	60.1	57.3 ± 9.4[b]	31.6 ± 5.2[b]		77.03 ± 42.03[b]	↑26.2%
Harte et al. [20] [4]	To evaluate the changes in circulating endotoxin after a high-saturated fat meal.	9 ND lean	62.9	39.9 ± 11.8[a]	24.9 ± 3.2[a]	5.9 ± 0.31%[a]	3.3 ± 0.15[a]	
		15 ND obese		43.8 ± 9.5[a]	33.3 ± 2.5[b]	5.9 ± 0.49[a]	5.1 ± 0.94[a]	↑ 54.5%
		12 IGT		41.7 ± 11.3[a]	32.0 ± 4.5[b]	6.3 ± 0.47[b]	5.7 ± 0.1[b]	↑ 72.7%
		18 T2DM		45.4 ± 10.1[a]	30.3 ± 4.5[c]	7.5 ± 1.12%[b]	5.3 ± 0.54[b]	↑ 60.6%
Hawkesworth et al. [21] [2]	To investigate metabolic endotoxemia in Gambian women.	31 ND lean	0	41.5 ± 6.2	20.8 ± 1.8	5.0 (4.8, 5.2)	3.89 (3.20, 4.73)[a]	
		33 ND obese		43.4 ± 5.4	34.3 ± 4.5	5.5 (5.2, 5.8)	3.86 (3.30, 4.52)[a]	
		29 obese T2DM		45.1 ± 5.2	33.3 ± 5.7	9.2 (7.7, 10.9)	5.19 (3.43, 7.87)[b]	↑ 33.4%
Peraneva et al. [22]	To detect serum bacterial DNA in subjects with high LPS activity.	200 ND	48.5	46 ± 12[a]	25.9 ± 3.8[a]	7.8 ± 1.0[a]	66 (54, 93)[a]	
		200 T1DM	47	36 ± 11[b]	23.8 ± 2.8[b]	5.1 ± 0.3[b]	55 (42, 71)[b]	↑ 20%
Jayashree et al. [23] [6]	To compare serum LPS levels in T2DM patients vs. healthy controls.	45 ND	55.5	46 ± 9[a]	26.9 ± 3.9 [a]	5.6 ± 0.4[a]	0.47 ± 0.02[a]	
		45 T2DM	51.5	51 ± 6[b]	27.2 ± 6.0[a]	8.0 ± 2.2[b]	0.57 ± 0.028[b]	↑ 21.3%

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Table 1 *(continued)*

Author (year)	Study aim	Sample size	Males (%)	Age (years)	BMI (kg/m^2)	HbA1c (%) or glycemia (mmol/L) [2]	Fasting endotoxin (EU/mL) or LBP (μg/ml) [1] levels at baseline	% change in LPS or LBP levels vs. ND
Verges et al. [24]	To evaluate lipoprotein kinetics and plasma LPS distribution.	14 ND 16 T2DM	64 31	29.6 ± 11.5[a] 55.8 ± 9.2[b]	22.4 ± 1.8[a] 31.8 ± 4.0[b]	NA 7.4 ± 1.5	0.94 ± 0.66[a] 0.92 ± 0.66[a]	No significant difference
Al-Disi et al. [25]	To determine the influence of a high-fat meal on changes in endotoxin levels.	18 ND 24 overweight/ obese 50 T2DM	0 0 0	24.4 ± 7.9 [a] 32.0 ± 7.8[b] 41.5 ± 6.2[c]	22.2 ± 2.2[a] 28.5 ± 1.5 [b] 35.2 ± 7.7[c]	4.8 ± 0.9[a] 4.7 ± 0.4[a] 7.9 ± 2.7[b]	1.5 ± 0.1[a] 3.0 ± 0.5[b] 3.4 ± 0.8[b]	 ↑ 100% ↑ 126.7%
Zaman and Zaman [26]	To assess postprandial endotoxemia in nonobese postmenopausal women and diabetic patients.	80 ND 80 T2DM	0 0	48 ± 5 48 ± 6	23 ± 1.4 24 ± 2.0	5.1 ± 0.6 9.1 ± 2.1	0.37 ± 0.02 0.39 ± 0.03	No significant difference

Data are means ± SD or median (interquartile range). Different letters indicate significant differences between groups in the same study.

Abbreviations: AER, Albumin Excretion Rate; HOMA-IR, Homeostasis Model Assessment-Estimated Insulin Resistance; IR, insulin resistance; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; Met, Metformin; MetS, Metabolic Syndrome; MVC, Microvascular Complications; NA, Not Available; ND, Non-Diabetic subjects; RSG, Rosiglitazone; T1DM, Type 1 Diabetic subjects; T2DM, Type 2 Diabetic subjects.

[1] Study that evaluated only LBP levels.

[2] Studies that evaluated glycemia (not HbA1c levels).

[3] Endotoxin, inv. log EU/ml (geometric mean).

[4] Endotoxin Log transformed before comparisons.

[5] Endotoxin (nmol/l).

[6] Endotoxin (μg/ml).

Table 2 – Geographic distribution, type of study, quality assessment and risk of bias of the selected studies.

Author, year	Country	Type of study	Representativeness	Measurement validity	Reliability	Overall risk of bias
Gubern et al. (2006) [6]	Spain	Cross-sectional	Yes	Yes	Yes	Low risk (8/9)
Creely et al. (2007) [14]	England	Cohort	Unknown	Unknown	Yes	Moderate risk (9/13)
Attas et al. (2009) [15]	Saudi Arabia	Cross-sectional	Yes	Yes	Unknown	Moderate risk (7/9)
Devaraj et al. (2009) [16]	United States of America	Cross-sectional	Yes	Unknown	Unknown	Moderate risk (7/9)
Lassenius et al. (2011) [17]	Finland	Cross-sectional	Yes	Unknown	Yes	Low risk (8/9)
Okorokov et al. (2001) [18]	Russia	Cross-sectional	Unknown	Unknown	Unknown	High risk (4/9)
Pussinen et al. (2011) [19]	Finland	Prospective cohort	Yes	Yes	Unknown [a]	Moderate risk (9/13)
Harte et al. (2012) [20]	United Kingdom	Clinical trial	Yes	Yes	Yes	Low risk (10/12)
Hawkesworth et al. (2013) [21]	Africa	Cross-sectional	No	Yes	Unknown	High risk (4/9)
Peraneva et al. (2013) [22]	Finland	Cross-sectional	Yes	Yes	Unknown	Moderate risk (6/9)
Jayashree et al. (2014) [23]	India	Cross-sectional	Unknown	Unknown	Yes	Moderate risk (6/9)
Verges et al. (2014) [24]	France	Cross-sectional	Yes	Yes	Unknown	Moderate risk (6/9)
Al-Disi et al. (2015) [25]	Saudi Arabia	Clinical trial	Yes	Yes	Yes	Moderate risk (9/12)
Zaman and Zaman (2015) [26]	Saudi Arabia	Clinical trial	Yes	Yes	Yes	High risk (6/12)

[a] Only interassay coefficient of variation is showed.

concentrations 21.3% higher) and the greatest difference among diabetic women in the study of Al-Disi et al. [25] (LPS concentrations 126.7% higher).

Although in the study of Verges et al. [24] fasting LPS concentrations in T2DM patients were not different from the control subjects, the postprandial LPS distribution in the two groups was different. T2DM subjects had higher LPS–very low-density lipoprotein (VLDL), LPS–high-density lipoprotein (HDL), free (nonlipoprotein bound) LPS and lower LPS–low-density lipoprotein (LDL) [24]. In another study in which fasting plasma LPS did not differ in T2DM patients and controls, diabetics had higher increase in LPS concentrations four hours after a meal [26]. However, the authors did not describe the type of meal provided to the participants (e.g. high-fat meal) [26]. Similarly, the other two clinical trials included in this review observed higher increase in postprandial LPS concentrations in T2DM patients compared with healthy controls after the consumption of a meal containing 75 g of fat [20,25].

Gubern et al. [6] verified higher LBP concentration and lower bactericidal/permeability-increasing protein (BPI) in T2DM patients and subjects with impaired glucose tolerance compared with non-diabetic subjects. BPI competes with LBP for the binding of endotoxin, but BPI–LPS complexes (in contrast to LBP and LPS) do not activate immune response. Interestingly, the treatment with metformin increased BPI concentrations in T2DM patients, although their values remained lower than the control ones [6].

Other studies also reported the influence of antidiabetic medication on endotoxemia. Rosiglitazone (RSG) decreased fasting serum insulin and plasma LPS in T2DM subjects [14,15]. Creely et al. [14] detected lower LPS concentrations in T2DM subjects who were treated with oral hypoglycemics and/or insulin (n = 14) compared with those treated with diet alone (n = 11). Attas et al. [15] examined LPS concentrations in non-diabetic subjects (n = 67) and T2DM subjects treated with: diet-controlled (n = 36), metformin (n = 141), RSG (n = 22), combined fixed dose of metformin/RSG (n = 100), and insulin (n = 47). LPS concentrations were higher in T2DM subjects compared to non-diabetic ones. Among T2DM subjects, those who were treated with RSG and insulin had lower and higher LPS concentrations, respectively, compared with other treatments.

In general, the most cited variables that correlated with LPS or LBP concentrations were triglycerides [6,15,17,19,20,23,25], fasting glycemia [6,15,21,23], insulinemia [6,14,15,17], CRP [6,17,19,22], HbA1c [6,16,23], and total cholesterol [15,19,25] (Table 3). In five studies separate clinical variables for diabetic and non-diabetic participants were correlated. Considering only diabetic subjects, insulinemia [6,14,15,17], triglyceridemia [6,15,17,25], glycemia [6,15], and HDL-c [6,15] were the most cited variables that correlated with LPS or LBP concentrations. Among control subjects, insulinemia [14,17], and triglyceridemia [15,25] were the most cited. The studies of Okorokov et al. [18] and Zaman & Zaman [26] did not correlate LPS with other variables.

Because HDL-c is the major factor involved in endotoxin neutralization, some authors used the LPS/HDL ratio as a functional measure of LPS activity [17,19]. LPS/HDL ratio was associated with increased risk of incident diabetes and was also correlated with metabolic syndrome components [17,19]. The risk was independent of other risk factors for DM (blood glucose, lipids, CRP, BMI, etc.) and was also independent of other factors that affect endotoxemia (cholesterol, HDL, smoking, etc.) [17,19].

4. Discussion

4.1. *Key Findings*

To our knowledge, no other systematic review has assessed the association between endotoxemia and diabetes. In general, we observed that diabetic subjects presented higher fasting and postprandial LPS concentrations compared to lean non-diabetic subjects and/or obese subjects. Fig. 2 shows the possible mechanisms explaining plasma LPS increase in diabetic subjects. Fasting endotoxin concentrations seem to change with nutritional and metabolic status (healthy, obese, diabetic, etc.) [14–23,25]. LPS was more elevated in diabetic patients with advanced complications, such as macroalbuminuria [17], and those treated with insulin [15]. These results suggest that metabolic disorder exacerbates endotoxemia. There is also

Table 3 – Variables that significantly correlated with LPS or LBP concentrations/activity.

Author (year)	Type of analyses	Main clinical variables that correlated with LPS or LBP concentrations	
		Non-diabetic subjects	Diabetic subjects
Gubern et al. [6] [a]	Pearson's correlation analysis	Positive: BMI (r = 0.13) Negative: BPI (r = −0.31)	Positive: BMI (r = 0.4), glucose (r = 0.37), HbA1c (r = 0.35), insulin (r = 0.30), TG (r = 0.31), CRP (r = 0.33) Negative: HDL (r = −0.21)
Creely et al. [14]	Pearson's correlation analysis	Positive: Insulin (r = 0.68); HOMA-IR (r = 0.69)	Change in insulin levels after RSG treatment (r = 0.673)
Attas et al. [15]	Multiple regression analysis	Positive: TG (R^2 = 0.192); total cholesterol (R^2 = 0.163)	Positive: TG (R^2 = 0.42); total cholesterol (R^2 = 0.10), glucose (R^2 = 0.076) and insulin (R^2 = 0.032) Negative: HDL-c (R^2 = 0.055)
Devaraj et al. [16]	Spearman's rank Correlation	**All subjects:** Positive with TLR4 (r = 0.56); HbA1c (= 0.64)	
Lassenius et al. [17]	Multivariate linear regression analyses Pearson's correlation analysis	Positive: TG (β = 0.69), diastolic blood pressure (β = 0.10); TG (r = 0.396); Insulin (r = 0.312), CRP (r = 0.272) Negative: age at onset of diabetes (β = −0.14) **LPS activity** [b]: Positive correlation with HOMA (r = 0.121); TG (r = 0.505); insulin (r = 0.370); CRP (r = 0.331); BMI (r = 0.199)	Positive: ThG (r = 0.73) HOMA (r = 0.213), Insulin (r = 0.25); TG (r = 0.325) (patients with IgAGN) **LPS activity** [b]: Positive correlation with HOMA (r = 0.230); TG (r = 0.496); insulin (r = 0.251); BMI (r = 0.343) (patients with IgAGN)
Pussinen et al. [19]	Two-tailed Pearson correlation	**All subjects:** Positive with CRP, cholesterol, and TG Negative: HDL-c	
Harte et al. [20]	Pearson's correlation analysis	**All subjects:** Positive with TG (r = 0.303)	
Hawkesworth et al. [21]	Simple linear regression analysis	**All subjects:** Positive with Log fasting glucose (β = 0.24)	
Peraneva et al. [22]	Spearman's rank correlation test	**All subjects:** Positive with CRP (r = 0.221)	
Jayashree et al. [23]	Pearson's correlation analysis	**All subjects:** Positive with ZO-1(r = 0.252), fasting plasma glucose (r = 0.229), 2 h post glucose (r = 0.341), HbA1c (r = 0.334), TG (r = 0.353), TNF-alpha (r = 0.407), IL-6 (r = 0.542) Negative: HDL-c (r = −0.531)	
Verges et al. [24]	Multivariable linear regression and Pearson's correlation analysis	**All subjects:** VLDL-LPS was associated with HDL-LPS (r = 0.740); LDL-LPS was associated with VLDL-LPS (r = 0.464); HDL-LPS was associated with free LPS (r = 0.592) and VLDL-LPS (0.322); free LPS was associated with HDL-LPS (r = 0.819).	
Al-Disi et al. [25]	Spearman bivariate correlations	**All subjects:** Positive with LDL-c at 3 h after a high-fat meal (R = 0.38) **Overweight/obese subjects:** Positive with TG (R = 0.63) and total cholesterol (R = 0.71) at baseline.	**T2DM subjects:** Positive with TG at 3 and 4 h postprandial (R = 0.52 and 0.50, respectively)

BPI, Bactericidal/Permeability-increasing Protein; BMI, Body Mass Index; CRP, C-Reactive Protein; HbA1c, Glycated Hemoglobin; HDL, High-density Lipoprotein; HOMA, Homeostasis Model Assessment-Estimated Insulin Resistance; IgAGN, IgA Glomerulonephritis; LBP, Lipopolysaccharide binding protein; LDL, Low-density Lipoprotein; LPS, lipopolysaccharide; RSG, rosiglitazone; TG, triglycerides; TLR4, Toll-like receptor 4; TNF-alpha, Tumor Necrosis Factor alpha; VLDL, Very-low-density Lipoprotein.

[a] Study that evaluated only LBP concentrations.

[b] LPS activity measured by LPS/HDL ratio.

evidence that endotoxin is involved in the onset of T1DM, since LPS concentrations were higher at the disease onset [17,18].

Moreover, diabetics, IGT, and obese subjects showed higher increase in postprandial endotoxemia after a high-fat meal intake compared to healthy subjects, indicating an exacerbated metabolic response [20,25]. Harte et al. [20] emphasized that the intake of three daily high-fat meals (75 g of saturated fat) could result in constant elevated endotoxin concentrations, as each fat meal can increase plasma LPS concentrations for up to four hours.

Intestinal LPS can reach the bloodstream by two main pathways: direct diffusion due to increased intestinal paracellular permeability or by uptake and incorporation of LPS to chylomicrons (chylomicron-driven transport of LPS)

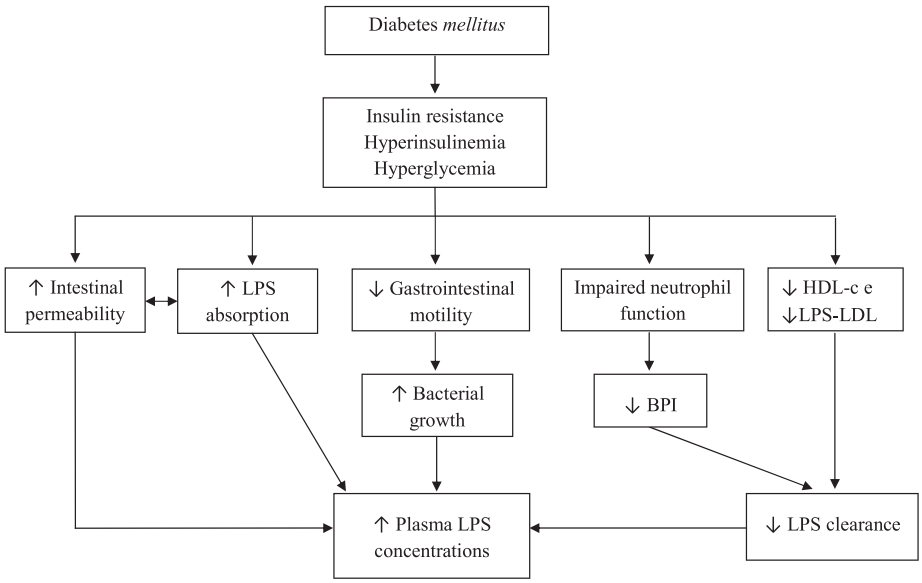

Fig. 2 – Possible mechanisms explaining high-LPS concentrations in diabetic subjects. LPS, lipopolysaccharide; BPI, bactericidal/permeability-increasing protein; HDL-c, high-density lipoprotein cholesterol.

[27]. Both pathways seem to contribute to increased plasma LPS concentrations in diabetic patients, since diabetics seem to have increased intestinal permeability [28] and higher LPS absorption after a high-fat meal [20,25]. Once in blood circulation, LPS binds to the CD14/TLR4 present on the macrophages and so induces the production of proinflammatory cytokines and impairs pancreatic β-cell function [27,28].

Bacteria or bacterial components are effectively cleared from circulation via reticuloendothelial system. In liver, Kupffer cells and specialized macrophages recognize and remove bacterial products from circulation [22]. Hyperinsulinemia and IR impair the functionality of polymorphonuclear neutrophils and Kupffer cells [14,29]. Similarly, hyperglycemia affects the functions of macrophages and other monocytes, suppressing the bactericidal activity of leukocytes [30,31]. Therefore, due to hyperglycemia and hyperinsulinemia, diabetic subjects seem to have lower clearance of LPS and consequently, increased LPS concentrations. Additionally, hypertriglyceridemia, hyperglycemia, and hyperinsulinemia, commonly observed in DM, are indirect sources of endotoxemia because individuals with these problems are more susceptible to developing infections, reducing jejunum motility and increasing gastrointestinal transit time, favoring bacterial overgrowth in the small intestine and increasing gut permeability [15,32]. Therefore, these conditions may explain not only the higher endotoxemia in diabetic patients, but also the positive correlations between LPS, triglycerides, insulin and glucose concentrations [14,15,17–21,23,25]. However, these correlations were not observed in all studies included in this review, as some authors mentioned the correlation of LPS with one or two of these variables (triglycerides, glucose, and insulin) [14,21,23,25], while others did not mention and/or did not assess the correlation between plasma LPS and such variables [16,19,20,22,24]. One possible explanation for these differences in correlation is the fact that the antidiabetic medication varied greatly among the studies [14–17,19–23,25]. In general, the antidiabetic drugs reduce insulin concentrations, improve insulin sensitivity, increase BPI and HDL-c concentrations, leading to lower plasma LPS concentrations [6,14,15]. The reduction of insulinemia and increase of insulin sensitivity enhance the functionality of neutrophils and increase the clearance of LPS [6,29]. BPI reduces LPS-activated immune response, since it reduces the binding of LPS to LBP [6]. HDL-c favors LPS detoxification [24,33]. Therefore, the antidiabetic medication seems to reduce the pro-inflammatory effects of LPS. RSG had greater effect on endotoxemia compared with other antidiabetic medication, which may partially explain the anti-inflammatory effects of this drug [15]. Another difference is that some authors used regression models to assess their data [15,17,21,24], which is considered more robust than other methods, such as simple correlations, used to do this type of analyses [34,35]. Using these models, one can study several independent variables, their relationships and the effects they have on dependent variables [34,35].

After the consumption of a high-fat meal, the insoluble fraction of LPS (lipid A) is incorporated into the micelles and absorbed with chylomicrons [32,36]. The binding of LPS to lipoproteins seems to inhibit endotoxin activity, and this ability seems to be dependent on the composition of the lipoproteins. Human reconstituted HDL, containing purified apoprotein A-I (apoA-I), phosphatidylcholine and cholesterol, neutralizes endotoxin in the blood more effectively than other lipoproteins [33]. Verges et al. [24] examined the metabolism of LPS in different lipoproteins and suggested a catabolic pathway for LPS. After reaching the liver and being removed from chylomicrons, free LPS transfers first to HDL, and then to VLDL [24]. The LPS-bound LDL fraction (LDL-LPS) seems to be mainly derived from VLDL catabolism. Diabetic patients had lower LDL-LPS concentrations due to reduced VLDL catabolism, which may represent an impaired catabolic pathway [24]. Furthermore, it is common for diabetics to present reduced HDL concentrations [17], which contribute to reduce

LPS clearance and increase the inflammatory status, exacerbated by high concentrations of endotoxin in diabetic subjects [20]. Serum LPS activity (measured by LPS/HDL ratio) seems to strongly correlate with metabolic syndrome components, such as triglycerides, fasting glucose, and HDL concentrations [17,19]. Therefore, high serum LPS activity combined with common metabolic abnormalities in DM may contribute to the development of macrovascular and microvascular complications, and so it is a potential tool to assess the metabolic risk profile in diabetic patients [17,19].

In summary, DM and its metabolic abnormalities characterized by insulin resistance, hyperinsulinemia, and hyperglycemia, lead to increased intestinal permeability and higher LPS absorption, increasing plasma LPS concentrations. Concomitantly, reduced intestinal motility commonly observed in patients with DM, favors bacterial growth, exacerbating intestinal integrity breakdown and increasing endotoxemia. Furthermore, reduced HDL-c and impaired neutrophil function, also common situations in DM, lead to reduced BPI concentrations and consequently lower LPS clearance. Altogether, these mechanisms seem to negatively affect endotoxemia, which may worsen DM control.

4.2. *Limitations*

Due to the observational nature of most of the studies included in this review, they describe only associations and not causalities. As the study of subclinical endotexemia is relatively recent, several articles did not meet the inclusion criteria we adopted. Furthermore, the heterogeneity of the articles and the different units of measurement of the variables used to assess endotoxemia did not allow us to perform a meta-analysis. Our findings also indicate that there was a considerable variability in quality and risk of bias among the studies included. That is another reason why we did not conduct a meta-analysis and the reason why it was difficult to make strong inferences from the results obtained in the included studies. However, regardless of these limitations these findings highlight the importance of LPS metabolism in patients with diabetes. Therefore, the analyzed data allowed us to propose possible mechanisms that could be investigated in future research.

Although LAL test is widely used to assess endotoxemia, this test is not capable of discriminating “toxic LPS” (diphosphoryl) and “nontoxic” (monophosphoryl) LPS [37] and its use has not been approved for clinical use [38]. Another disadvantage of this test is its indeterminate interlaboratory variability [38], as noted by the very different LPS concentrations among the articles included in this review. Such limitations to assessing plasma LPS in humans tend to hinder the establishment of the true relationship between clinical variables. However, there is no “gold-standard” test recommended to assess endotoxemia yet.

5. Conclusion

T1DM and T2DM seem to increase metabolic endotoxemia. Hyperglycemia and hyperinsulinemia cause increased intestinal permeability, decreased functionality of neutrophils and antimicrobial factors such as BPI, as well as impaired LPS catabolic pathway mediated by lipoproteins, leading to a lower LPS clearance and higher concentrations of circulating endotoxin in diabetic patients.

This systematic review reveals what is known to date about the influence of endotoxemia on DM. This evidence is novel and it suggests that elevated LPS concentrations could be an important factor affecting glucose metabolism and could be implicated in complications associated with DM. Thus, specific strategies for modifying endotoxemia could be useful for treating DM. Future research on this topic must be well designed to reduce bias risk and should infer causality. Double-blind, randomized, controlled trials that assess the effect of changing LPS concentrations (by dietary and/or medication modifications, for example) on glucose homeostasis will hopefully help address these issues. Future studies should also elucidate the complex mechanisms related to the action of LPS on diabetes, since DM is a multifactorial disease and various confounders such as diet, age, medication, smoking, and obesity influence both DM and endotoxemia. Thus, a better understanding of the interaction of these factors is still needed.

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Conflict of Interests/Financial Disclosure

None for all authors.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2016.12.009.

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