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Ezetimibe Reduces Plant Sterol Accumulation and Favorably Increases Platelet Count in Sitosterolemia

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Abstract

Objective—To assess if ezetimibe (EZE), a sterol-absorption inhibitor, improves platelet (PLT) count and size relative to its effect on plasma plant sterol (PS) in patients with sitosterolemia (STSL).

Study design—Patients with STSL (5 males, 3 females, 16 to 56 years of age) receiving EZE intervention as part of their routine care participated in this study. EZE was discontinued for 14 weeks (off) and then resumed for another 14 weeks (on). Hematology variables along with plasma and red blood cells (RBC) PS and total cholesterol (TC) levels were measured at the end of each phase.

Results—EZE increased PLT count ($23 \pm 9\%$) and decreased mean PLT volume (MPV; $10 \pm 3\%$, all $P < .05$). In patients off EZE, PLT counts inversely correlated ($r = -0.96$ and $r = -0.91$, all $P < .01$) with plasma and RBC PS to TC ratio (PS/TC), and MPV positively correlated ($r = 0.91$, $P = .03$ and $r = 0.93$, $P = .02$) with plasma and RBC PS/TC. EZE reduced plasma and RBC

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sitosterol (-35 ± 4 and $-28 \pm 3\%$), total PS (-37 ± 4 and $-28 \pm 3\%$, all $P < .0001$) levels and PS/TC (-27 ± 4 and $-28 \pm 4\%$, $P < .01$).

Conclusion—EZE reduces plasma and RBC PS levels, and increasing PLT count and decreasing MPV, and thereby may reduce the risk for bleeding in STSL. Plasma PS levels and *ABCG5*/*ABCG8* genes should be analyzed in patients with unexplained hematologic abnormalities.

Keywords

phytosterols; macrothrombocytopenia; sterol absorption inhibitor

Sitosterolemia (STSL) is a rare autosomal recessive condition caused by mutations in either of *ABCG5* or *ABCG8*, two adenosine triphosphate-binding cassette (ABC) transporter genes located in a head-to-head orientation on chromosome 2p21.¹ The transporters are primarily expressed in the intestine and the liver, and participate in the removal and excretion of absorbed dietary sterols, thus preventing their accumulation in blood and tissues.^{1–3} In STSL, the plasma levels of plant sterols (PS), mainly sitosterol, campesterol and stigmasterol are elevated. In contrast, plasma total cholesterol (TC) levels are usually normal or moderately elevated^{4,5} although in some cases TC can be extremely high.^{6–8} Clinical features of STSL include tendon xanthomas, premature coronary artery disease, hemolytic anemia, macrothrombocytopenia, (large platelets [PLT] present in reduced quantity), and bleeding.^{2,9–11} Severe bleeding episodes, resulting from macrothrombocytopenia, have been reported in STSL, which necessitate PLT transfusion.¹¹ Other hematologic abnormalities include hemolytic anemia with stomatocytes; deformed red blood cells (RBC).⁹

Ezetimibe (EZE), a sterol absorption inhibitor commonly used to treat hypercholesterolemia¹² is also used to treat STSL. In STSL, EZE effectively decreases plasma levels of TC, low-density lipoprotein (LDL)-cholesterol,¹³ and PS.¹⁴ Importantly, EZE has been shown to improve thrombocytopenia in *ABCG5*-knockout (KO) mice,^{11,15} although such effects are not consistently observed in patients with STSL.^{16,17} Abnormal liver function has been reported in patients with STSL receiving EZE,¹⁸ but overall EZE is considered safe in patients with STSL.^{14,19,20} We sought to investigate if EZE improves PLT abnormalities in STSL, and if any effects correlate with PS reduction. The safety of EZE on liver and kidney function was also examined.

METHODS

Eight patients (5 males and 3 females, between 16 and 56 years of age) were recruited from Hutterite colonies in Manitoba, Canada ($n = 4$) and South Dakota, US ($n = 4$). All patients were identified as having homozygous *ABCG8* S107X mutation (NM_022437.2:c.320C>G) and are related to a proband previously reported by Mymin et al^{21,22} and Chong et al.²³ All procedures involving human patients were approved by the University of Manitoba Biomedical Ethics board and written informed consent was obtained from all patients. The trial was conducted by investigators of the Sterol & Isoprenoid Research (STAIR) consortium (<https://rarediseasesnetwork.epi.usf.edu/STAIR/>) after approval by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD).

The study was a single-site pilot interventional study of 8 patients with STSL conducted at the Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba. It was designed as a two 14-week phase (off and on EZE) study. After consenting, patients were taken off EZE for 14 weeks with blood samples collected before and at the end of the phase. On the last day of blood draw, patients were instructed to resume EZE (10 mg/day) for another 14 weeks and follow their usual diet. Blood was collected at the end of the 14 weeks treatment period with EZE. Plasma sterol and lipid concentrations, and complete blood counts were measured at baseline, after 14 weeks off EZE and 14 weeks on EZE. Serum, plasma and RBC fractions were separated by centrifugation at 3000 rpm for 20 minutes at 4°C, and stored at -80°C until further analysis. Participants were asked to monitor and report any adverse experiences including headaches, chest pain, or dizziness. Physical examinations and markers of liver and kidney function were measured at baseline and 14 weeks off and on EZE.

The levels of plasma and RBC TC and total PS (sitosterol, campesterol, stigmasterol and brassicasterol) were measured by gas-liquid chromatography equipped with a flame ionization detector and an auto sampler system (Varian 430-GC; Agilent Technologies, Santa Clara, CA, US). For sterol quantification, an internal standard of 5 α -cholestane was used in combination with sterol standard curve for each individual sterol, using authentic standards (Sigma-Aldrich Ltd. Oakville, ON, Canada) and (MJS BioLynx Inc, Brockville, ON, Canada). Briefly, 5 α -cholestane (50 ug) was added to each 0.5 mL plasma or 0.5 g RBC sample, and saponified with 4 mL methanolic potassium hydroxide for 2 h at 100°C. The unsaponifiable portion (sterols) were extracted twice from the mixture with 4 mL petroleum ether, derivatized with 0.1 mL TMS reagent (pyridine–hexamethyldisilazane–trimethylchlorosilane; 9:3:1 by volume), re-suspended in 0.4 mL hexane and injected (1 μ L) onto a 30-m SAC-5 column (Sigma-Aldrich Ltd. Oakville, ON, Canada). The column temperature was set to 280°C, with isothermal running conditions maintained for 30 minutes per sample. The injector and detector temperatures were set at 295°C and 300°C, respectively. The carrier gas (helium) flow rate was 1 mL/minute, with the inlet split set at 40:1.²⁴ Complete blood count was measured using automated hematology analyzer. Serum markers of RBC hemolysis including lactate dehydrogenase (LDH) and total bilirubin and serum lipids were measured using automated enzymatic methods. Serum LDL-cholesterol levels were calculated by the Friedewald equation.²⁵

Statistical Analyses

Statistical analyses were performed using SPSS 21.0 (SPSS, Inc., Chicago, IL, US). All data are presented as mean $\pm$ SEM. Statistical significance was set at $P < .05$. Linear mixed-model analysis was used where treatment, sex and site were specified as fixed factors, and age was specified as a covariate in the model. Significant values for treatment effect were analyzed by including the treatment term as a main effect in the linear mixed-model ANOVA. Percentage change from baseline for each phase was analysed using two-tailed paired Student *t* test. Relationships between two variables were assessed with stepwise multiple linear regression analysis unless otherwise stated. Data that were not normally distributed, as determined by a Shapiro-Wilk test, were log or inverse transformed before statistical analysis.

RESULTS

All eight initially recruited patients completed the study (Table I). Mean values of serum TC and LDL-cholesterol indicated mild hypercholesterolemia. All patients had elevated plasma and RBC levels of PS (Table I). The plasma and RBC concentrations of sitosterol and total PS were similar on average ($P > .11$), but the PS to TC ratio (PS/TC) tended to be higher in RBC than in plasma ($P = .08$; Table I). One subject had mild anemia [hemoglobin (Hb) = 11.7 g/dL; hematocrit (HCT) = 37.7%], and stomatocytes were noted in another, otherwise RBC and white blood cells (WBC) indices were within normal ranges (Table II). In contrast, three subjects had decreased PLT counts ($< 140 \times 10^3/\mu\text{L}$), four had increased PLT size (MPV > 12 fL) and two had both large PLT size and low PLT counts. One of our subjects had a clinically significant hematologic disorder, namely, a past history of chronic hemolysis, which was no longer manifested at the time of this study. None of the other subjects had any history of bleeding diathesis or atherosclerotic manifestations, heart murmurs, or vascular bruits. Baseline kidney function tests for all patients were within normal limits as measured by serum creatinine levels (Data not shown). Patients lacked evidence of abnormal liver function except two patients who had mild elevations in one liver enzyme (gamma-glutamyltransferase) or all (gamma-glutamyltransferase, alanine transaminase, aspartate transaminase and alkaline phosphatase) (Data not shown). All patients had normal bilirubin levels (0.42 ± 0.06 mg/dL). EZE tends to lower plasma TC ($-12 \pm 6\%$, $P = .09$) after 14 weeks but did not affect RBC TC ($0.3 \pm 3\%$, $P = 1.0$) (Figure 1, **A** and **B**). In patients off EZE, RBC TC was negatively correlated to RBC sitosterol ($r = -0.77$, $P = .03$), total PS ($r = -0.79$, $P = .02$) and PS/TC ($r = -0.86$, $P = .006$). Regardless of EZE treatment, RBC consistently had lower ($P < .001$) TC levels than plasma (-39 ± 3 and $-30 \pm 4\%$). EZE dramatically reduced plasma and RBC sitosterol (-35 ± 4 and $-28 \pm 3\%$, all $P < .0001$) and total PS (-37 ± 4 and $-28 \pm 3\%$, all $P < .0001$) (Figure 1, **C–F**). When total PS were expressed against TC concentrations (i.e., PS/TC ratio) in plasma and RBC these reductions were significant ($-27 \pm 4\%$, $P = .002$ and $-28 \pm 4\%$, $P = .003$). Furthermore, the decrease in the PS/TC in plasma after EZE correlated with that of tissue (RBC). After removing an outlier, the correlation became significantly stronger ($r = 0.99$, $P < .0001$) than when the outlier was included ($r = 0.72$, $P = .05$).

EZE increased ($P = .03$) PLT count ($23 \pm 9\%$) compared with 14 weeks off EZE (170 ± 14 vs $144 \pm 16 \times 10^3/\mu\text{L}$) (Table II). In patients off EZE, PLT count showed insignificant inverse relationships with plasma and RBC total PS ($r = -0.47$, $P = .24$ and $r = -0.68$, $P = .06$) and PS/TC ($r = -0.64$, $P = .09$ and $r = -0.68$, $P = .06$). However, after removing the data for one patient who had the highest PLT counts, these correlations became statistically significant for PS ($r = -0.77$, $P = .04$ and $r = -0.91$, $P = .004$) and PS/TC ($r = -0.96$, $P = .001$, and $r = -0.91$, $P = .005$) (Figure 2, **A**). Likewise, EZE reduced MPV ($-10 \pm 3\%$) compared with baseline (12 ± 0.4 vs 13 ± 0.4 fL, $P = .04$) (Table II). Because of technical problems MPV could not be determined on samples obtained at 14 weeks off EZE. These measures were therefore only conducted on baseline samples, where the MPV for five patients was measured. Baseline plasma and RBC PS/TC were positively correlated with MPV ($r = 0.91$, $P = .03$ and $r = 0.93$, $P = .02$, $n = 5$) (Figure 2, **B**). After 14 weeks on EZE no significant differences were observed in RBC indices including Hb, HCT, mean

corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width standard deviation (RDW-SD) and red cell distribution width coefficient of variation (RDW-CV), and WBC, neutrophils, lymphocytes, monocytes, eosinophils and basophils compared with off EZE (Table II). However, MCHC, which reflects the proportion of Hb in RBC, slightly increased ($P = .03$) after EZE relative to off EZE (34 ± 0.2 vs. 33 ± 0.3 g/dL). MCHC inversely associated with RBC sitosterol ($r = -0.86$, $P < .01$), total PS ($r = -0.91$, $P < .01$), and PS/TC ($r = -0.88$, $P < .01$) before EZE. Similarly, plasma sitosterol ($r = -0.51$, $P = .19$), total PS ($r = -0.68$, $P = .07$) and PS/TC ($r = -0.90$, $P = .003$) were negatively correlated with MCHC in patients off EZE, suggesting that when plasma PS increase in patients off EZE, MCHC decreases.

DISCUSSION

The major finding of this study is that blocking intestinal absorption of PS with EZE in patients with STSL increases PLT number and decreases PLT size, and measures were correlated with reductions in blood PS concentrations. The minimal effect of EZE on blood cholesterol suggests that the improvements in PLT count and size were primarily due to improved PS homeostasis. The effect of EZE on PLT may potentially ameliorate the bleeding tendency in STSL.

Mutations in *ABCG5/8* genes cause STSL, which is characterized by increased tissue retention of PS. We previously showed that the presence of intact *ABCG5/8* protects against PS accumulation and hemolysis in hamsters²⁶ and hypercholesterolemic subjects.²⁷ Total PS represents 0.2% of total plasma sterols in healthy subjects²⁸ but these levels increase 30-fold in STSL. The *ABCG5/8* genes are not expressed in blood cells, and therefore these cells could be a target for the toxic effect of PS.²⁹ Macrothrombocytopenia represents a clinical hematologic feature of STSL, which can lead to clinically problematic bleeding episodes.^{11, 30, 31}

Bile acid sequestrants reduce PS levels but do not normalize macrothrombocytopenia in patients with STSL who presented with moderate bleeding.³⁰ EZE is a sterol absorption inhibitor that has been used successfully in STSL,^{32, 33} but new data on improvement in hematologic variables have been reported.¹⁶ In a mouse model of STSL, EZE reversed macrothrombocytopenia³⁴ compared with bone marrow transplantation, indicating that low PLT count in STSL is caused by the build-up of PS rather than intrinsic hematopoietic defects. Recently, EZE failed to decrease plasma PS levels and improve thrombocytopenia but corrected hemolytic anemia after one year in a patient with STSL, suggesting that EZE may improve some but not all phenotypic features of STSL.¹⁶ In the current study, plasma and RBC total PS (free and esterified) and PS/TC were reduced by approximately 30%. Furthermore, our analysis of free and esterified sterols showed that most of the plasma PS are esterified ($85 \pm 3\%$ of total PS) but those of RBC are free ($57 \pm 5\%$ of total PS), similar to previous reports.^{35,36} EZE reduced both plasma free ($-32 \pm 4\%$, $P = .006$) and the esterified form of PS ($-38 \pm 7\%$, $P = .009$). In RBC, EZE decreased free PS ($-35 \pm 4\%$, $P < .0001$) but not the ester form ($-20 \pm 9\%$, $P = .10$) (data not shown). The decrease in plasma PS/TC strongly correlated with the decrease in RBC PS/TC ratio, suggesting tissue stores of

PS represented by RBC sterols were reduced with EZE. Measurement of PS in plasma reflects PS levels from the most recent meals, and PS levels in RBC indicate a longer-term average of plasma levels and better reflection for tissue stores. Moreover, PS are carried in plasma by lipoproteins, mainly LDL-cholesterol, and any change in plasma TC concentration will also change PS levels. Thus, PS levels were expressed either as absolute concentrations or expressed as ratios relative to TC. EZE increased PLT count by 23% and decreased MPV by 10%. This is of clinical importance because decrease in PLT count may increase bleeding, however EZE therapy in patients with STSL may normalize PLT function through improved PLT count and size. It is known that build-up of PS in arterial endothelium impairs endothelial function and increases risk of atherosclerosis. Overall, EZE treatment was effective in lowering plasma and RBC PS levels with a favorable increase in PLT count.

The slight increase in RBC MCHC index, together with its negative correlations with plasma and RBC PS suggest that EZE may have increased MCHC by reducing PS levels. Similarly, a high RDW reflects an increased variability in the size of RBC (anisocytosis) and indicates body's iron status. RDW increases with accumulation of PS levels.^{31, 37} Plasma and RBC total PS levels in patients on EZE tend to positively correlate with anisocytosis ($r = 0.64$, $P = .09$ and $r = 0.67$, $P = .07$, respectively), denoting a possible lower variation in RBC size after EZE.

EZE tended to lower plasma TC but did not affect RBC TC levels. In this study, similar to previous reports,^{35,36} most of the plasma cholesterol is esterified ($90 \pm 2\%$ of TC), and that of RBC is free ($56 \pm 6\%$ of TC). EZE decreased free ($-24 \pm 5\%$ and $-18\% \pm 6$, all $P < .05$) cholesterol but not the ester form ($-11 \pm 9\%$, $P = .11$ and $+16 \pm 13$, $P = .56$) in plasma and RBC, respectively (data not shown). RBC TC concentrations are similar to plasma TC levels in healthy humans.³⁸ Despite EZE treatment, the current patients with STSL had at least 30% lower TC levels in RBC than plasma on both study phases. RBC TC levels inversely correlated with RBC sitosterol and total PS, suggesting reciprocity between cholesterol and PS. Decreased RBC TC levels are likely due to increased RBC membrane PS incorporation³⁹ and may indicate decreased tissue cholesterol content.⁴⁰ Miettinen et al showed that RBC and plasma TC values were not related to each other in hypercholesterolemic children compared with the high respective correlations of PS.⁴¹ Herein, RBC TC tends to correlate with plasma TC after EZE ($r = 0.71$, $P = .05$), and RBC PS were strongly correlated with plasma PS ($r = 0.90$, $P = .003$), and had a linear relation when adjusted for TC ($r = 1.0$, $P < .0001$). Comparable PS levels in plasma and RBC indicate a rapid exchange of PS between the two compartments, suggesting that large amounts of PS in plasma could probably replace cholesterol in the RBC membrane,⁴² weaken interactions between the molecules,⁴³ and increase cell fragility.^{9, 10, 37} A mouse model of STSL has shown that PS incorporate directly into the PLT membrane, disrupting lipid asymmetry, resulting in the formation of hyper-activatable PLT that lose the ability to aggregate.¹¹

A number of limitations should be noted regarding the present study. The number of patients was relatively small; however, it should be noted that STSL is an extremely rare disease with fewer than 100 cases reported worldwide. Furthermore, this study included patients of

only a single ethnic group, the Hutterites, all with a single genotype and, as such, the results from this study cannot be generalized to other populations. The current study was also limited by short duration. Long-term follow-up is needed to fully assess the ability of EZE to further reduce plasma and tissue PS levels. Given that RBC have a long life span (e.g., 120 days) compared with PLT, which have an estimated turnover of 9–10 days⁴⁴ longer duration of treatment may be required to see a marked effect on RBC indices. Also three patients were on EZE prior to the start of the study, which may have blunted the effect despite the washout period. Similar to previous reports,¹⁷ EZE therapy over 14 weeks was well tolerated with no adverse effects on serum markers of kidney and liver functions, or muscle damage (data not shown). Overall, the study findings indicate that EZE is effective in reducing plasma and RBC PS levels and improving PLT indices in patients with STSL, thereby potentially ameliorating bleeding tendency in STSL. The data also suggest that plasma PS levels and *ABCG5/A BCG8* genes should be analyzed in patients with unexplained hematologic abnormalities.

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List of abbreviations

EZE	ezetimibe
PS	plant sterols
STSL	sitosterolemia
RBC	red blood cells
MPV	mean platelet volume
PLT	platelets
TC	total cholesterol
MCHC	mean corpuscular hemoglobin concentration
PS/TC ratio	total PS to total cholesterol ratio.

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Figure 1.

Plasma and RBC total cholesterol (TC), sitosterol and total plant sterols (PS) in patients with STSL (n = 8) after 14 weeks off ezetimibe (Off EZE) and 14 weeks on ezetimibe (On EZE).

Data are presented as mean $\pm$ SEM. *: P < .05; **: P < .01; ***: P < .0001. Total PS is the sum of sitosterol, campesterol, stigmasterol and brassicasterol values.

Figure 2.

A and B, Correlations of PLT counts and MPV with plasma PS/TC. C, Correlation of PLT counts with plasma PS/TC (Off EZE, $n = 7$, one patient who had the highest PLT counts was an outlier and not included). D, Correlation of MPV with plasma PS/TC (baseline, $n = 5$).

Table IBaseline characteristic of sitosterolemia patients (mean $\pm$ SEM)

<i>Demographics (n = 8)</i>	
Age (year)	28 $\pm$ 6
Body weight (kg)	72 $\pm$ 6
Height (cm)	166 $\pm$ 2
Body mass index (kg/m ²)	26 $\pm$ 2
<i>Lipid analyses (n = 7)*</i>	
TC (mg/dL)	211 $\pm$ 17
LDL-cholesterol (mg/dL)	131 $\pm$ 14
HDL-cholesterol (mg/dL)	55 $\pm$ 3
Triglyceride (mg/dL)	122 $\pm$ 27
Plasma TC [‡] (mg/dL)	156 $\pm$ 13
RBC TC [‡] (mg/dL)	101 $\pm$ 6
Plasma sitosterol (mg/dL)	6 $\pm$ 0.7
RBC sitosterol (mg/dL)	5 $\pm$ 0.5
Plasma total PS [†] (mg/dL)	9 $\pm$ 1
RBC total PS (mg/dL)	8 $\pm$ 0.7
Plasma PS/TC ratio	0.06 $\pm$ 0.01
RBC PS/TC ratio	0.08 $\pm$ 0.01

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoproteins; PS, plant sterols; RBC, red blood cells; TC, total cholesterol; PS/TC ratio, plant sterol to cholesterol ratio.

* n=7, biochemical data was not available for one patient.

[‡]Plasma TC and RBC TC were determined by gas chromatography.

[†]Total PS is the sum of sitosterol, campesterol, stigmasterol and brassicasterol.

Table II

Hematology indices at baseline, 14 weeks off ezetimibe (off EZE) and 14 weeks on ezetimibe (on EZE) (mean $\pm$ SEM)

Variable	Baseline	14 week off EZE	14 weeks on EZE	Mean % change	P
PLT (10^3 /mL) [§]	164.3 $\pm$ 11.9	143.6 $\pm$ 15.8	170.3 $\pm$ 13.7	+23 $\pm$ 8.9	.03
MPV (fL)	13.0 $\pm$ 0.4 [¶]	-	12.4 $\pm$ 0.4 [§]	-10 $\pm$ 2.6	.04
WBC (10^3 /mL) [§]	5.6 $\pm$ 0.5	5.6 $\pm$ 0.3	6.3 $\pm$ 0.5	+11 $\pm$ 5.3	.07
RBC (10^6 /mL) [§]	4.8 $\pm$ 0.1	4.6 $\pm$ 0.2	4.7 $\pm$ 0.1	+1 $\pm$ 1.4	.57
Hb (g/dL) [§]	14.2 $\pm$ 0.5	13.8 $\pm$ 0.5	14.0 $\pm$ 0.6	+1.3 $\pm$ 1.0	.24
Hematocrit (%) [§]	42.6 $\pm$ 1.3	41.4 $\pm$ 1.5	41.4 $\pm$ 1.5	+0.1 $\pm$ 1.1	.98
MCV (fL) [§]	89.7 $\pm$ 0.8	89.5 $\pm$ 0.9	88.8 $\pm$ 1.2	-0.8 $\pm$ 0.7	.32
MCH (pg/cell) [§]	30.0 $\pm$ 0.4	29.8 $\pm$ 0.4	29.9 $\pm$ 0.6	+0.4 $\pm$ 0.8	.61
MCHC (g/dL) [§]	33.4 $\pm$ 0.4	33.3 $\pm$ 0.3	33.7 $\pm$ 0.2 [*]	+1.3 $\pm$ 0.5	.03
RDW-SD (fL) [†]	43.3 $\pm$ 0.8	45.0 $\pm$ 0.9	44.6 $\pm$ 0.7	-0.8 $\pm$ 1.0	.37
RDW-CV (%) [§]	13.6 $\pm$ 0.1	14.2 $\pm$ 0.2	14.1 $\pm$ 0.4	-0.8 $\pm$ 2.0	.86
Neutrophils (%) [§]	55.5 $\pm$ 2.2	56.2 $\pm$ 2.0	53.7 $\pm$ 3.0	-4 $\pm$ 4.0	.31
Lymphocytes (%) [§]	32.2 $\pm$ 2.8	33.3 $\pm$ 2.1	34.5 $\pm$ 3.1	+3 $\pm$ 5.9	.59
Monocytes (%) [§]	9.7 $\pm$ 1.4	8.1 $\pm$ 0.8	8.7 $\pm$ 0.9	+10 $\pm$ 9.7	.42
Eosinophils (%) [§]	2.2 $\pm$ 1.5	2.1 $\pm$ 0.6	2.6 $\pm$ 0.6	+20 $\pm$ 10.7	.07
Basophils (%) [§]	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1	+33 $\pm$ 19.8	.36

P < .05.

[§] (n = 8),

[¶] (n = 5), and

[†] (n = 4).

Because of technical problems MPV could not be determined on the samples obtained at 14 weeks off EZE. MPV measurements were therefore done on the baseline samples, where the mean MPV value represents measurement for five patients.

Abbreviations: Hb, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; RBC, red blood cells; RDW-CV, red cell distribution width coefficient of variation; RDW-SD, red cell distribution width standard deviation; WBC, white blood cells.