

Relationship between plasma levels of zonulin, bacterial lipopolysaccharides, D-lactate and markers of inflammation in haemodialysis patients

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Background: Accelerated atherosclerosis is one of the main causes of cardiovascular morbidity and mortality in haemodialysis (HD) patients with chronic kidney disease. Chronic systemic inflammation, partially related to increased permeability of the intestinal wall may contribute to the increased cardiovascular risk in HD patients [1]. Impaired structure and function of the intestinal barrier related to dysbiosis may increase the penetration of bacteria derived toxins (including lipopolysaccharides), or even viable bacteria into the blood and can induce systemic inflammation [2]. The presence of bacterial DNA was also associated with significantly higher levels of highly sensitive CRP, IL-6, and D-lactate [3]. Recently zonulin, a new marker potentially useful for the evaluation of the permeability of intestinal wall, was described [4]. The aim of this study was to evaluate the association between markers of intestinal permeability and inflammation in HD patients.

Methods: Plasma concentration of zonulin, haptoglobin, TNF- α , IL-6, D-lactates and bacterial lipopolysaccharides (LPS) were assessed in blood samples obtained after overnight fast before midweek morning HD session in 150 stable, prevalent HD patients (Tab. 1 - demographic and clinical characteristics). Daily intake of energy and macronutrients was assessed on the basis of a food frequency questionnaire (Tab. 2).

Results: Inflammatory markers (serum hsCRP, plasma TNF α) were slightly elevated (Tab. 3). Serum hsCRP level was increased in over 70% of patients. Plasma levels of zonulin, IL-6, and TNF α were significantly higher in HD than in normal weight healthy subjects [5] [11.6(10.9 – 12.3) vs 5.4(4.8 – 6.8) ng/mL, 6.2(1.0 – 10.3) vs 1.0(0.8 – 2.1) pg/mL, 5.9(2.9 – 11.8) vs 1.3(0.9 – 1.6), respectively]. Our study shows elevated plasma levels of zonulin in HD patients (Fig. 1) even after comparison to obese subjects [8.2 (7.1-8.4) ng/mL]. The greatest severity of inflammation was found in the highest tertile of zonulin, LPS, and D-lactates concentrations (Tab. 4), only the correlation between plasma IL-6 and D-lactate acid and LPS, but not zonulin was significant. D-lactates level was weakly associated with IL-6 (R=0.175; p=0.03) (Fig. 2) and also a borderline correlation between plasma levels of zonulin and serum hsCRP concentration (R=0.159; p=0.07) was found. There was no correlation between concentrations of LPS, D-lactates, and zonulin and daily energy, as well as macronutrient intake.

Table 1. Demographic and clinical characteristics of 150 haemodialysis patients (mean & 95% CI).

Age (years)	62 (59 - 64)
Gender (male/female)	92 / 58
Body mass index (kg/m ²)	26.1 (25.2-26.9)
Obesity (BMI \geq 30 kg/m ²) (n/%)	28 / 18.7
Primary cause of CKD (n/%)	
Diabetes	42 / 28.0
Hypertension	17 / 11.3
Nephrolithiasis	8 / 5.3
Autosomal Dominant Polycystic Kidney Disease (ADPKD)	10 / 6.7
Ischemic nephropathy	2 / 1.3
Glomerulonephritis	24 / 16.0
Interstitial nephritis	13 / 8.5
Other or unknown	34 / 22.7
Time on dialysis (months)	48 (41-56)
Kt/V (per HD session) [^]	1.05 (1.01-1.08)
Co-morbidity (%)	
Hypertension	136 / 90.7
Diabetes	55 / 36.7
Coronary artery disease	83 / 55.3
Stroke	12 / 8.0
Past kidney transplantation	11 / 7.3
Pharmacotherapy (n/%)	
Antihypertensive	136 / 90.7
No of antihypertensive drugs (n)	2.0 (1.8-2.2)
Oral anti-diabetic	19 / 34.5*
Insulin	36 / 65.5*
Antiplatelet	77 / 51.3
Statins	60 / 40.0
Fibrates	0
Oral phosphorous binders	129 / 86.0
Carbonate calcium dose (g/day)	3.8 (3.4-4.3)
Sevelamer hydrochloride	4 / 2.6
Cinacalcet	17 / 11.3
Cinacalcet dose (mg/day)	79 (60-98)
Alfacalcidol	31 / 20.7

[^] mean value from last 6 months
* percentage of patients with diabetes

Table 2. Energy and macronutrients intake in 107 participants, who returned filled questionnaire (mean & 95% CI).

Daily energy intake (kcal/day)	1608 (1434-1781)
Daily energy intake (kcal/kg/day)	22.7 (20.3-25.1)
Carbohydrates (g/day)	202 (181-224)
Fat (g/day)	62 (54-70)
Proteins (g/day)	64 (57-71)
Proteins (g/kg/day)	0.91 (0.81-1.01)
Fiber (g/1000 kcal)	9.3 (8.8-9.9)

Table 3. Biochemical characteristics and the study parameters [(mean & 95% CI or *median (25 – 75 percentile)].

Haemoglobin (g/dL) [^]	10.8 (10.6 – 11.0)
Total cholesterol (mg/dL)	169 (160 – 178)
LDL cholesterol (mg/dL)	90 (84 – 95)
HDL cholesterol (mg/dL)	28 (26 – 29)
Triglycerides (mg/dL)	169 (160 – 178)
Calcium (mg/dL) [^]	5.77 (5.52 – 6.02)
Phosphorous (mmol/L) [^]	8.57 (8.44 – 8.70)
Parathyroid hormone (pg/mL)	449 (380 – 519)
Hs-CRP (mg/L)*	4.82 (2.33 – 11.60)
Interleukin-6 (pg/mL)*	6.20 (1.01 – 10.32)
Tumour necrosis factor- α (pg/mL)*	5.94 (2.93 – 11.80)
Bacterial lipopolysaccharides (ng/mL)	27.7 (22.1 – 36.9)
D-lactates (μ g/mL)*	1.47 (0.94 – 2.34)
Zonulin (ng/mL)	11.6 (10.9 – 12.3)
Haptoglobin (μ g/mL)*	1.37 (0.82 – 2.03)

[^] mean value from last 6 months

Table 4. Biochemical characteristics and the study parameters in tertile subgroups of zonulin, bacterial lipopolysaccharides and D-lactates [(mean & 95% CI or *median (25 – 75 percentile)].

	Zonulin (ng/mL)		
	Low tertile <9.8	Medium tertile	High tertile >13.1
Haemoglobin (g/dL)	10.6 (10.3 – 10.9)	10.8 (10.5 – 11.2)	10.9 (10.5 – 11.3)
Total cholesterol (mg/dL)	169 (155 – 183)	161 (146 – 176)	177 (161 – 193)
LDL cholesterol (mg/dL)	89 (80 – 99)	85 (75 – 94)	95 (84 – 106)
HDL cholesterol (mg/dL)	28 (26 – 30)	28 (26 – 31)	27 (24 – 30)
Triglycerides (mg/dL)	158 (134 – 182)	158 (120 – 196)	162 (134 – 190)
Hs-CRP (mg/L)*	3.96 (1.58 – 10.32)	4.04 (2.20 – 8.78)	5.76 (3.58 – 13.20) [^]
Interleukin-6 (pg/mL)*	6.39 (1.01 – 9.66)	4.08 (0.78 – 9.70)	8.35 (1.28 – 11.31)
Tumour necrosis factor- α (pg/mL)*	4.41 (2.36 – 8.26)	7.07 (3.12 – 15.60) [^]	7.01 (3.16 – 11.80) [^]
Bacterial lipopolysaccharides (ng/mL)*	26.9 (22.2 – 37.0)	28.5 (18.3 – 35.6)	27.0 (22.9 – 37.3)
D-lactates (μ g/mL)*	1.77 (0.96 – 2.62)	1.29 (0.91 – 2.21)	1.42 (0.98 – 2.30)
Haptoglobin (μ g/mL)*	1.37 (0.74 – 1.91)	1.27 (0.84 – 2.36)	1.64 (0.91 – 2.30)
	Bacterial lipopolysaccharides (ng/mL)		
	Low tertile <24.1	Medium tertile	High tertile >32.6
Haemoglobin (g/dL)	10.6 (10.3 – 10.9)	10.9 (10.6 – 11.2)	10.9 (10.4 – 11.3)
Total cholesterol (mg/dL)	171 (153 – 188)	176 (163 – 191)	159 (146 – 173)
LDL cholesterol (mg/dL)	87 (76 – 97)	95 (86 – 104)	87 (77 – 98)
HDL cholesterol (mg/dL)	27 (25 – 29)	29 (25 – 31)	27 (25 – 30)
Triglycerides (mg/dL)	178 (137 – 219)	153 (129 – 177)	146 (123 – 170)
Hs-CRP (mg/L)*	3.73 (1.80 – 11.04)	4.84 (3.42 – 9.08)	5.48 (2.58 – 15.92)
Interleukin-6 (pg/mL)*	3.48 (0.78 – 8.38)	6.29 (1.20 – 9.52)	9.11 (1.09 – 11.80) [^]
Tumour necrosis factor- α (pg/mL)*	4.94 (2.31 – 10.10)	6.46 (3.06 – 12.30)	8.26 (3.15 – 12.90)
D-lactates (μ g/mL)*	1.65 (0.97 – 2.38)	1.33 (0.95 – 2.34)	1.47 (0.94 – 2.21)
Haptoglobin (μ g/mL)*	1.43 (0.84 – 2.36)	1.33 (0.83 – 2.30)	1.37 (0.73 – 1.92)
Zonulin (ng/mL)	10.8 (9.7 – 11.9)	12.2 (11.2 – 13.2)	11.8 (10.1 – 13.4)
	D-lactates (μ g/mL)		
	Low tertile <1.14	Medium tertile	High tertile >2.0
Haemoglobin (g/dL)	10.7 (10.4 – 11.1)	10.7 (10.4 – 11.1)	10.9 (10.5 – 11.2)
Total cholesterol (mg/dL)	165 (153 – 177)	170 (153 – 186)	173 (156 – 189)
LDL cholesterol (mg/dL)	89 (80 – 98)	85 (76 – 95)	94 (83 – 106)
HDL cholesterol (mg/dL)	28 (25 – 30)	28 (25 – 30)	27 (25 – 30)
Triglycerides (mg/dL)	150 (126 – 174)	172 (137 – 208)	156 (124 – 187)
Hs-CRP (mg/L)*	4.84 (2.58 – 12.60)	5.20 (2.61 – 10.32)	3.87 (2.15 – 11.04)
Interleukin-6 (pg/mL)*	2.78 (0.86 – 9.66)	6.54 (0.68 – 10.33)	8.27 (1.95 – 10.80) [^]
Tumour necrosis factor- α (pg/mL)*	6.33 (3.15 – 15.63)	7.07 (2.48 – 12.20)	4.68 (2.59 – 10.20)
Bacterial lipopolysaccharides (ng/mL)*	27.5 (21.4 – 37.6)	27.7 (23.3 – 36.6)	28.0 (21.3 – 34.4)
Haptoglobin (μ g/mL)*	1.00 (0.71 – 1.73)	1.64 (1.03 – 2.33) [^]	1.39 (0.89 – 2.38) [^]
Zonulin (ng/mL)	11.4 (10.4 – 12.3)	12.7 (11.1 – 14.4)	10.7 (9.6 – 11.8)

[^]p<0.05; [^]p<0.01 vs first tertile

Conclusions:

1. Plasma zonulin concentration is elevated in HD patients. 2. The weak association between plasma D-lactate, but not zonulin and IL-6 levels indicates that intestinal flora overgrowth rather than increased intestinal permeability contribute very slightly to the chronic inflammation observed in HD patients

Figure 1. Plasma zonulin concentration in 150 haemodialysis patients and 30 normal weight healthy subjects [5].

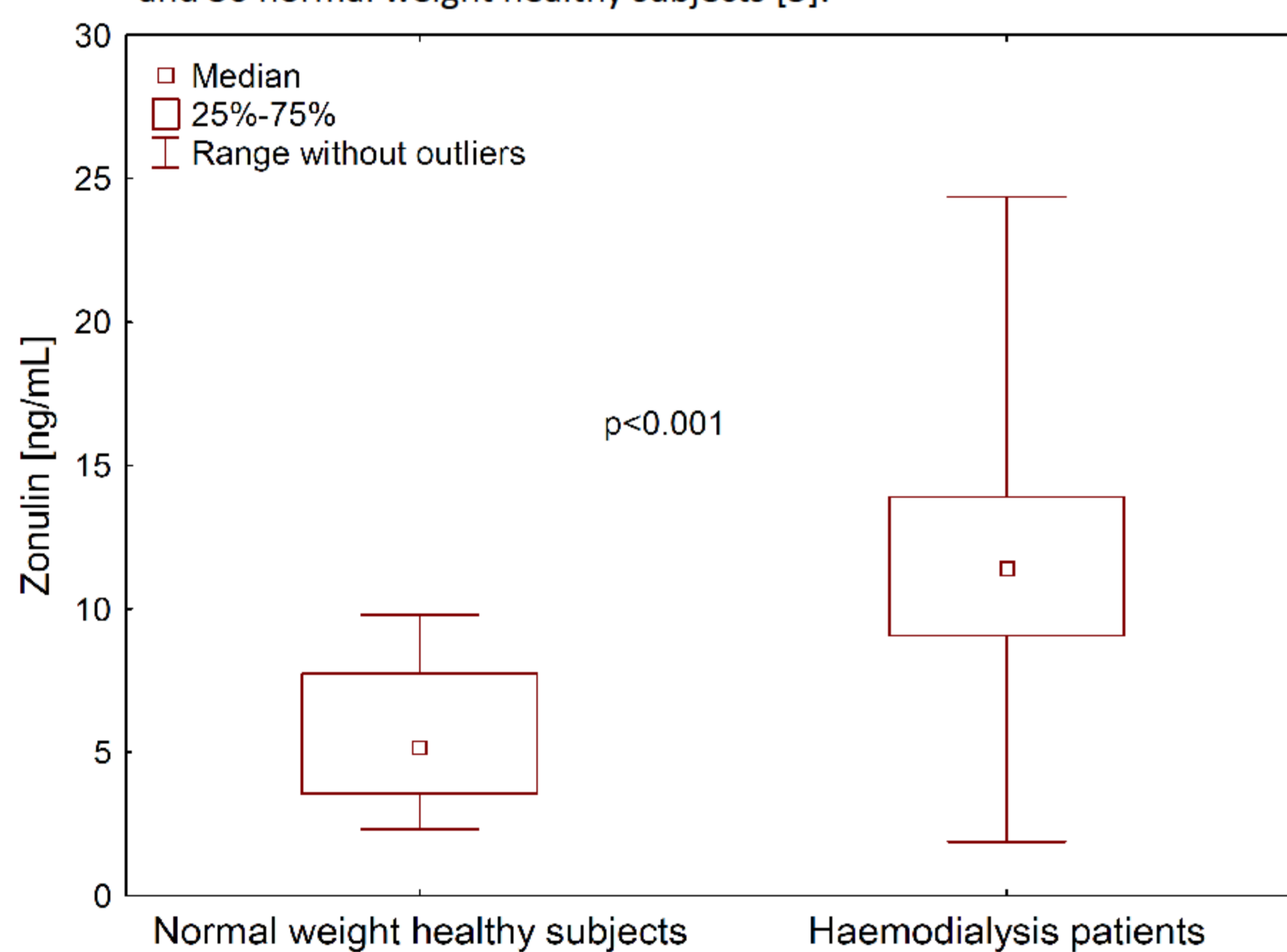
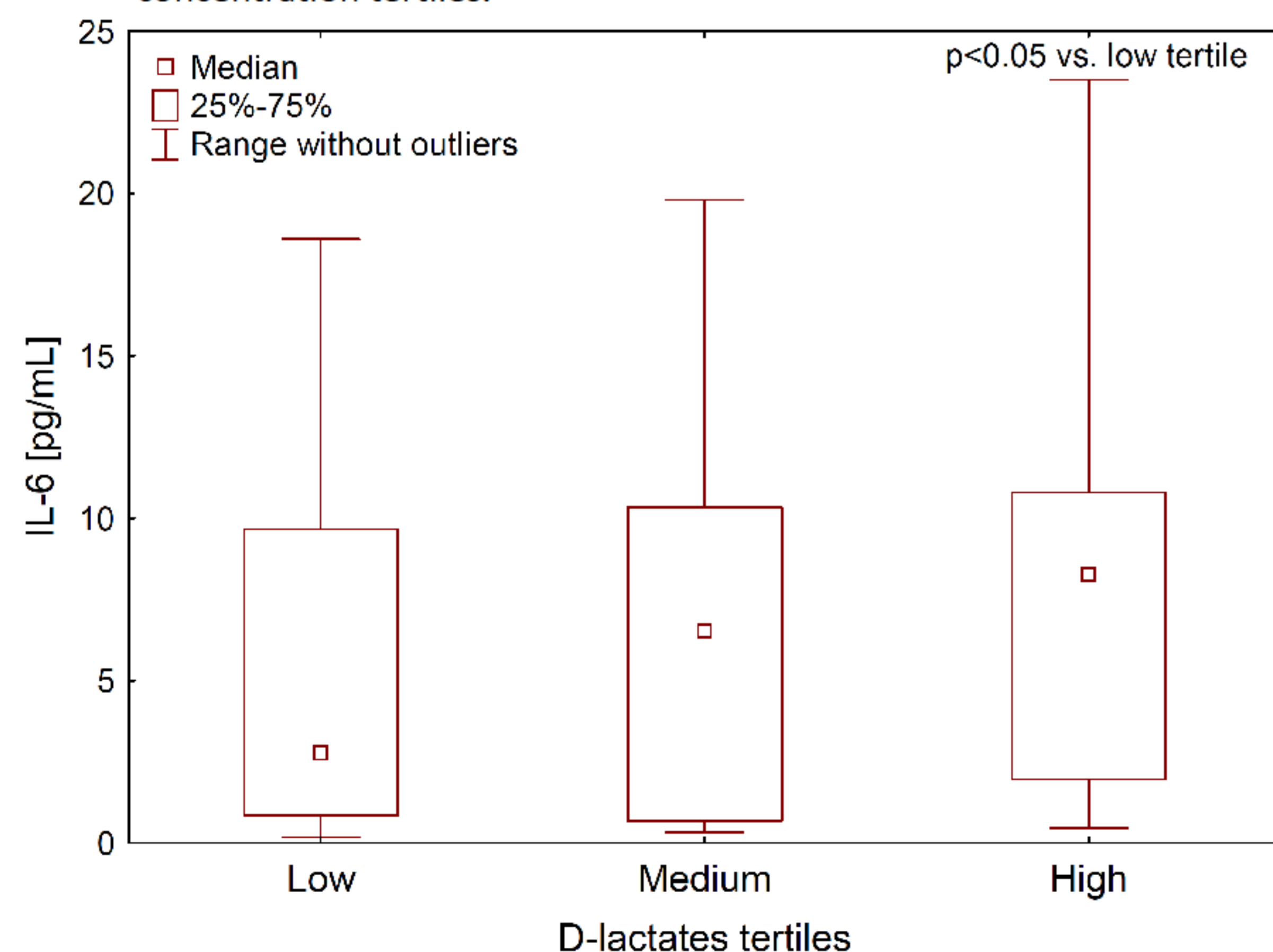


Figure 2. Plasma interleukin-6 concentration in subsequent D-lactates concentration tertiles.



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