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ORIGINAL CONTRIBUTION

Higher plasma lipopolysaccharide concentrations are associated with less favorable phenotype in overweight/obese men

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Abstract
Purpose Lipopolysaccharide (LPS) from the outer membrane of gram-negative bacteria might be an inflammation trigger in adipose tissue. It has recently been proposed that there is a link between adipose tissue distribution and blood LPS. However, the number of studies on this topic is scarce, and further investigation in humans is required. In this study, we explored the association between plasma LPS concentrations and body fat distribution, as well as the biochemical parameters that may indicate the presence of metabolic disorders.
Methods Sixty-seven young adult men with body mass index of 26–35 kg/m^2 were evaluated. Anthropometry, body composition and body fat distribution, blood pressure, energy expenditure, physical activity level, dietary intake, and biochemical parameters were assessed.
Results Men with median plasma LPS $\geq$ 0.9 EU/mL presented higher sagittal abdominal diameter, trunk fat percentage, and android fat percentage, and mass, insulin and alanine aminotransferase concentrations, homeostasis model assessment of insulin resistance (HOMA-IR), and beta cell dysfunction (HOMA-B) than those with lower plasma LPS. LPS correlated positively with the trunk fat percentage, and android fat percentage, and mass, insulin, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase concentrations, as well as HOMA-IR and HOMA-B.
Conclusion Our results suggest that a higher plasma LPS concentration is associated with a less favorable phenotype as characterized by higher central adiposity, higher values of HOMA-IR, and beta cell function impairment in overweight/obese men.

Keywords Lipopolysaccharide · Abdominal obesity · Insulin · Homeostasis model assessment

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Introduction

Adipose tissue functions extend beyond the storage of fat. The endocrine, paracrine, and autocrine signals secreted by adipose tissue regulate the metabolism in other cells. Both the lack of (lipoatrophy) and excess adipose tissue are detrimental to metabolic equilibrium, which is also dependent on the functionality of this tissue. It has been proposed that each person may have a threshold level of adiposity beyond which dysfunctionality occurs [1].

Hyperglycemia, dyslipidemia, and hypertension are metabolic disorders that often occur together and characterize a

phenotype frequently associated with obesity [1, 2]. However, obesity per se [body mass index (BMI) higher than 30 kg/m^2] is not necessarily associated with these metabolic disorders in clinical practice [3]. It may require the co-occurrence of insulin resistance [4–7].

Body fat distribution and adipocyte size, rather than total adiposity, are key physical characteristics that influence the functionality of adipose tissue and occurrence of insulin resistance. Visceral fat mass, in particular, is a strong and independent predictor of the adverse health outcomes of obesity [1, 8, 9]. One of the mechanistic explanations is that expansion of visceral adipose tissue mass without adequate vascularization support might lead to hypoxia, which, in turn, activates signaling to recruit immune cells. These cells increase the expression of inflammatory molecules that can impair signaling of the insulin receptor, creating resistance to the effects of insulin [8, 10]. In this manner, inflammatory activation links insulin resistance and visceral adiposity [11, 12].

The well-known involvement of inflammatory cytokines in insulin resistance has encouraged research aimed at identifying inflammatory triggers. Lipopolysaccharides (LPSs), a molecule derived from the outer membrane of gram-negative bacteria, are potent triggers of inflammatory responses through interaction with toll-like receptor 4 (TLR4) [10, 13].

LPS is capable of influencing adipogenesis. Chronic exposure to low doses of LPS induces adiposity, as well as chronic inflammation, insulin resistance, hyperglycemia, and dyslipidemia in mice [14]. Muccioli et al. [15] reported that LPS may serve as a master switch to control adipose tissue metabolism in vivo and ex vivo through inhibition of cannabinoid-driven adipogenesis. There are also data showing that LPS suppresses adipogenesis in 3T3-L1 preadipocytes [16]. Therefore, it remains poorly understood as to whether LPS induces or inhibits adipogenesis, and how this may affect metabolic control. LPS might trigger inflammation in adipose tissue since expression of TLR4 has been identified in adipose tissue and isolated adipocytes [17]. In addition, LPS infusion has been shown to alter expression of inflammatory markers in subcutaneous adipose tissue, and to cause insulin resistance in humans [18].

The presence of higher blood LPS concentrations in obese and diabetics patients when compared to apparently healthy/lean subjects [12, 19–23] raises the possibility that adipose tissue functionality and expansion may be affected by LPS concentration. However, evidence of the relationship between adipose tissue distribution and circulating concentrations of LPS are still emerging [12] and requires further investigation in humans.

Since obese subjects with similar BMIs are categorize as 'healthy' or 'unhealthy' based on their biochemical characteristics (especially a higher degree of insulin resistance) [24], our goal was to investigate whether subjects with a similar BMI and total adiposity would present a distinguishable phenotype based on plasma LPS concentrations.

Experimental methods

Subjects

Written advertisements and social networks were used for recruitment. One hundred and fifty men were screened. The inclusion criteria were: BMI between 26 and 35 kg/m^2, age between 18 and 50 years, non-smoker, no food allergy, and ethanol consumption lower than 168 g/week. Body weight changes over 3 kg, following weight loss diet, the use of drugs that affect the biochemical parameters evaluated in the study, and the presence of acute or chronic diseases were the exclusion criteria. Sixty-seven young adult men were included in the study (mean age 27.1 $\pm$ 0.9 years).

This study complies with the guidelines set out in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethical Committee in Human Research from Universidade Federal de Viçosa, Brazil (protocol number 185/2011). All subjects provided written informed consent. After an overnight fasting, the assessments were performed using standardized protocols and environmental conditions.

Anthropometrics and body composition

Anthropometric data and body composition were assessed by a single trained technician. Neck circumference was measured at the middle point of the neck's height [25]. Waist circumference was measured at the midpoint between the iliac crest and the last rib. The sagittal abdominal diameter was measured with an abdominal caliper (Holtain Kahn Abdominal Caliper®). With the subject in supine position, the sagittal abdominal diameter was measured at the same position in waist circumference. Hip circumference was measured at the largest point between the waist and thigh. Thigh circumference was measured at the midpoint between the inguinal crease and the proximal border of the patella with the subject in the standing position, and with the right leg slightly bent [25]. The conicity index and the sagittal index were calculated according to Valdez et al. [26] and Kahn et al. [27], respectively.

$$\text{Conicity index} = \text{waist circumference (cm)}/0.109 \times \sqrt{\text{weight (kg)}}/\text{height (cm)}$$

$$\text{Sagittal index} = \text{sagittal abdominal diameter (cm)}/\text{thigh circumference (cm)}$$

Body fat distribution was measured by the dual-energy X-ray absorptiometry (DXA; Lunar Prodigy Advance DXA System, GE Lunar), which gives a report differentiating discriminating trunk, android, and gynoid adiposity. The trunk region included the neck, chest, abdominal, and pelvic areas. The android region was the area between the ribs and the pelvis, and was completely enclosed by the trunk region. The gynoid region included the hips and upper thighs, and overlapped both the leg and trunk regions.

Energy expenditure

Subjects were instructed to abstain from caffeine and alcohol consumption, to refrain from heavy physical activity, and to maintain a regular sleep–wake schedule (8 h/night) during the 72 h before test day. Respiratory gas exchange was measured over 30 min under fasting conditions by indirect calorimetry using a ventilated respiratory canopy (Deltatrac II, MBM-200; Datex Instrumentarium Corporation) in compliance with the manufacturer guidelines. Then, the resting energy expenditure (REE) was obtained. The subject's daily energy requirement was calculated by multiplying the measured REE by a physical activity factor [28]. The physical activity factor was determined through the Portuguese version of the International Physical activity Questionnaire validated by Pardini et al. [29].

Dietary intake assessment

Subjects provided 3-day food records (2 non-consecutive week days and 1 weekend day). A dietitian reviewed the food records with the subjects to check for errors or omissions. All of the food records were analyzed by the same dietitian using Dietpro 5.2i software (Agromídia, Viçosa, Brazil).

Biochemical analysis

Blood samples were drawn from an antecubital vein after a 12-h overnight fast. Plasma-EDTA and serum were separated from blood through centrifugation (2.200×*g*, 15 min, 4 °C) and stored at −80 °C.

Serum glucose, total cholesterol, HDL cholesterol, triglycerides, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase were analyzed through enzymatic colorimetric methods using commercial kits (Quibasa-Química Básica, Brazil) in an autoanalyzer (COBAS MIRA Plus; Roche Diagnostic Systems). The high-sensitivity C-reactive protein was also analyzed using a commercial kit (Quibasa-Química Básica, Brazil) in an autoanalyzer by an immunoturbidimetric assay.

LDL cholesterol and VLDL cholesterol concentrations were calculated according to the Friedewald et al. [30] formula. Serum insulin concentrations were analyzed through an electrochemiluminescence immunoassay (Elecsys-Modular E-170, Roche Diagnostics Systems). The homeostasis model assessment of insulin resistance (HOMA-IR) and the homeostasis model assessment of beta cell function (HOMA-B) were calculated according to Matthews et al. [31].

Plasma LPS concentrations were determined through a chromogenic method using a Limulus Amebocyte Lysate (LAL) commercial kit (Hycult Biotech, The Netherlands). Undiluted plasma samples were heated at 75 °C for 5 min to neutralize endotoxin inhibitors. Aliquots (50 µl) of plasma and standards were added to the pyrogen-free microplate. The LAL reagent (50 µl) was added to each well. After a 30-min incubation, the absorbance at 405 nm was read (Multiskan Go, Thermo Scientific, USA). When the optical density of the 10 and 4 EU/ml standards differed by <10 %, the reaction was interrupted by adding 50 µl of the stop solution (acetic acid) and the absorbance was read again. Since absorbance is directly proportional to the concentration of endotoxin, a standard curve was used to calculate the LPS concentration in the samples. The concentration of LPS was expressed as endotoxin units per milliliter (EU/ml).

Statistical analysis

Statistical analyses were performed using SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA). Parametric and nonparametric tests were used based on the results from the Shapiro–Wilk tests of normality. A 5 % level of significance was used. Data are presented as mean ± SEM. To determine the influence of LPS, subjects were divided into two groups according to median plasma LPS values: greater than and equal to 0.9 EU/ml or lower than 0.9 EU/ml. The Student's *t* test or Mann–Whitney test were used to compare the groups. Spearman correlation analyses were run to study the correlation between the variables. After data analysis, the statistical power of the comparisons was calculated and values >99 % were found, thus confirming that the number of volunteers was sufficient to ensure the statistical power needed.

Results

General characteristics of the subjects

Overall anthropometric, body composition, and biochemical parameters are shown in Table 1. From the total group ($n = 67$), 61.2 % ($n = 41$) of the volunteers were overweight and the others were obese. Furthermore, 94 % ($n = 63$) of the volunteers had total body fat >25 %. The

Table 1 Clinical, anthropometric, body composition, and biochemical characteristics of the study participants ($n = 67$)

	Mean ± SEM
Daily energy requirements (kcal/day)	3,049.8 ± 56.9
Physical activity level	1.6 ± 1.5
Systolic blood pressure (mmHg)	120.0 ± 2.0
Diastolic blood pressure (mmHg)	70.6 ± 2.2
BMI (kg/m^2)	29.7 ± 0.3
Waist circumference (cm)	101.4 ± 0.9
Body fat (%)	34.0 ± 0.6
Total fat mass (kg)	31.3 ± 0.9
Total fat-free mass (kg)	63.5 ± 0.7
Total lean mass (kg)	60.0 ± 0.7
Glucose (mmol/l)	5.1 ± 0.1
Insulin (μU/ml)	9.7 ± 8.6
HOMA-IR	2.3 ± 0.2
HOMA-B	120.3 ± 7.9
Total cholesterol (mmol/l)	4.9 ± 0.1
VLDL cholesterol (mmol/l)	0.7 ± 0.0
LDL cholesterol (mmol/l)	3.1 ± 0.1
HDL cholesterol (mmol/l)	1.1 ± 0.0
Triglycerides (mmol/l)	1.6 ± 0.1
Uric acid (μmol/l)	338.6 ± 11.9
AST (UI/l)	38.3 ± 2.1
ALT (UI/l)	26.5 ± 1.8
Alkaline phosphatase (UI/l)	55.8 ± 2.6
hsCRP (mg/dl)	1.5 ± 0.2
LPS (EU/ml)	1.3 ± 0.1

majority (95.5 %, $n = 64$) had an abnormal waist circumference higher than 90 cm. The biochemical profile revealed that 47.8 % ($n = 32$) of the men had HDL cholesterol concentrations <1.0 mmol/l, 34.3 % ($n = 23$) had triglycerides ≥1.7 mmol/l, and 20.9 % ($n = 14$) had elevated fasting blood glucose (≥5.55 mmol/l). In addition, 25.4 % ($n = 17$) had high blood pressure (systolic ≥130 and/or diastolic ≥85 mmHg). Metabolic syndrome occurred in 37.3 % ($n = 25$) of the subjects according to the International Diabetes Federation criteria [32].

Characterization of subjects below and above the median LPS concentration

Plasma LPS was classified as above/equal or below the median (0.9 EU/ml) concentration presented by the subjects. The subjects' anthropometrics, body compositions, clinical and biochemical characteristics, and energy intake, macronutrient, and dietary fiber consumption are shown in Tables 2, 3, and 4.

The group with the higher LPS concentration had significantly higher sagittal abdominal diameter, trunk fat percentage, android fat percentage, and android fat mass, and lower android fat-free mass, and android lean mass. They also had higher insulin, HOMA-IR, HOMA-B, and ALT than the group with lower plasma LPS. The energy intake, macronutrient, and fiber consumption was similar between the groups ($p > 0.05$). Blood pressure, physical activity level, and daily energy requirements were not significantly different between the groups (data not shown).

Correlation among LPS concentration, fat distribution, and biochemical variables

Correlations were calculated between LPS and the outcome variables. Significant associations are reported in Table 5. The plasma LPS concentration correlated positively with trunk fat percentage, android fat mass, and percentage. In addition, the plasma LPS concentration correlated positively with serum insulin, AST, ALT, alkaline phosphatase, HOMA-IR, and HOMA-B. The other biochemical parameters, as well as anthropometric, body composition, REE, and dietary intake data, were not significantly correlated with LPS concentrations.

Discussion

The impact of total adiposity and of different locations of fat depots over metabolic abnormalities is difficult to characterize [33]. However, for subjects with similar BMIs, it is still possible to identify those considered 'metabolically healthy obese' from the 'at risk' subjects. Visceral adipose tissue, degree of insulin sensitivity, and expression of inflammatory markers are determinants for the distinction of these categories [34]. Since LPS is involved in inflammatory activation and may influence intra-abdominal fat expansion [35], we investigated whether LPS concentrations could be used to discriminate the phenotype presented by obese individuals with similar BMIs and total adiposity.

The data reveal that despite having similar weights, BMIs, waist circumferences, and total body fat, subjects with higher plasma LPS concentrations presented a less favorable phenotype than subjects with lower LPS concentrations. The 'less favorable phenotype' was characterized by higher android and trunk adiposity, and higher fasting insulin, HOMA-IR, and HOMA-B, and ALT.

Traditionally, waist circumference and BMI are indices that reflect excess abdominal and overall adiposity, respectively, and are usually considered good predictors of cardiometabolic risk factors [36]. In the present study, these indices were similar between subjects with slightly different phenotypes, especially regarding the insulin resistance markers. Contradictory results regarding the association of these indices with LPS concentration have been reported

Table 2 Anthropometric and body composition characteristics of excessive body weight men according to plasma LPS concentration

	LPS < 0.9 EU/ml ($n = 33$)	LPS ≥ 0.9 EU/ml ($n = 34$)	p value
Body weight (kg)	94.9 ± 2.0	93.5 ± 1.7	0.589
BMI (kg/m^2)	29.8 ± 0.4	29.7 ± 0.4	0.468
Neck (cm)	41.0 ± 0.4	40.8 ± 0.4	0.757
Waist circumference (cm)	101.3 ± 1.3	101.4 ± 1.3	0.960
Sagittal abdominal diameter (cm)	22.5 ± 0.4	23.4 ± 0.4	0.045
Hip (cm)	108.8 ± 1.0	108.8 ± 0.8	0.981
Thigh (cm)	57.6 ± 0.6	57.5 ± 0.6	0.498
Waist-to-height	56.8 ± 0.7	57.3 ± 0.8	0.387
Waist-to-hip	0.9 ± 0.0	0.9 ± 0.0	0.947
Waist-to-thigh	1.8 ± 0.0	1.8 ± 0.0	0.382
Conicity index	127.5 ± 0.8	128.2 ± 1.0	0.623
Sagittal index	0.4 ± 0.0	0.4 ± 0.0	0.056
Body composition (DXA)			
Total body fat (%)	33.3 ± 0.9	34.7 ± 0.9	0.285
Total fat mass (kg)	31.0 ± 1.3	31.7 ± 1.2	0.687
Total fat-free mass (kg)	64.6 ± 1.1	62.4 ± 1.0	0.132
Total lean mass (kg)	61.1 ± 1.0	58.9 ± 1.0	0.127
Trunk fat (%)	35.5 ± 1.1	38.8 ± 1.0	0.035
Trunk fat mass (kg)	15.5 ± 0.8	17.0 ± 0.8	0.179
Trunk fat-free mass (kg)	28.3 ± 0.5	27.4 ± 0.5	0.222
Trunk lean mass (kg)	27.2 ± 0.5	26.3 ± 0.5	0.204
Gynoid fat (%)	39.4 ± 1.0	40.0 ± 0.9	0.661
Gynoid fat mass (kg)	5.3 ± 0.2	5.3 ± 0.2	0.864
Gynoid fat-free mass (kg)	8.7 ± 0.2	8.3 ± 0.2	0.153
Gynoid lean mass (kg)	8.2 ± 0.2	7.9 ± 0.2	0.185
Android fat (%)	33.3 ± 1.3	38.4 ± 1.2	0.004
Android fat mass (kg)	2.1 ± 0.1	2.5 ± 0.1	0.043
Android fat-free mass (kg)	4.3 ± 0.1	4.0 ± 0.1	0.035
Android lean mass (kg)	4.2 ± 0.1	3.9 ± 0.1	0.034

Data presented as mean ± SEM

Table 3 Biochemical characteristics of excessive body weight men according to plasma LPS concentration

	LPS < 0.9 EU/ml ($n = 33$)	LPS ≥ 0.9 EU/ml ($n = 34$)	p value
Glucose (mmol/l)	5.1 ± 0.1	5.2 ± 0.1	0.358
Insulin (μU/ml)	8.4 ± 0.9	10.9 ± 1.0	0.022
HOMA-IR	1.9 ± 0.2	2.3 ± 0.6	0.027
HOMA-B	106.1 ± 11.2	134.0 ± 10.8	0.017
Total cholesterol (mmol/l)	4.6 ± 0.2	5.0 ± 0.2	0.128
VLDL cholesterol (mg/dl)	0.6 ± 0.1	0.7 ± 0.0	0.056
LDL cholesterol (mmol/l)	2.9 ± 0.2	3.2 ± 0.2	0.261
HDL cholesterol (mmol/l)	1.1 ± 0.1	1.1 ± 0.0	0.221
Triglycerides (mmol/l)	1.5 ± 0.2	1.8 ± 0.2	0.064
Uric acid (μmol/l)	338.6 ± 11.9	338.6 ± 11.9	0.356
AST (UI/l)	34.9 ± 2.1	41.6 ± 3.6	0.139
ALT (UI/l)	22.2 ± 2.2	30.7 ± 2.8	0.001
Alkaline phosphatase (UI/l)	56.0 ± 4.4	55.7 ± 3.0	0.217
hsCRP (mg/dl)	1.3 ± 0.2	1.6 ± 0.3	0.183

Data presented as mean ± SEM

Table 4 Energy intake, macronutrient, and dietary fiber consumption of excessive body weight men according to plasma LPS concentration

	LPS < 0.9 EU/ml ($n = 33$)	LPS ≥ 0.9 EU/ml ($n = 34$)	p value
Energy (kcal/day)	2,726.9 ± 137.6	2,863.2 ± 95.1	0.118
Carbohydrate (%)	52.7 ± 1.1	51.8 ± 1.0	0.579
Protein (%)	16.7 ± 0.6	16.4 ± 0.4	0.741
Fat (%)	30.7 ± 1.0	31.7 ± 0.9	0.430
Dietary fiber (g/day)	27.6 ± 2.4	26.9 ± 1.3	0.224

Data presented as mean ± SEM

Table 5 Correlation coefficient between plasma LPS concentration, body composition, and biochemical variables

	LPS	
	r	p
Trunk fat (%)	0.26	0.033
Android fat mass (kg)	0.26	0.034
Android fat (%)	0.34	<0.004
Insulin (μU/ml)	0.28	0.021
HOMA-IR	0.27	0.029
HOMA-B	0.30	0.013
AST (UI/l)	0.26	0.030
ALT (UI/l)	0.41	<0.001
Alkaline phosphatase (UI/l)	0.25	0.048

Correlations were determined by Spearman's correlation test

previously [21, 37]. We showed that sagittal abdominal diameter, an unusual indicator of abdominal obesity, distinguished subjects with different concentrations of plasma LPS. It has been claimed that sagittal abdominal diameter measured in the supine position reflects mainly visceral adipose tissue [25]. As previously mentioned, regional adipose tissue distribution is a relevant physical characteristic to be considered in the clinical evaluation of an individual's metabolic risk, especially because visceral (or intra-abdominal) fat depots are functionally and metabolically different from subcutaneous depots. The visceral depot is considered more hazardous and is associated with metabolic alterations, including insulin resistance [38, 39]. The present finding that plasma LPS concentrations were not associated with total body fat, but were positively associated with android and trunk fat (central region) reinforces the possible influence of LPS on fat distribution. Unfortunately, the use of BMI, waist circumference, sagittal abdominal diameter, and adiposity measurements using DXA does not allow proper discrimination between subcutaneous and visceral adipose tissue at the central location.

Trøseid et al. [12] presented evidences that plasma LPS concentrations were more strongly correlated with intra-abdominal fat than with subcutaneous fat volumes. The mechanisms underlying this association are unclear. The cross-sectional nature of most studies, including ours, does not allow for determination of whether visceral depots increase due to excessive energy intake or to higher gut-derived LPS. They also preclude determining whether a higher degree of insulin resistance arises before or after visceral adiposity accumulation. Since subjects from the present study had similar energy and macronutrient intakes, it is possible that higher LPS concentrations influence the accumulation of central fat and insulin resistance. A current hypothesis holds that translocation of gut-derived molecules to adipose tissue localized in close proximity to the gut, such as mesenteric fat, would trigger macrophage infiltration and inflammation, which, in turn, would stimulate expansion of this visceral depot [12, 35]. The downstream signaling of the insulin receptor can be impaired by inflammatory signals, which can be directly induced by LPS stimulation [5, 6]. Mesenteric fat expresses higher concentrations of proinflammatory chemokines than other sites of adipose tissue in obese mice [40]. It is also still unclear whether inflammatory activation occurs before or after specific adipose tissue expansion and insulin resistance establishment.

Interestingly, subjects with higher LPS concentrations had simultaneously higher central fat, fasting insulin, HOMA-IR, and HOMA-B. In addition, a positive correlation between plasma LPS and markers of insulin resistance was observed. This finding is in agreement with other studies reporting a relationship between plasma LPS and biomarkers of insulin signaling [19, 21, 23]. The visceral localization and hypertrophy of intra-abdominal adipocytes are often related to the development of systemic insulin resistance through higher delivery of fatty acids to ectopic sites, such as the liver, and muscles. The induced lipotoxicity in these sites would, in turn, impair proper insulin signaling [39]. The pathophysiological sequence of events that leads to insulin resistance based on regional fat distribution and an interactive influence of LPS remains to be established in humans.

It should be noted that we were expecting that the prevalence of metabolic syndrome would be higher in the group with the higher LPS concentration. However, the prevalence did not differ (data not shown) between groups. Since the volunteers are young adults, it is possible that the less favorable phenotype associated with higher LPS concentrations would increase the prevalence of metabolic syndrome in the long term. Longitudinal studies would be of great interest to test this hypothesis.

Finally, we verified that AST, ALT, and alkaline phosphatase enzymes were positively associated with plasma LPS, even though the mean values observed remained within normal ranges. AST and ALT are markers of liver

injury [41]. Since the liver is responsible for circulating LPS clearance, higher LPS concentrations may negatively influence hepatic cells and increase the release of hepatic enzymes [42, 43]. The injection of LPS in animals increases concentrations of ALT and AST in the blood [44]. In addition, the fatty liver, as a part of unfavorable adipose tissue distribution, may explain the association of these enzymes with plasma LPS. The correlation between alkaline phosphatase and LPS may be explained by the function of this enzyme in the dephosphorylation of the LPS, reducing the toxicity of lipid A by 100-fold [45, 46].

In conclusion, our data indicate that for men with a similar body size and total fatness, there are overweight/obese men with less favorable phenotypes as predicted by LPS concentrations. This is characterized by higher central adiposity, higher values of HOMA-IR, and beta cell function impairment. Our study design does not permit the establishment of causality between LPS, central obesity, and insulin resistance. However, it corroborates the view that fat distribution, in particular android/trunk fat, is a useful clinical marker to identify overweight/obese subjects who are potentially at increased risk for metabolic abnormalities in the long term. The potential role of LPS on adipose tissue distribution and expansion, and how this may impact insulin sensitivity, needs further investigation.

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