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Reliability of plasma lipopolysaccharide-binding protein (LBP) from repeated measures in healthy adults

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Abstract

Plasma lipopolysaccharide-binding protein (LBP), a measure of internal exposure to bacterial lipopolysaccharide, has been associated with several chronic conditions and may be a marker of chronic inflammation; however, no studies have examined the reliability of this biomarker in a healthy population. We examined the temporal reliability of LBP measured in archived samples from participants in two studies. In Study one, 60 healthy participants had blood drawn at two time points: baseline and follow-up (either three, six, or nine months). In Study two, 24 individuals had blood drawn three to four times over a seven-month period. We measured LBP in archived plasma by ELISA. Test–retest reliability was estimated by calculating the intraclass correlation coefficient (ICC). Plasma LBP concentrations showed moderate reliability in Study one (ICC 0.60, 95 % CI 0.43–0.75) and Study two (ICC 0.46, 95 % CI 0.26–0.69). Restricting the follow-up period improved reliability. In Study one, the reliability of LBP over a three-month period was 0.68 (95 % CI: 0.41–0.87). In Study two, the ICC of samples taken seven days apart was 0.61 (95 % CI 0.29–0.86). Plasma LBP concentrations demonstrated moderate test–retest reliability in healthy individuals with reliability improving over a shorter follow-up period.

Keywords

Lipopolysaccharide-binding protein (LBP); Reliability; ICC

Introduction

Lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria, has been implicated as an underlying factor of obesity-driven low-grade inflammation [1]. However, LPS, as measured by the limulus amebocyte lysate (LAL) assay, is limited by its lack of sensitivity [2]. In addition, technical difficulties with the assay, the need to collect samples

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under LPS-free conditions, and fluctuations in LPS throughout the day make it difficult to measure circulating LPS in large-scale studies [2, 3]. Alternatively, lipopolysaccharide-binding protein (LBP), an endogenous protein that binds to LPS and transfers LPS monomers to “Cluster of Differentiation 14” (CD14), has been used as a proxy to assess chronic endotoxemia status and immune responses to it [4, 5]. Recent studies have suggested that LBP concentrations are associated with high-fat diets, obesity, and chronic diseases [5–11]. Despite these promising results suggesting that LBP may be a marker for disease risk of a range of pathologic conditions, no studies have examined the temporal reliability of the biomarker. It is important to establish the reliability of LBP, given that the biomarker is an acute-phase protein and may be influenced by short-term effects, such as diet or infection. Thus, the purpose of this study was to examine the reliability of LBP within healthy adults over time.

Methods

The analyses were conducted using stored plasma samples collected as part of two completed studies: the Multiethnic Cohort (MEC) Reliability Study and the “Enzyme Activation Trial two” (2EAT). The MEC includes men and women primarily from five different racial-ethnic groups (African Americans, Japanese Americans, Latinos, Native Hawaiians, and whites in Hawaii and California) aged 45–75 at recruitment and aged 60 and older at blood draw. The MEC Reliability Study (designated Study one) was an observational study designed to collect fasting repeat blood samples from a group of volunteers of the same ethnic and age groups as MEC participants in Hawaii [12]. Sixty healthy volunteers had blood drawn after an overnight fast at two different time points: baseline and follow-up (either at three, six, or nine months) and evenly distributed as much as possible by sex and time interval.

The 2EAT study (designated Study two) was a randomized, crossover, controlled feeding study designed to test the effects of vegetable diets on biotransformation enzyme activity and other biomarkers of cancer susceptibility in healthy adults [13]. Men and women, aged 20–40 years of white and Asian ethnicity, were recruited and randomized to four different controlled diet periods. Exclusions were made for health conditions known to influence inflammation [e.g., chronic disease, medication use, heavy alcohol consumption, smoking, and obesity (body mass index (BMI) $>30 \text{ kg/m}^2$)]. There was a washout period of 21 days or longer between each controlled diet period. Blood was drawn in the morning after a 12 h, overnight fast and plasma was obtained, aliquoted, and stored at -80°C . For the current reliability study, we used blood samples drawn at baseline and at the end of each washout period (before the start of each controlled intervention period).

LBP concentrations were measured using a commercial ELISA kit (Cell Sciences Inc), samples were diluted 1:1000, and the assay was conducted according to kit protocol with a standard curve of 5–50 $\mu\text{g/mL}$. Samples from different time points were run in the same batch to control for temporal measurement differences. Additionally, samples were run in duplicate. Based on blinded testing of quality control of samples, the median duplicate intra- and inter-assay coefficient of variation (CV) in our laboratory was 4.7 and 12.5 %, respectively.

Statistical analysis

LBP concentrations were log-transformed to normalize distributions. The temporal reliability was estimated by the intraclass correlation coefficient (ICC). We used the interpretation by Rosner, where the ICC is considered high at >0.75 , moderate at $0.4\text{--}0.74$, and poor at <0.40 [14, 15]. The ICC was calculated using a multi-level measurement model where blood draws were nested within individuals. We also calculated the number of repeated measures that would be needed to yield a reliability measure (Cronbach α) of 0.75 . All analyses were conducted using Stata, v12.0 (StataCorp).

Results

Table 1 gives the demographic characteristics of the participants in Studies one and two. Table 2 gives LBP concentrations at each blood draw and the associated ICC coefficients. Plasma LBP concentrations showed moderate temporal reliability in both Study one (ICC 0.60 , 95 % CI $0.43\text{--}0.75$) and Study two (ICC 0.46 , 95 % CI $0.26\text{--}0.69$) (Table 2). In Study two, the mean time between baseline visit and follow-up visits one, two, and three was 18 days (range 3–109 days), 71 days (range 39–158), and 117 days (range 76–196 days), respectively. Restricting the followup period to the shortest time period in each study (i.e., three months for Study one and seven days for Study two) improved reliability. In Study one, the test–retest reliability of LBP over a three-month period was 0.68 (95 % CI $0.41\text{--}0.87$). In Study two, the ICC of samples taken seven days apart was 0.61 (95 % CI $0.29\text{--}0.86$). Three measures of LBP would need to be averaged to achieve a reliability coefficient of 0.75 based on the results from Study one, and four measures would be needed to obtain a reliability coefficient of 0.75 based on Study two.

Discussion

Plasma LBP concentrations demonstrated moderate test–retest reliability in healthy adults, with reliability appearing to be better in multiple blood samples collected over a shorter follow-up period. To our knowledge, this is the first study to measure the longer-term temporal reproducibility of LBP in a healthy population.

Circulating LBP concentrations vary in both acute (e.g., infection, injury) and chronic inflammatory conditions (e.g., autoimmune disease, obesity, cardiovascular disease, cancer). Therefore, it is important to determine whether circulating concentrations of the marker reflect only the short-term physiologic state or whether they represent an individual's average concentration over time, relative to other individuals. LBP concentrations are thought to be affected by a wide range of covariates, including age, body mass, smoking, alcohol use, diet, particularly saturated fat and dietary fiber intake, physical activity level, and infection. While variations in LBP concentrations due to acute conditions are a concern when attempting to capture long-term levels of LBP, in our study, obtaining blood from healthy individuals after an overnight fast helped to minimize the influence of short-term factors which may affect LBP concentrations.

Advantages of this study include use of two studies, which included participants of different ethnicities and ages and followed individuals for varying time periods. While Study one

followed individuals for a longer time period, Study two benefited from having three or more blood draws over varying time periods for each participant. Additionally, fasting samples were obtained for healthy individuals, at the same time of day, thus reducing the likelihood of obtaining a blood sample influenced by diet. The main limitation of our results is that the studies analyzed provided information on the temporal reliability of LBP over weeks and months, while in certain types of studies, particularly long-term prospective studies, one needs to understand whether the measures used reflect biomarker levels over years or decades. Moreover, not all participants completed all four diet periods in Study two, resulting in fewer participants at the beginning of the fourth diet period. Stratified analyses examining reliability by race and sex were not possible given the small sample sizes in the studies. As such, additional studies in larger populations are warranted. Lastly, given that the population demographics differed by study, there is a chance that these underlying differences may have contributed to differences in the mean concentrations and temporal variations of LBP between studies.

In conclusion, we found that circulating concentrations of LBP showed moderate temporal reliability up to a nine-month period. This suggests that a single measurement of these biomarkers may be used for risk assessment in short-term studies; longitudinal studies capturing exposure over a multiple-year period may be needed in order to obtain more stable estimates. Future studies are needed on the reliability of LBP measures over a longer duration, as are studies of the potential of LBP as a marker of chronic inflammation and cancer risk.

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Table 1

Personal and study characteristics of participants from Study one and Study two

	<i>n</i> (%)
Study one (<i>n</i> = 60)	
Sex	
Male	29 (48.3)
Female	31 (51.7)
Race/ethnicity	
White	46 (76.7)
Japanese	12 (20.0)
Hawaiian	2 (3.3)
Age, years	
65	30 (50)
>65	30 (50)
Study two (<i>n</i> = 24)	
Sex	
Male	8 (33.3)
Female	16 (66.7)
Race/ethnicity	
White	12 (50)
Asian	8 (33.3)
Other	4 (16.7)
Age, years	
30	11 (45.8)
>30	13 (54.2)

Table 2

Mean concentrations and intraclass correlation (ICC) of circulating lipopolysaccharide-binding protein (LBP) at baseline and follow-up

	Mean concentrations (sd) µg/mL	Range µg/mL	ICC (95 % CI)	Within-person CV	Between person CV
Study one					
Overall (<i>n</i> = 60)			0.60 (0.43–0.75)	0.22 (0.18–0.26)	0.27 (0.21–0.34)
Baseline	34.5 (13.4)	13.8–94.3			
Follow-up	34.4(13.9)	14.7–97.3			
Three months (<i>n</i> = 18)			0.68 (0.41–0.87)	0.22 (0.16–0.31)	0.32 (0.22–0.49)
Baseline	38.6 (18.2)	13.8–94.4			
Follow-up	37.6 (14.0)	17.0–76.2			
Six months (<i>n</i> = 19)			0.51 (0.21–0.81)	0.21 (0.16–0.30)	0.22 (0.13–0.36)
Baseline	30.3 (8.3)	15.1–47.0			
Follow-up	30.9 (11.8)	14.7–66.8			
Nine months (<i>n</i> = 23)			0.52 (0.25–0.78)	0.22 (0.16–0.29)	0.23 (0.15–0.36)
Baseline	34.8 (12.0)	16.1–64.4			
Follow-up	34.8 (15.2)	21.0–97.3			
Study two					
Overall (<i>n</i> = 24)			0.46 (0.26–0.69)	0.26 (0.22–0.31)	0.25 (0.17–0.36)
Baseline	27.3 (10.1)	15.8–50.0			
Follow-up visit one (<i>n</i> = 24)	24.6 (8.6)	13.5–37.6			
Follow-up visit two (<i>n</i> = 24)	28.2 (14.3)	14.4–79.3			
Follow-up visit three (<i>n</i> = 21)	27.6 (14.2)	13.3–77.8			
Visits Seven days apart (<i>n</i> = 14)			0.61 (0.29–0.86)	0.21 (0.14–0.30)	0.26 (0.16–0.43)
Baseline	26.7 (9.5)	15.8–49.0			
Follow-up visit one	25.1 (8.7)	14.1–37.6			