Journal of Nutrigenetics and Nutrigenomics

J Nutrigenet Nutrigenomics 2013;6:283–304
DOI: 10.1159/000357947
Received: March 1, 2013
Accepted: December 11, 2013
Published online: February 4, 2014

1661–6499/14/0066–0283$39.50/0
www.karger.com/jnn

**Original Paper**

# The Influence of Erythropoietin (EPO T→G) and α-Actinin-3 (ACTN3 R577X) Polymorphisms on Runners' Responses to the Dietary Ingestion of Antioxidant Supplementation Based on Pequi Oil (*Caryocar brasiliense* Camb.): A Before-After Study

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**Key Words**
Exercise-induced oxidative injuries · Antioxidant supplementation · Sport nutrition · Nutrigenomics · Nutrigenetics

**Abstract**
***Background/Aims:*** As diet can affect an individual's genes and these can affect response to supplementation, we aimed to investigate the influence of erythropoietin (EPO T→G) and α-actinin-3 (ACTN3 R577X) polymorphisms on plasma lipid peroxidation, hemogram and biochemical dosages of creatine kinase, aspartate aminotransferase, alanine aminotransferase and C-reactive protein (including high-sensitivity C-reactive protein) of runners (n = 123) before and after 14 days of 400 mg pequi oil supplementation, a natural carotenoid-rich oil, after races under closely comparable conditions. ***Methods/Results:*** Blood samples were taken immediately after racing to perform the tests. Before pequi oil supplementation, EPO polymorphism influenced erythrogram and plateletgram results, suggesting an aerobic advantage for the TG genotype and a disadvantage for the GG genotype as regards possible microvascular complications, while no association was found for ACTN3 polymorphism with endurance performance. Both polymorphisms influenced the runners' response to pequi oil: significant responses were observed for the EPO TT genotype in erythrocyte, hematocrit, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration values, and for the TT and TG genotypes in red blood cell distribution width values. Significant differences were also observed in the plateletgram for the TT and TG genotypes. ACTN3 mainly

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influenced aspartate aminotransferase and creatine kinase values: heterozygotes had a significant reduction in aspartate aminotransferase values and homozygous individuals (XX) in creatine kinase values after pequi oil supplementation. ***Conclusion:*** These results emphasize the importance of studying nutrigenomic effects on athletes' performance.



## Introduction

Regular physical activity is known to increase the concentration of antioxidant enzymes and, consequently, resistance to oxidative stress. However, despite its potential beneficial effects, exercise, especially above habitual intensity or training, may exceed the endogenous antioxidant system's capacity and often results in oxidative stress and injuries, even in trained individuals [1–3]. This oxidative overload, although felt most intensely in skeletal muscles, can lead to an increase in plasma lipid peroxidation plus oxidative damage in many other organs and body systems responsible for regulating and maintaining homeostasis, including the heart, liver, erythrocytes and immune system [1, 3, 4]. These damaging effects, with their consequent inflammatory processes, may jeopardize performance and lead to overtraining syndrome [4]. Such concerns have led to research into whether antioxidant supplementation could prevent the damaging effects of reactive oxygen and nitrogen species and thereby improve performance [1, 5].

Considering that reactive oxygen and nitrogen species can act as signals that regulate molecular events of cellular adaptation to exercise, the practical consequence is that antioxidant supplements can inhibit beneficial adaptive responses associated with improved athletic performance [1, 6]. Therefore, the prudent recommendation for physically active individuals is a diet rich in antioxidants from natural foods [7], and the recommendation of the use of antioxidant supplements should be made only for those cases in which exhaustive exercise causes oxidative stress and cell damage [1, 8]. Thus, research on natural antioxidants has increased greatly since the 1980s, and thenceforth there has been a considerable broadening of the search for natural antioxidants that can be included in the diet as substitutes for synthetic antioxidants [1, 8]. In this context, it has been demonstrated that the carotenoid-rich oil extracted from pequi pulp (*Caryocar brasiliense* Camb.), a typical fruit found in the Brazilian Cerrado, has anti-inflammatory properties, besides reducing arterial pressure, exercise-induced DNA and tissue damages, and anisocytosis in runners. Despite these protective effects of pequi oil, some of the responses of the runners were influenced by genetic polymorphisms related to oxidative stress [2–4, 9].

Genetic polymorphisms have also been implicated in athletic performance, and the number of genes and markers that have a possible association with one or more phenotype traits related to performance grows every year [10–13]. Among them, the single nucleotide polymorphism (SNP) R577X in the α-actinin-3 (ACTN3, SNP rs1815739) gene has been reported to favor endurance performance [14, 15], while the GG genotype of the SNP rs1617640 in the promoter region of the erythropoietin (EPO) gene has been associated with decreased EPO expression [16], suggesting it could negatively affect endurance performance. Although the EPO polymorphism has been reported as G/T, the ancestral allele is referred to as the T allele by the official website [17]. Therefore, the mutation is that which is involved in decreased EPO expression. Moreover, a G-to-A transition at nucleotide 6002 (G6002A, SNP rs121918116) in exon 8 of the erythropoietin receptor (EPOR) gene, which results in a trp439-to-ter substitution (W439X) causing a premature termination of the receptor cytoplasmic region, has been associated with dominant familial erythrocytosis, high hemoglobin levels and low serum EPO, being reported as favoring aerobic Olympic performance [18, 19].

As common dietary chemicals can act on the human genome, either directly or indirectly, to alter gene expression [20, 21], knowledge on how individual genetic differences can affect response to antioxidant supplementation and how diet interacts with the human genome to influence performance, health and disease is of unquestionable importance to the athlete's performance and health [4]. Thus, we aimed to investigate the influence of SNPs rs1815739 in the ACTN3 gene and rs1617640 in the EPO gene on plasma lipid peroxidation [thiobarbituric acid reactive substances (Tbars) assay], complete hemogram and biochemical dosages of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and C-reactive protein [CRP and high-sensitivity CRP (hs-CRP)] of runners, before and after the ingestion of 400 mg pequi oil in capsules supplied daily for 14 consecutive days. In other words, we aimed to evaluate how individual genetic differences in the EPO and ACTN3 genes can affect response to antioxidant supplementation with pequi oil under the oxidative stress experienced by runners during the race, and how the diet with pequi oil interacts with the human genome to influence this. As the G6002A mutation in the EPOR gene (SNP rs121918116) has also been reported as a 'de novo' event [19], it was also tested in this work. The tests were performed after races in the same environment and under closely comparable conditions, according to the type, intensity and length of weekly training of the athletes.

## Materials and Methods

*Study Design and Participants*

The trial was conducted after preclinical and toxicological tests in mice [22]. Initially, trained street runners of both genders (53 females and 86 males) of different age groups (15–67), who had a run performance of at least 4,000 m, were recruited in high schools, colleges, universities, clubs and companies in Brasília (Federal District, Brazil), according to previously reported criteria [2–4, 9, 23].

In general, only trained athletes were included in the study, and although they had a varying degree of training intensity, the amount of weekly training was similar (in number of days and hours of training). The recruited group of athletes ran at different times (from August 2007 to April 2008). They were separated by the distance covered (4–21 km), all of them being monitored by different members of our group, within a route previously defined by us. The distance chosen and the time required to cover it were chosen according to the type, intensity and duration of weekly training, to ensure no additional physical stress beyond what they were accustomed to.

The volunteers participated in two races under the same environment, type, intensity and length of weekly training conditions, before (control group) and after (treatment group) the ingestion of 400 mg of pequi oil in capsules supplied daily for 14 consecutive days; no significant change in the daily routine, training or lifestyle of any runner occurred between the first race and second race, except for the ingestion of pequi oil capsules. The daily ingestion took into account data from the pequi literature and the maximum daily dose of provitamin A carotenoids (25 mg) recommended by the National Agency for Sanitary Surveillance (ANVISA). Both races were run outdoors on flat tracks, under the same environmental conditions, and the same route was covered within the same time by each athlete to finish the two races. The athletes could choose the distance that they would cover (4–21 km), according to the type, intensity and length of their weekly training, to guarantee no additional physical stress beyond what they were accustomed to, in order to avoid differences in training amount or intensity and consequent increased oxidative stress.

Each athlete participated in the control and treatment group, being compared in the statistical tests with him/herself, and only those athletes who followed the instructions correctly and participated in both races were enrolled in the study, which totaled 125 athletes (49 females and 76 males). Afterwards, subjects were stratified by age according to their inclusion in one of the following groups: adolescents (15- to 19-year-olds), young adults (20- to 40-year-olds) and middle-aged adults (41- to 58-year-olds), following the age criteria for reference values of biochemical parameters; for clinical purposes, some reference values are different for ages up to 19 years old [24]. Because there were only 2 runners aged above 58 years, they were removed from the sample size. Thus, the final sample size studied here was 123 (49 females and 74 males).

**Table 1.** Relative composition of pequi (*C. brasiliense* Camb.) pulp oil capsules

| | |
|---|---|
| Fatty acids [25; present study], % | |
| Saturated | |
| Palmitic ($C_{16:0}$) | 41.78 |
| Stearic ($C_{18:0}$) | 1.28 |
| Araquinic ($C_{20:0}$) | 0.12 |
| Monounsaturated | |
| Oleic ($C_{18:1}$; ω–9) | 54.28 |
| Palmitoleic ($C_{16:1}$; ω–7) | 0.67 |
| Polyunsaturated | |
| Linoleic ($C_{18:2}$; ω–6) | 1.36 |
| Linolenic ($C_{18:3}$; ω–3) | 0.51 |
| Carotenoids [26–28], mg/100 g of pequi fruit pulp | |
| Provitamin A | 6.26–11.5 |
| Lycopene | 1.12–2.08 |
| Total | 6.75–28.66 |
| Vitamin E [29], μg/100 g of pequi fruit pulp | >110.52 |

The omega nomenclature (ω), which is defined according to the carbon numeration associated with the first double bonds (3rd, 6th, 7th or 9th) from the methyl group, is correlated with the unsaturated fatty acids.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and the procedures were approved by the Ethics Committee of the University of Brasília and by the National Commission for Ethics in Research (CONEP), No. 0.001668/2005-18. Written informed consent was obtained from all subjects.

*Preparation of Capsules*

Pequi oil, whose relative composition is shown in table 1, was extracted by cold maceration using chloroform as a solvent [25], incorporated in Aerosil (colloidal silicon dioxide) q.s.p. as previously reported [2–4, 9, 23]. The capsule production was patented as No. PI0601631-6 (National Institute of Industrial Property – INPI).

*Procedures and Measurements*

Peripheral blood samples collected immediately after the two races in Vacutainer tubes containing EDTA were used to obtain a complete hemogram and for genotyping of the polymorphisms, while serum samples were submitted to Tbars assay and to examine dosages of CK, AST, ALT and CRP and hs-CRP.

*Biochemical Analyses and Hemogram*

Hemogram was carried out using the automated analyzer Cell-Dyn 3700 (Abbott Diagnostics), considering the reference values previously reported [9]. Serum CK, AST, ALT and CRP analyses were run on an automated chemistry analyzer (ADVIA 1650, Bayer Diagnostics), and hs-CRP was measured by an immunometric assay (Immulite 2000, DPC, Medlab), using the appropriate chemical reagents, controls and protocols of the manufacturers. Assessments also took into account the reference values reported in the literature for clinical purposes and for athletes, as follows: although reference values for CK are up to 171 U/l for males and up to 145 U/l for females [24] for clinical purposes, athletes have higher CK reference values than nonathletes, these being 82–1,083 U/l for males and 47–513 U/l for females [30]. For clinical purposes, reference values for AST are in the range of 15–37 U/l for males and 13–31 U/l for females; for ALT they are in the range of 10–40 U/l for males and 7–35 U/l for females [24, 31]. Because of the higher CK values for athletes [30], the upper reference limits for AST are expected, since the serum level of this enzyme can increase with muscular injuries [3]. As for CRP and hs-CRP, both tests measure the same molecule in the blood, but hs-CRP has been developed to detect CRP at lower levels, being much more sensitive for diagnosis [32]. Although according to the American Heart Association and US Center for Disease Control reference values for CRP are <3 mg/l [33], women usually have lower values than men [32].

The Tbars assay was carried out according to Wasowicz et al. [34], with slight modifications, as previously described [3].

*Genotyping of the Polymorphisms*

Genomic DNA was isolated from the buffy coat layer using the Blood Genomic Prep Mini Spin Kit (GE Healthcare, Buckinghamshire, UK). DNA samples were quantified in a Nanovue spectrophotometer (GE Healthcare), diluted in Milli-Q water to a final concentration of 50 ng/μl and stored at –20°C until analysis.

DNA samples underwent amplification in an MJ PTC-100 (MJ Research Inc., Waltham, Mass., USA). The ACTN3 R577X genotypes were determined as described by Clarkson et al. [35], using the restriction enzyme *Dde*I (New England Biolabs, Inc.). Primers used for the SNP genotyping of the EPO region (rs1617640) followed Tong et al. [36], and the PCR was performed by denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 45 s and a final extension step of 10 min at 72°C. The amplified fragment subsequently underwent digestion by *Bse*RI (New England Biolabs, Inc.) restriction enzyme in a condition recommended by the supplier. The point mutation G6002A in exon 8 of the EPOR (rs121918116) gene was screened using the EPOR primer pair 3 as reported by De La Chapelle et al. [18], with the following PCR conditions: 30 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1 min and extension at 72°C for 30 s. The 295-bp PCR product was then digested by 1 U of the *Nco*I restriction enzyme (Promega Brazil Ltd.) under the conditions recommended by the manufacturer.

The PCR and the PCR-digested products were separated by electrophoresis in 6% nondenaturing polyacrylamide gels and visualized by staining with silver nitrate.

*Statistical Analyses*

The minimum sample size was estimated through a power analysis from the statistical analysis of quantitative data, considering the maximum tolerable sampling error (standard error or sampling error) ranging from 0.05 to 0.20, according to a higher or lesser variation in the results of tests carried out and the samples after stratifications of the total group, according to the formula:

$$\left(\sqrt{n}\right)^2 = (1.96)^2 \, x \, \frac{(\sigma)^2}{(E)^2} \rightarrow n = (1.96)^2 \, x \, \frac{\sigma^2}{E^2}.$$

Where: n = sample size; σ = standard deviation; E = maximum tolerable error = 1.96 × $\sigma_{\bar{x}}$, where $\sigma_{\bar{x}}$ = standard error of mean (SEM). Thus,

$$E = 1.96 \times \frac{\sigma}{\sqrt{n}},$$

because the confidence interval = $\bar{x} \pm 1.96 \, \sigma_{\bar{x}}$, where $\bar{x}$ = mean.

This formula was used for all calculations, including those arising from the stratification of the sample by sex, age, distance covered and genotypes of the studied polymorphisms. As a cautionary measure, additional care is required with an oversized sample, which may reduce the confidence intervals of the estimates and permit the detection of differences between subgroups that, although statistically significant, have no clinical relevance [37]. Therefore, quantitative variables that naturally have reference values with very large intervals were not considered in the calculation of the sample size.

Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences) version 17.0. Data were expressed as mean ± SEM and values of $p < 0.05$ were considered statistically significant. The continuous variables were tested for normal distribution with the Shapiro-Wilk test. For the analyzed parameters, possible differences between the sexes were evaluated by the t test or the Mann-Whitney U test (nonnormalized data), while differences among age groups, distance covered and genotypes were evaluated by ANOVA or the Kruskall-Wallis test (data not normally distributed), followed respectively by the Tukey or the Mann-Whitney U tests. To verify differences in the comparison of before-after pequi oil supplementation, the statistical significance was assessed by the paired-samples t test or the Wilcoxon matched pairs test (when the data were not normally distributed). The possible correlations between the parameters sex/age groups, sex/distance covered, age groups/distance covered, genetic polymorphisms/sex, genetic polymorphisms/age groups and genetic polymorphisms/distance covered were analyzed through the $\chi^2$ correlation test.

The genotype distributions were tested for Hardy-Weinberg equilibrium (HWE) by the $\chi^2$ test, using the Genepopweb Statistical Program version 4.1 (http://genepop.curtin.edu.au). Values of $p > 0.05$ were considered in HWE. The same program was used to calculate the allelic and genotypic frequencies of each locus, genetic diversity parameters such as observed heterozygosity, expected heterozygosity and inbreeding coefficient. For each polymorphism, the distribution frequencies of the genotypes between sexes were also tested through the Mann-Whitney U test, and values of $p < 0.05$ were considered statistically significant.

**Table 2.** Values of CK, AST, ALT, CRP, hs-CRP and Tbars assays for the total and sex groups before and after pequi oil supplementation

| | Total | Female | Male | p values |
|---|---|---|---|---|
| Participants, n (%) | 123 (100) | 49 (39.8) | 74 (60.2) | |
| CK, U/l | | | | |
| Before | 285.86±31.21 | 149.92±16.99 | 379.68±48.51 | 0.000 |
| After | 237.62±20.90* | 150.76±18.07 | 297.56±31.23* | 0.000 |
| AST, U/l | | | | |
| Before | 29.81±0.80 | 27.84±1.32 | 31.15±0.98 | 0.006 |
| After | 28.20±0.66 | 24.92±0.89* | 30.43±0.84 | 0.000 |
| ALT, U/l | | | | |
| Before | 23.02±1.04 | 22.04±1.78 | 23.69±1.27 | 0.246 |
| After | 21.55±0.82* | 19.24±0.90* | 23.12±1.21 | 0.078 |
| CRP, mg/dl | | | | |
| Before | 0.33±0.03 | 0.26±0.03 | 0.38±0.05 | 0.121 |
| After | 0.37±0.03 | 0.34±0.04 | 0.38±0.04 | 0.695 |
| hs-CRP, mg/dl | | | | |
| Before | 1.60±0.21 | 1.05±0.16 | 2.02±0.34 | 0.030 |
| After | 1.54±0.17 | 1.48±0.26 | 1.59±0.23 | 0.407 |
| Tbars, nmol/ml of MDA | | | | |
| Before | 0.0266±0.001 | 0.0271±0.001 | 0.0264±0.001 | 0.608 |
| After | 0.0264±0.001 | 0.0271±0.001 | 0.0260±0.001 | 0.350 |

Data are expressed as mean ± SEM.

p values for Tbars assay (before and after) were generated by the t test, while the other p values were generated by the Mann-Whitney U test.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

## Results

In all estimates of the minimum sample size (total group and stratifications by sex, age, distance covered and genotypes), the supplementation with pequi capsules reduced the variability of the sample and hence the required sample size. Thus, both in the total group and in the stratifications, the final sample size was a little higher than calculated.

Except for values of ALT and CRP before as well as after pequi oil supplementation, significant differences between the sexes were observed in both biochemical dosages and erythrogram for the same parameters described in the literature, with different reference values for females and males [9, 24, 30–33] (tables 2, 3a). However, after pequi oil supplementation these differences disappeared for hs-CRP. The significant reduction in CK values after pequi oil supplementation was related particularly to males, while for AST and ALT, it was mainly related to females (table 2). Significant differences in the comparisons before-after pequi oil supplementation were also observed for red blood cells (RBC), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets for both sexes, while a decrease in red blood cell distribution width (RDW) and plateletcrit was significant for males (table 3a, c).

The age group of 41–58 years had significantly lower CK values than the age group of 15–19 years after pequi supplementation, while before pequi supplementation the same occurred with hs-CRP values. Tbars assay values were significantly higher for the age groups of 20–40 and 41–58 years when compared with the age group of 15–19 years. However, significant differences in the comparisons before-after pequi oil supplementation were only

**Table 3.** Hemogram of the total and sex groups before and after pequi oil supplementation

| | Total | Female | Male | p values |
|---|---|---|---|---|
| **a** *Erythrogram* | | | | |
| RBC, $\times 10^6$/mm$^3$ | | | | |
| Before | 5.23±0.05 | 4.83±0.05 | 5.48±0.05 | 0.000 |
| After | 5.15±0.05* | 4.75±0.05* | 5.41±0.05* | 0.000 |
| HGB, g/dl | | | | |
| Before | 14.44±0.28 | 13.88±0.33 | 14.79±0.40 | 0.000 |
| After | 14.38±0.28 | 13.81±0.33 | 14.74±0.40 | 0.000 |
| HCT, % | | | | |
| Before | 44.98±0.35 | 41.98±0.46 | 46.85±0.34 | 0.000 |
| After | 44.31±0.34* | 41.17±0.42* | 46.27±0.31* | 0.000 |
| MCV, fl | | | | |
| Before | 86.18±0.35 | 86.99±0.49 | 85.67±0.47 | 0.054 |
| After | 86.22±0.35 | 86.89±0.47 | 85.80±0.49 | 0.133 |
| MCH, pg | | | | |
| Before | 29.43±0.14 | 29.68±0.21 | 29.27±0.19 | 0.154 |
| After | 29.76±0.14* | 30.08±0.19* | 29.56±0.19* | 0.068 |
| MCHC, g/% or g/dl | | | | |
| Before | 34.15±0.09 | 34.12±0.16 | 34.16±0.11 | 0.973 |
| After | 34.52±0.06* | 34.63±0.09* | 34.45±0.08* | 0.118 |
| RDW, % | | | | |
| Before | 14.83±0.09 | 14.65±0.14 | 14.95±0.12 | 0.107 |
| After | 14.25±0.10* | 14.38±0.18 | 14.17±0.11* | 0.331 |
| **b** *Leukogram* | | | | |
| WBC, /mm$^3$ | | | | |
| Before | 7,399.13±209.85 | 7,542.22±386.90 | 7,307.14±240.53 | 0.977 |
| After | 7,398.26±178.37 | 7,593.33±294.76 | 7,272.86±223.96 | 0.421 |
| Lymphocytes, /mm$^3$ | | | | |
| Before | 2,654.92±89.99 | 2,562.58±150.41 | 2,713.45±112.24 | 0.227 |
| After | 2,609.55±92.99 | 2,552.58±154.55 | 2,645.66±116.82 | 0.419 |
| Segmented, /mm$^3$ | | | | |
| Before | 3,998.68±161.73 | 4,255.47±285.74 | 3,835.93±191.58 | 0.243 |
| After | 3,949.05±133.72 | 4,172.67±203.47 | 3,807.32±175.39 | 0.103 |
| Rods, /mm$^3$ | | | | |
| Before | 26.70±7.81 | 21.22±8.91 | 30.21±11.52 | 0.807 |
| After | 26.96±7.94 | 27.71±12.89 | 26.46±10.14 | 0.791 |
| Basophils, /mm$^3$ | | | | |
| Before | 85.19±4.77 | 91.09±6.86 | 81.45±6.46 | 0.357 |
| After | 98.34±4.39 | 92.71±6.85 | 101.92±5.71* | 0.624 |
| Eosinophils, /mm$^3$ | | | | |
| Before | 140.22±9.93 | 122.82±12.84 | 151.24±13.95 | 0.241 |
| After | 148.47±10.52 | 136.31±14.08 | 156.17±14.70 | 0.544 |
| Monocytes, /mm$^3$ | | | | |
| Before | 492.64±20.52 | 477.47±29.81 | 502.25±27.81 | 0.577 |
| After | 542.04±18.45* | 538.40±28.22 | 544.35±24.41 | 0.812 |
| **c** *Plateletgram* | | | | |
| Platelets, $\times 10^3$/mm$^3$ | | | | |
| Before | 335.24±6.44 | 332.89±10.5 | 336.71±8.22 | 0.722 |
| After | 312.12±6.01* | 316.29±10.19* | 309.51±7.45* | 0.644 |
| Plateletcrit, % | | | | |
| Before | 0.36±0.01 | 0.37±0.02 | 0.35±0.01 | 0.395 |
| After | 0.33±0.01* | 0.35±0.01 | 0.31±0.01* | 0.025 |
| MPV, fl | | | | |
| Before | 10.54±0.15 | 10.88±0.27 | 10.34±0.19 | 0.079 |
| After | 10.36±0.16 | 10.99±0.26 | 9.98±0.18 | 0.001 |
| PDW, % | | | | |
| Before | 18.01±0.11 | 18.04±0.18 | 18.00±0.14 | 0.843 |
| After | 18.09±0.10 | 18.32±0.15 | 17.95±0.13 | 0.062 |

Data are expressed as mean ± SEM.

HGB = Hemoglobin; MCV = mean corpuscular volume; WBC = white blood cells.

p values for RBC, HCT, MCV, MCH and RDW were generated by ANOVA, while the other p values were generated by the Mann-Whitney U test.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

**Table 4.** Values of CK, AST, ALT, CRP, hs-CRP and Tbars assays for the age groups before and after pequi oil supplementation

| Age groups | 15–19 years (teenager) | 20–40 years (young adult) | 41–58 years (middle age) | p values |
|---|---|---|---|---|
| Participants, n (%) | 20 (16.3) | 78 (63.4) | 25 (20.3) | |
| CK, U/l | | | | |
| Before | 433.85±138.84 | 276.82±30.97 | 191.17±28.63 | 0.181 |
| After | 327.75±59.00 | 229.39±27.06* | 188.54±30.60 [a] | 0.080 |
| AST, U/l | | | | |
| Before | 32.55±2.17 | 29.19±0.99 | 29.50±1.70 | 0.378 |
| After | 27.60±1.59* | 27.96±0.82 | 29.46±1.63 | 0.874 |
| ALT, U/l | | | | |
| Before | 21.70±1.43 | 22.35±1.42 | 26.29±2.26 | 0.031 |
| After | 21.25±2.06 | 20.60±1.03 | 24.88±1.74 | 0.044 |
| CRP, mg/dl | | | | |
| Before | 0.29±0.06 | 0.34±0.04 | 0.33±0.05 | 0.747 |
| After | 0.38±0.08 | 0.38±0.04 | 0.31±0.04 | 0.859 |
| hs-CRP, mg/dl | | | | |
| Before | 1.35±0.57 | 1.85±0.30 | 1.11±0.23[a] | 0.004 |
| After | 1.66±0.59 | 1.70±0.22 | 1.03±0.16 | 0.120 |
| Tbars, nmol/ml of MDA | | | | |
| Before | 0.0219±0.001 | 0.0274±0.001[a] | 0.0279±0.002[a] | 0.008 |
| After | 0.0238±0.002 | 0.0267±0.001 | 0.0277±0.001 | 0.115 |

Data are expressed as mean ± SEM.

p values for Tbars assay (before and after) were generated by ANOVA, while the other p values were generated by the Kruskal-Wallis test.

The lower-case letters indicate significant differences among age groups, with [a] = significant compared to 15–19 years.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

observed for 15–19 years in AST values and for 20–40 years in CK values (table 4). The age group of 41–68 years also differed from 15–19 and 20–40 years in RBC, mean corpuscular volume and MCH values before pequi supplementation, as well as in MCHC values both before and after pequi supplementation. Significant differences in the comparisons before-after pequi oil supplementation were observed mainly for 15–19 and 20–40 years in RBC, some hematimetric indices and monocytes, and for 20–40 years in platelet and plateletcrit values, although RDW significantly decreased for the age groups of 20–40 and 41–58 years (table 5).

Significant differences among the distances covered were also observed in the biochemical dosages, erythrogram and leukogram, but not in the plateletgram (tables 6, 7), and pequi oil was particularly efficient in reducing CK and AST values for the distance of 6–7 km and RDW values up to 8–10 km (tables 6, 7a).

Despite the observed differences, all were inside the cited reference values. Moreover, although a significant (p = 0.000) positive correlation (0.368) between age group and distance covered was found, no correlation among the analyzed parameters (sex, age and distance covered) and the polymorphisms was shown. Since for EPOR (rs121918116) all runners presented the wild-type genotype, only the results of EPO and ACTN3 polymorphisms will be presented. For EPO, significant differences between TT and TG genotypes were observed for CRP after pequi supplementation (p = 0.023) (table 8) and for MCHC before pequi supplementation (0.018) (table 9a), and between TT and GG genotypes for rods (p = 0.031) (table 9b)

**Table 5.** Hemogram of the age groups before and after pequi oil supplementation

| Age groups | 15–19 years | 20–40 years | 41–58 years | p values |
|---|---|---|---|---|
| **a** *Erythrogram* | | | | |
| RBC, $\times 10^6$/mm$^3$ | | | | |
| Before | 5.35±0.12 | 5.28±0.06 | 4.97±0.07[a, b] | 0.017 |
| After | 5.17±0.13* | 5.21±0.06* | 4.95±0.07 | 0.076 |
| HGB, g/dl | | | | |
| Before | 15.50±0.27 | 13.96±0.42 | 15.07±0.26 | 0.295 |
| After | 15.07±0.29* | 13.97±0.42 | 15.10±0.25 | 0.955 |
| HCT, % | | | | |
| Before | 45.35±0.83 | 45.24±0.45 | 43.80±0.72 | 0.248 |
| After | 43.71±0.88* | 44.71±0.43* | 43.53±0.67 | 0.292 |
| MCV, fl | | | | |
| Before | 84.92±0.80 | 85.91±0.46 | 88.12±0.52[a, b] | 0.011 |
| After | 84.84±0.78 | 86.03±0.47* | 88.04±0.54[a] | 0.016 |
| MCH, pg | | | | |
| Before | 29.05±0.34 | 29.25±0.18 | 30.33±0.26[a, b] | 0.006 |
| After | 29.28±0.33 | 29.65±0.18* | 30.54±0.25[a, b] | 0.010 |
| MCHC, g/% or g/dl | | | | |
| Before | 34.18±0.16 | 34.05±0.12 | 34.41±0.19 | 0.366 |
| After | 34.51±0.13 | 34.47±0.08* | 34.69±0.13 | 0.332 |
| RDW, % | | | | |
| Before | 14.25±0.19 | 14.96±0.12[a] | 14.93±0.17 | 0.015 |
| After | 14.26±0.24 | 14.22±0.13* | 14.31±0.18* | 0.946 |
| **b** *Leukogram* | | | | |
| WBC, /mm$^3$ | | | | |
| Before | 7,180.00±348.88 | 7,611.11±284.56 | 6,926.09±461.53 | 0.574 |
| After | 7,420.00±398.85 | 7,691.67±236.91 | 6,460.87±292.58[b] | 0.040 |
| Lymphocytes, /mm$^3$ | | | | |
| Before | 2,750.35±207.54 | 2,734.51±116.10 | 2,319.35±186.42 | 0.172 |
| After | 2,740.75±292.04 | 2,669.63±110.66 | 2,304.78±176.91 | 0.227 |
| Segmented, /mm$^3$ | | | | |
| Before | 3,693.65±222.97 | 4,104.26±228.92 | 3,928.83±320.03 | 0.785 |
| After | 3,799.15±258.84 | 4,138.11±188.40 | 3,479.35±190.61 | 0.149 |
| Rods, /mm$^3$ | | | | |
| Before | 26.65±24.24 | 32.81±10.39 | 7.61±5.26 | 0.286 |
| After | 33.47±24.39 | 27.71±9.98 | 19.22±13.86 | 0.817 |
| Basophils, /mm$^3$ | | | | |
| Before | 83.95±12.40 | 82.33±5.94 | 95.35±10.56 | 0.786 |
| After | 81.50±7.38 | 102.71±5.87* | 99.13±9.79 | 0.298 |
| Eosinophils, /mm$^3$ | | | | |
| Before | 129.15±21.55 | 152.82±13.51 | 109.83±16.91 | 0.199 |
| After | 173.75±24.36* | 151.90±14.33 | 115.57±16.28 | 0.164 |
| Monocytes, /mm$^3$ | | | | |
| Before | 490.75±45.69 | 501.70±27.89 | 465.52±37.57 | 0.805 |
| After | 587.35±45.92* | 561.18±22.72* | 441.91±37.91[a, b] | 0.013 |
| **c** *Plateletgram* | | | | |
| Platelets, $\times 10^3$/mm$^3$ | | | | |
| Before | 322.80±16.34 | 341.57±8.01 | 325.70±14.57 | 0.494 |
| After | 320.95±16.42 | 309.95±7.36* | 311.43±13.58 | 0.797 |
| Plateletcrit, % | | | | |
| Before | 0.33±0.02 | 0.37±0.01 | 0.34±0.02 | 0.303 |
| After | 0.33±0.02 | 0.32±0.01* | 0.33±0.02 | 0.654 |
| MPV, fl | | | | |
| Before | 10.23±0.29 | 10.67±0.22 | 10.37±0.25 | 0.767 |
| After | 10.33±0.30 | 10.33±0.22 | 10.47±0.26 | 0.595 |
| PDW, % | | | | |
| Before | 17.76±0.24 | 18.16±0.15 | 17.75±0.21 | 0.357 |
| After | 18.02±0.22 | 18.14±0.13 | 18.01±0.19 | 0.984 |

Data are expressed as mean ± SEM.

HGB = Hemoglobin; MCV = mean corpuscular volume; WBC = white blood cells.

p values for RBC, HCT, MCV and RDW were generated by ANOVA, while the other p values were generated by the Kruskal-Wallis test.

The lower-case letters indicate significant differences among age groups, with [a] = significant compared to 15–19 years; [b] = significant compared to 20–40 years.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

**Table 6.** Values of CK, AST, ALT, CRP, hs-CRP and Tbars assays for the distance covered (km) before and after pequi oil supplementation

| Distance | 4–5 km | 6–7 km | 8–10 km | 16–21 km | p values |
|---|---|---|---|---|---|
| Participants, n (%) | 50 (40.7) | 38 (30.9) | 28 (22.8) | 7 (5.7) | |
| CK, U/l | | | | | |
| Before | 219.46±31.03 | 348.14±76.76[a] | 295.68±63.65 | 372.71±98.86[a] | 0.032 |
| After | 205.73±23.82 | 242.14±33.66* | 267.46±63.35 | 313.00±84.85[a] | 0.131 |
| AST, U/l | | | | | |
| Before | 27.65±1.15 | 29.49±1.40 | 32.89±1.86[a] | 34.29±2.93[a] | 0.013 |
| After | 26.86±1.07 | 27.24±1.36* | 30.64±0.90[a] | 32.86±2.03[a, b] | 0.002 |
| ALT, U/l | | | | | |
| Before | 20.12±1.18 | 24.43±1.77[a] | 24.64±2.91 | 29.43±5.00[a] | 0.036 |
| After | 19.43±1.28 | 21.95±1.26 | 23.07±2.02 | 28.29±2.29[a, b] | 0.015 |
| CRP, mg/dl | | | | | |
| Before | 0.29±0.05 | 0.30±0.03 | 0.41±0.08 | 0.43±0.11 | 0.271 |
| After | 0.38±0.05 | 0.35±0.05 | 0.37±0.05 | 0.34±0.04 | 0.920 |
| hs-CRP, mg/dl | | | | | |
| Before | 1.30±0.35 | 1.85±0.32[a] | 1.80±0.50 | 1.51±0.74 | 0.024 |
| After | 1.53±0.33 | 1.54±0.24 | 1.68±0.34 | 0.99±0.25 | 0.305 |
| Tbars, nmol/ml of MDA | | | | | |
| Before | 0.0245±0.001 | 0.0302±0.001[a] | 0.0256±0.001[b] | 0.0271±0.003 | 0.005 |
| After | 0.0255±0.001 | 0.0282±0.001[a] | 0.0259±0.001 | 0.0253±0.002 | 0.170 |

Data are expressed as mean ± SEM.

p values were generated by the Kruskal-Wallis test.

The lower-case letters indicate significant differences among distance covered detected by the Mann-Whitney U test, with [a] = significant compared to 4–5 km; [b] = significant compared to 6–7 km.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

and platelets (p = 0.026) after pequi supplementation and for mean platelet volume (MPV) (p = 0.006) and platelet distribution width (PDW) (p = 0.003) before pequi supplementation. MPV also presented significant differences between TG and GG genotypes before pequi supplementation (p = 0.034) (table 9c). For ACTN3, the XX genotype presented significantly decreased values of mean corpuscular volume compared to the RR (p = 0.039) and RX (p = 0.005) genotypes before pequi supplementation, the same occurring after pequi supplementation with MCH compared to the RX genotype (p = 0.038) (table 10a). The XX genotype also presented increased lymphocytes before (p = 0.014) and after (p = 0.015) pequi supplementation compared to the RX genotype.

In the before-after comparisons, the EPO polymorphism mainly influenced the results of the erythrogram, where significant responses to the pequi oil were observed only for the wild-type genotype (TT) in the RBC (p = 0.000), HCT (p = 0.001), MCH (p = 0.000) and MCHC (p = 0.001) values, and for the TT and TG genotypes (p = 0.000 for both) in the RDW values (table 9a). The ACTN3 polymorphism mainly influenced AST and CK values, where RX heterozygotes had a significant reduction in AST values (p = 0.037), and homozygous XX individuals in CK values (p = 0.010) after pequi oil supplementation (table 8).

Both genetic polymorphisms were in HWE, and the distribution of their allele and genotype frequencies, as well as the genetic diversity parameters and HWE data for the $\chi^2$ test, are shown in table 11. No significant differences were observed for the distribution frequencies of the ACTN3 and EPO genotypes between sexes.

**Table 7.** Values of hemogram for the distance covered (km) before and after pequi oil supplementation

| Distance | 4–5 km | 6–7 km | 8–10 km | 16–21 km | p values |
|---|---|---|---|---|---|
| **a** *Erythrogram* | | | | | |
| RBC, $\times 10^6$/mm$^3$ | | | | | |
| Before | 5.32 ± 0.08 | 5.12 ± 0.07 | 5.25 ± 0.11 | 5.05 ± 0.13 | 0.238 |
| After | 5.21 ± 0.08* | 5.08 ± 0.07 | 5.16 ± 0.09* | 5.07 ± 0.18 | 0.664 |
| HGB, g/dl | | | | | |
| Before | 13.81 ± 0.56 | 15.10 ± 0.19 | 14.68 ± 0.55 | 14.81 ± 0.58 | 0.950 |
| After | 13.66 ± 0.57 | 15.10 ± 0.20 | 14.65 ± 0.53 | 14.97 ± 0.58 | 0.816 |
| HCT, % | | | | | |
| Before | 45.53 ± 0.57 | 44.29 ± 0.50 | 45.31 ± 0.79 | 43.03 ± 1.58 | 0.239 |
| After | 44.55 ± 0.56* | 44.09 ± 0.58 | 44.41 ± 0.66* | 43.26 ± 1.77 | 0.826 |
| MCV, fl | | | | | |
| Before | 85.74 ± 0.61 | 86.84 ± 0.48 | 86.43 ± 0.64 | 85.09 ± 2.11 | 0.499 |
| After | 85.76 ± 0.61 | 87.04 ± 0.52 | 86.25 ± 0.64 | 85.41 ± 2.14* | 0.470 |
| MCH, pg | | | | | |
| Before | 29.30 ± 0.24 | 29.59 ± 0.18 | 29.49 ± 0.30 | 29.30 ± 0.87 | 0.917 |
| After | 29.59 ± 0.25* | 29.82 ± 0.19 | 30.04 ± 0.25* | 29.56 ± 0.76 | 0.672 |
| MCHC, g/% or g/dl | | | | | |
| Before | 34.16 ± 0.13 | 34.10 ± 0.19 | 34.12 ± 0.21 | 34.40 ± 0.33 | 0.952 |
| After | 34.50 ± 0.10 | 34.26 ± 0.12 | 34.83 ± 0.09[a,*] | 34.64 ± 0.26 | 0.006 |
| RDW, % | | | | | |
| Before | 14.54 ± 0.14 | 14.80 ± 0.15 | 15.01 ± 0.17 | 16.34 ± 0.38[a–c] | 0.000 |
| After | 13.98 ± 0.13* | 14.20 ± 0.18* | 14.41 ± 0.18* | 15.69 ± 0.64[a–c] | 0.001 |
| **b** *Leukogram* | | | | | |
| WBC, /mm$^3$ | | | | | |
| Before | 7,372.92 ± 253.95 | 6,936.36 ± 402.97 | 7,757.14 ± 533.82 | 8,483.33 ± 951.99 | 0.269 |
| After | 7,737.50 ± 270.30 | 6,863.64 ± 278.01[a] | 7,517.86 ± 418.77 | 7,066.67 ± 857.39 | 0.153 |
| Lymphocytes, /mm$^3$ | | | | | |
| Before | 2,818.94 ± 134.56 | 2,284.48 ± 160.56[a] | 2,753.50 ± 197.93[b] | 2,892.83 ± 267.33[b] | 0.026 |
| After | 2,785.47 ± 144.47 | 2,494.85 ± 151.15 | 2,423.32 ± 223.90* | 2,672.83 ± 207.53 | 0.280 |
| Segmented, /mm$^3$ | | | | | |
| Before | 3,793.84 ± 163.81 | 3,908.42 ± 347.97 | 4,285.96 ± 393.05 | 4,827.33 ± 1,039.38 | 0.597 |
| After | 4,099.14 ± 198.04 | 3,445.73 ± 242.85 | 4,332.29 ± 262.16[b] | 3,703.17 ± 777.14 | 0.065 |
| Rods, /mm$^3$ | | | | | |
| Before | 8.14 ± 4.69 | 49.39 ± 18.49 | 26.00 ± 18.95 | 56.50 ± 56.50 | 0.022 |
| After | 23.81 ± 12.57 | 27.21 ± 14.26 | 38.22 ± 18.19 | 0.00 ± 0.00 | 0.307 |
| Basophils, /mm$^3$ | | | | | |
| Before | 85.76 ± 8.11 | 78.58 ± 7.68 | 91.29 ± 9.71 | 88.50 ± 20.52 | 0.713 |
| After | 102.47 ± 7.36 | 87.85 ± 7.20 | 102.54 ± 9.05 | 102.83 ± 16.37 | 0.491 |
| Eosinophils, /mm$^3$ | | | | | |
| Before | 163.45 ± 16.54 | 141.06 ± 17.12 | 114.29 ± 18.47[a] | 66.83 ± 30.26[a] | 0.043 |
| After | 158.80 ± 15.80 | 176.15 ± 24.07 | 109.14 ± 14.83 | 95.33 ± 26.44 | 0.218 |
| Monocytes, /mm$^3$ | | | | | |
| Before | 514.04 ± 34.47 | 459.73 ± 37.93 | 481.39 ± 39.16 | 551.33 ± 46.10 | 0.678 |
| After | 602.20 ± 27.39* | 489.18 ± 38.65[a] | 509.68 ± 32.84[a] | 492.50 ± 47.82 | 0.020 |
| **c** *Plateletgram* | | | | | |
| Platelets, $\times 10^3$/mm$^3$ | | | | | |
| Before | 324.94 ± 8.97 | 328.39 ± 10.42 | 364.89 ± 16.30 | 321.00 ± 24.82 | 0.372 |
| After | 310.16 ± 8.12 | 302.33 ± 10.54* | 334.04 ± 14.63* | 284.29 ± 28.56 | 0.241 |
| Plateletcrit, % | | | | | |
| Before | 0.35 ± 0.01 | 0.34 ± 0.01 | 0.38 ± 0.02 | 0.35 ± 0.02 | 0.579 |
| After | 0.32 ± 0.01* | 0.32 ± 0.01* | 0.36 ± 0.02 | 0.31 ± 0.03 | 0.459 |
| MPV, fl | | | | | |
| Before | 10.68 ± 0.28 | 10.52 ± 0.26 | 10.35 ± 0.26 | 10.28 ± 0.42 | 0.982 |
| After | 10.17 ± 0.22* | 10.51 ± 0.31 | 10.41 ± 0.34 | 10.76 ± 0.74 | 0.714 |
| PDW, % | | | | | |
| Before | 18.06 ± 0.18 | 17.98 ± 0.21 | 17.89 ± 0.20 | 18.42 ± 0.58 | 0.901 |
| After | 18.08 ± 0.15 | 17.97 ± 0.20 | 18.23 ± 0.18 | 18.22 ± 0.38 | 0.577 |

Data are expressed as mean ± SEM.

HGB = Hemoglobin; MCV = mean corpuscular volume; WBC = white blood cells.

p values for HGB, HCT, MCV and RDW were generated by ANOVA, while the other p values were generated by the Kruskal-Wallis test.

The lower-case letters indicate significant differences among distance covered detected by the Mann-Whitney U test, with [a] = significant compared to 4–5 km; [b] = significant compared to 6–7 km; [c] = significant compared to 8–10 km.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

**Table 8.** Influence of EPO (SNP rs1617640) and ACTN3 R577X (SNPs rs1815739) polymorphisms on the CK, AST, ALT, CRP, hs-CRP and Tbars assay values before and after pequi oil supplementation

| | EPO | | | p values | ACTN3 R577X | | | p values |
|---|---|---|---|---|---|---|---|---|
| | TT | TG | GG | | RR | RX | XX | |
| CK, U/l | | | | | | | | |
| Before | 338.53±58.19 | 243.64±29.94 | 209.40±59.22 | 0.329 | 342.86±70.53 | 229.77±28.14 | 317.50±67.19 | 0.216 |
| After | 277.19±37.28 | 193.58±19.55 | 245.40±78.68 | 0.252 | 257.59±30.89 | 243.20±36.49 | 178.05±25.24* | 0.162 |
| AST, U/l | | | | | | | | |
| Before | 29.95±1.18 | 29.72±1.22 | 29.50±2.70 | 0.993 | 29.25±1.13 | 30.67±1.27 | 28.60±2.12 | 0.591 |
| After | 28.28±1.10 | 27.70±0.79 | 30.40±2.75 | 0.804 | 28.48±1.10 | 28.53±1.03* | 26.65±1.31 | 0.756 |
| ALT, U/l | | | | | | | | |
| Before | 22.21±1.42 | 23.43±1.71 | 25.50±3.18 | 0.359 | 21.84±1.43 | 24.39±1.76 | 21.75±2.16 | 0.638 |
| After | 21.51±1.37 | 21.28±1.05 | 23.30±2.70 | 0.575 | 21.07±1.43 | 22.37±1.23 | 20.30±1.69 | 0.573 |
| CRP, mg/dl | | | | | | | | |
| Before | 0.36±0.05 | 0.31±0.04 | 0.28±0.08 | 0.815 | 0.33±0.05 | 0.30±0.03 | 0.42±0.11 | 0.612 |
| After | 0.44±0.05 | 0.29±0.03[a] | 0.33±0.07 | 0.069 | 0.33±0.04 | 0.36±0.03 | 0.46±0.09 | 0.279 |
| hs-CRP, mg/dl | | | | | | | | |
| Before | 1.68±0.31 | 1.68±0.34 | 0.81±0.19 | 0.669 | 1.78±0.41 | 1.20±0.18 | 2.37±0.73 | 0.287 |
| After | 1.99±0.30 | 1.09±0.15 | 1.16±0.58 | 0.121 | 1.61±0.29 | 1.46±0.22 | 1.64±0.54 | 0.704 |
| Tbars, nmol/ml of MDA | | | | | | | | |
| Before | 0.0271±0.001 | 0.0267±0.001 | 0.0237±0.003 | 0.388 | 0.0274±0.001 | 0.0268±0.001 | 0.0247±0.002 | 0.417 |
| After | 0.0274±0.001 | 0.0255±0.001 | 0.0258±0.002 | 0.258 | 0.027±0.001 | 0.0261±0.001 | 0.0262±0.001 | 0.751 |

Data are expressed as mean ± SEM.

For EPO, all p values were generated by the Kruskal-Wallis test; for ACTN3, p values of Tbars were generated by ANOVA, while p values of the other parameters were generated by the Kruskal-Wallis test.

The lower-case letters indicate significant differences detected between genotypes in the 2-to-2 comparisons, with [a] = significant compared to the first genotype of each genetic marker.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

## Discussion

There are several types of experimental studies, including randomized placebo-controlled, case-control, quasi-experimental and before-after studies. They are different and each one has its own characteristics. Because most exercise-induced physiological and biochemical changes have already been well documented [30, 38–45], and our study did not aim to evaluate such alterations, it was designed to be a before-after study, which differs from a randomized placebo-controlled study. Before-after studies have been validated in the scientific literature [46], and although they can present some limitations compared to randomized placebo-controlled studies, such as the lack of a placebo group, we followed all the steps to guarantee quality control and validation of our study [46, 47], as discussed below.

Physical training induces beneficial adaptations; however, exhaustive exercise can increase the generation of reactive oxygen species (ROS) and cause damage in muscles and in many other organs and body systems responsible for the regulation and maintenance of homeostasis, a fact that may compromise the athlete's performance and possibly lead to overtraining syndrome [1, 41, 44, 45]. Although it is difficult for any study to control all the involved variables, a number of them were controlled in this study: (1) only trained sportsmen/women were included; (2) although the athletes underwent a varying degree of training intensity, the amount of training per week was similar (in terms of number of days and hours of training); (3) the distance covered and the time required to cover it were chosen according to the type, intensity and duration of weekly training of each athlete, guaranteeing no additional physical stress beyond what they were accustomed to; (4) the volunteers ran the same

**Table 9.** Influence of EPO (SNP rs1617640) polymorphism on the hemogram before and after pequi oil supplementation

| | EPO genotypes | | | p values |
|---|---|---|---|---|
| | TT | TG | GG | |
| **a** *Erythrogram* | | | | |
| RBC, $\times 10^6$/mm$^3$ | | | | |
| Before | 5.23 ± 0.07 | 5.27 ± 0.07 | 5.04 ± 0.14 | 0.401 |
| After | 5.11 ± 0.07* | 5.22 ± 0.07 | 5.02 ± 0.13 | 0.369 |
| HGB, g/dl | | | | |
| Before | 14.12 ± 0.44 | 14.65 ± 0.41 | 15.11 ± 0.57 | 0.454 |
| After | 14.03 ± 0.44 | 14.60 ± 0.41 | 15.19 ± 0.58 | 0.485 |
| HCT, % | | | | |
| Before | 44.99 ± 0.48 | 45.20 ± 0.53 | 43.73 ± 1.58 | 0.534 |
| After | 44.03 ± 0.49* | 44.74 ± 0.48 | 43.59 ± 1.59 | 0.494 |
| MCV, fl | | | | |
| Before | 86.37 ± 0.54 | 85.88 ± 0.46 | 86.69 ± 1.49 | 0.725 |
| After | 86.46 ± 0.54 | 85.88 ± 0.49 | 86.66 ± 1.42 | 0.685 |
| MCH, pg | | | | |
| Before | 29.28 ± 0.21 | 29.49 ± 0.20 | 29.93 ± 0.64 | 0.279 |
| After | 29.79 ± 0.21* | 29.66 ± 0.19 | 30.16 ± 0.59 | 0.565 |
| MCHC, g/% or g/dl | | | | |
| Before | 33.90 ± 0.13 | 34.34 ± 0.14[a] | 34.52 ± 0.23 | 0.028 |
| After | 34.46 ± 0.09* | 34.53 ± 0.10 | 34.8 ± 0.17 | 0.317 |
| RDW, % | | | | |
| Before | 14.69 ± 0.14 | 14.94 ± 0.12 | 15.09 ± 0.42 | 0.303 |
| After | 14.11 ± 0.12* | 14.28 ± 0.15* | 14.84 ± 0.54 | 0.129 |
| **b** *Leukogram* | | | | |
| WBC, /mm$^3$ | | | | |
| Before | 7,037.04 ± 258.03 | 7,737.25 ± 351.87 | 7,630.00 ± 800.15 | 0.543 |
| After | 7,198.15 ± 259.08 | 7,582.35 ± 265.34 | 7,540.00 ± 677.94 | 0.699 |
| Lymphocytes, /mm$^3$ | | | | |
| Before | 2,595.04 ± 131.05 | 2,690.15 ± 133.40 | 2,795.10 ± 353.24 | 0.839 |
| After | 2,502.44 ± 125.11 | 2,722.54 ± 146.25 | 2,600.40 ± 373.40 | 0.574 |
| Segmented, /mm$^3$ | | | | |
| Before | 3,714.63 ± 195.74 | 4,283.83 ± 281.46 | 4,049.80 ± 478.14 | 0.426 |
| After | 3,885.31 ± 198.08 | 4,039.31 ± 193.88 | 3,823.90 ± 534.99 | 0.778 |
| Rods, /mm$^3$ | | | | |
| Before | 28.30 ± 11.40 | 23.67 ± 11.27 | 33.90 ± 33.90 | 0.780 |
| After | 15.64 ± 8.21 | 27.45 ± 11.90 | 84.40 ± 50.01[a] | 0.117 |
| Basophils, /mm$^3$ | | | | |
| Before | 79.30 ± 6.59 | 91.04 ± 7.31 | 86.60 ± 19.31 | 0.502 |
| After | 95.13 ± 6.76 | 105.00 ± 6.10 | 81.10 ± 15.66 | 0.249 |
| Eosinophils, /mm$^3$ | | | | |
| Before | 135.57 ± 13.38 | 141.54 ± 16.35 | 158.40 ± 31.54 | 0.801 |
| After | 133.74 ± 13.21 | 168.46 ± 18.29 | 124.00 ± 24.01* | 0.284 |
| Monocytes, /mm$^3$ | | | | |
| Before | 481.46 ± 33.07 | 509.33 ± 28.68 | 466.20 ± 55.75 | 0.426 |
| After | 535.43 ± 30.72 | 553.88 ± 23.77 | 516.20 ± 60.06 | 0.633 |
| **c** *Plateletgram* | | | | |
| Platelets, $\times 10^3$/mm$^3$ | | | | |
| Before | 333.00 ± 9.10 | 344.63 ± 10.26 | 298.70 ± 15.02 | 0.160 |
| After | 316.89 ± 8.50* | 314.17 ± 9.42* | 275.20 ± 16.87[a] | 0.098 |
| Plateletcrit, % | | | | |
| Before | 0.34 ± 0.01 | 0.37 ± 0.01 | 0.37 ± 0.03 | 0.176 |
| After | 0.32 ± 0.01 | 0.33 ± 0.01* | 0.32 ± 0.04 | 0.988 |
| MPV, fl | | | | |
| Before | 10.23 ± 0.19 | 10.65 ± 0.24 | 12.10 ± 0.66[a, b] | 0.022 |
| After | 10.18 ± 0.18 | 10.40 ± 0.26 | 11.37 ± 0.72 | 0.283 |
| PDW, % | | | | |
| Before | 17.67 ± 0.12 | 18.18 ± 0.17 | 19.43 ± 0.64[a] | 0.006 |
| After | 18.00 ± 0.11* | 18.09 ± 0.16 | 18.77 ± 0.53 | 0.440 |

Data are expressed as mean ± SEM.

HGB = Hemoglobin; MCV = mean corpuscular volume; WBC = white blood cells.

p values of RBC, HCT, MCV and RDW were generated by ANOVA, while the other p values were generated by the Kruskal-Wallis test.

The lower-case letters indicate significant differences detected between genotypes in the 2-to-2 comparisons, with [a] = significant compared to the first genotype; [b] = significant compared to the second genotype.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

**Table 10.** Influence of ACTN3 R577X (SNPs rs1815739) polymorphism on the hemogram before and after pequi oil supplementation

| | ACTN3 genotypes | | | p values |
|---|---|---|---|---|
| | RR | RX | XX | |
| **a** *Erythrogram* | | | | |
| RBC, $\times 10^6/mm^3$ | | | | |
| Before | 5.27 ± 0.07 | 5.13 ± 0.07 | 5.41 ± 0.14 | 0.078 |
| After | 5.17 ± 0.07 | 5.07 ± 0.07 | 5.34 ± 0.13 | 0.113 |
| HGB, g/dl | | | | |
| Before | 14.47 ± 0.46 | 14.19 ± 0.44 | 15.01 ± 0.51 | 0.425 |
| After | 14.4 ± 0.47 | 14.14 ± 0.45 | 14.97 ± 0.52 | 0.455 |
| HCT, % | | | | |
| Before | 45.34 ± 0.56 | 44.49 ± 0.54 | 45.43 ± 0.81 | 0.364 |
| After | 44.46 ± 0.54* | 43.91 ± 0.53 | 44.98 ± 0.75 | 0.432 |
| MCV, fl | | | | |
| Before | 86.27 ± 0.61 | 86.85 ± 0.46 | 84.23 ± 0.77[a, b] | 0.026 |
| After | 86.37 ± 0.60 | 86.73 ± 0.49 | 84.56 ± 0.85 | 0.138 |
| MCH, pg | | | | |
| Before | 29.38 ± 0.23 | 29.72 ± 0.19 | 28.78 ± 0.41 | 0.166 |
| After | 29.87 ± 0.23* | 29.93 ± 0.19 | 29.07 ± 0.39[b] | 0.099 |
| MCHC, g/% or g/dl | | | | |
| Before | 34.06 ± 0.15 | 34.22 ± 0.14 | 34.14 ± 0.23 | 0.608 |
| After | 34.59 ± 0.09* | 34.53 ± 0.10 | 34.36 ± 0.15 | 0.409 |
| RDW, % | | | | |
| Before | 14.89 ± 0.16 | 14.71 ± 0.13 | 15.03 ± 0.22 | 0.445 |
| After | 14.28 ± 0.20* | 14.17 ± 0.13* | 14.39 ± 0.18* | 0.667 |
| **b** *Leukogram* | | | | |
| WBC, $/mm^3$ | | | | |
| Before | 7,577.27 ± 367.35 | 7,074.51 ± 323.99 | 7,835.00 ± 339.37 | 0.147 |
| After | 7,450.00 ± 294.08 | 7,223.53 ± 276.27 | 7,730.00 ± 380.31 | 0.430 |
| Lymphocytes, $/mm^3$ | | | | |
| Before | 2,695.66 ± 150.15 | 2,513.50 ± 142.05 | 2,933.00 ± 155.61[b] | 0.065 |
| After | 2,594.68 ± 142.30 | 2,486.35 ± 149.18 | 2,962.60 ± 196.88[b] | 0.066 |
| Segmented, $/mm^3$ | | | | |
| Before | 49.37 ± 19.17 | 3,848.46 ± 206.96 | 4,073.70 ± 258.23 | 0.755 |
| After | 33.21 ± 15.46 | 3,916.94 ± 194.29 | 3,897.60 ± 260.05 | 0.935 |
| Rods, $/mm^3$ | | | | |
| Before | 49.37 ± 19.17 | 10.60 ± 4.45 | 19.80 ± 11.11 | 0.396 |
| After | 33.21 ± 15.46 | 30.40 ± 11.96 | 4.85 ± 4.85 | 0.517 |
| Basophils, $/mm^3$ | | | | |
| Before | 84.89 ± 8.24 | 83.71 ± 6.99 | 89.70 ± 10.86 | 0.638 |
| After | 101.80 ± 7.51 | 93.15 ± 6.41 | 104.25 ± 10.09 | 0.626 |
| Eosinophils, $/mm^3$ | | | | |
| Before | 145.05 ± 17.85 | 133.94 ± 14.87 | 145.90 ± 18.01 | 0.463 |
| After | 167.09 ± 20.66 | 130.25 ± 13.87 | 154.85 ± 18.24 | 0.232 |
| Monocytes, $/mm^3$ | | | | |
| Before | 457.64 ± 31.56 | 488.65 ± 26.56 | 580.00 ± 66.04 | 0.119 |
| After | 538.91 ± 28.32* | 516.25 ± 28.23 | 616.00 ± 44.83 | 0.161 |
| **c** *Plateletgram* | | | | |
| Platelets, $\times 10^3/mm^3$ | | | | |
| Before | 342.58 ± 10.68 | 327.35 ± 9.16 | 339.25 ± 16.94 | 0.535 |
| After | 308.80 ± 10.45* | 309.87 ± 8.59* | 325.45 ± 13.99 | 0.700 |
| Plateletcrit, % | | | | |
| Before | 0.36 ± 0.01 | 0.34 ± 0.01 | 0.38 ± 0.03 | 0.921 |
| After | 0.32 ± 0.01* | 0.32 ± 0.01* | 0.35 ± 0.02 | 0.279 |
| MPV, fl | | | | |
| Before | 10.51 ± 0.26 | 10.63 ± 0.22 | 10.30 ± 0.40 | 0.587 |
| After | 10.23 ± 0.25 | 10.48 ± 0.21 | 10.30 ± 0.53 | 0.564 |
| PDW, % | | | | |
| Before | 18.16 ± 0.19 | 17.98 ± 0.15 | 17.75 ± 0.32 | 0.306 |
| After | 18.16 ± 0.16 | 18.04 ± 0.14 | 18.07 ± 0.26 | 0.772 |

Data are expressed as mean ± SEM.

HGB = Hemoglobin; MCV = mean corpuscular volume; WBC = white blood cells.

p values of RBC and monocytes were generated by ANOVA, while p values of the other parameters were generated by the Kruskal-Wallis test.

The lower-case letters indicate significant differences detected between genotypes in the 2-to-2 comparisons, with [a] = significant compared to the first genotype; [b] = significant compared to the second genotype.

* Indicates significant differences in the comparison of before-after pequi oil supplementation.

**Table 11.** Distribution of EPO and ACTN3 allele frequencies, genetic diversity parameters, genotype frequencies and HWE data for the $\chi^2$ test

| Genetic markers | Chromosome location | Allele | Allele frequencies | Heterozygosity | | Inbreeding coefficient |
|---|---|---|---|---|---|---|
| | | | | observed | expected | |
| EPO T→G | 7q22.1 | T<br>G | 0.699<br>0.301 | 0.44 | 0.42 | –0.0396 |
| ACTN3 R577X | 11q13.2 | R<br>X | 0.598<br>0.402 | 0.46 | 0.48 | 0.0406 |
| | | Genotypes | Genotype frequencies | Individuals, n | | HWE test, p values |
| | | | | observed | expected | |
| EPO T→G | | TT<br>TG<br>GG | 48.0<br>43.9<br>8.1 | 59<br>54<br>10 | 60.1<br>51.8<br>11.1 | 0.8300 |
| ACTN3 R577X | | RR<br>RX<br>XX | 36.6<br>46.3<br>17.1 | 45<br>57<br>21 | 43.8<br>59.4<br>19.8 | 0.7092 |

p values were generated using the statistical program Genepopweb version 4.0 (http://genepop.curtin.edu.au).

route and distances in both races in the same sample time interval and under the same environmental conditions, and (5) the only change in the athletes' routine between the two races was the supplementation with pequi oil. Thus, although we cannot exclude possible differences in relative intensity experienced by the athletes completing a distance in both races with the same running time, because we did not measure oxygen uptake or heart rate parameters, the significant differences obtained in this study are more likely to be related to the pequi oil supplementation and/or its interaction with genetic factors than to differences in the relative intensity or amount of training. Our results support this assertion, since they did not exceed reference values determined for clinical purposes [9, 24, 31–33], and much less for athletes [30].

AST is used to evaluate muscular, myocardium, erythrocyte, hepatic, renal and pancreatic cell injuries [48], while increased ALT levels indicate hepatic lesions [49]. Total CK is the most widely used biochemical marker to evaluate muscular cell injury, especially for determining the effects of exhausting exercise in healthy individuals [43, 50]. Considering the general downward trend in the values of CK, AST, ALT, RDW, PLT and plateletcrit after supplementation with the capsules, our results suggest that pequi oil was effective in reducing exercise-induced oxidative cell damage. Results obtained with the minimum sample size calculations corroborate this suggestion, since the supplementation with pequi capsules reduced the variability of the sample and, consequently, the required sample size. Other reports with lower or equal intervention times have also shown physiological effects, with significant changes in the used markers [51–55].

Aerobic exercise regularly expands baseline plasma volume, and there is a high variability of change in this volume in the same individual and between different individuals, by virtue of physical performance [39]. Since (1) the athletes ran the same distance in both races, in the same time interval and under the same environmental conditions and (2) each one of

the athletes participated in the control and treatment group and they were compared with themselves in statistical tests, the general trend of reduced erythrocytes, hemoglobin and HCT after supplementation with capsules indicates that pequi oil favored a higher increase in plasma volume expansion. This is because after pequi supplementation there was also a general trend toward increased MCH and MCHC within the reference values, where MCH indicates the average amount of oxygen-carrying hemoglobin inside an RBC, i.e., the weight of hemoglobin in a medium RBC, and MCHC indicates the mean concentration of hemoglobin inside RBCs (with respect to 1 dl of erythrocytes) [56].

It is a well-known fact that blood parameters vary in accordance with stress, duration and type of exercise. There can be changes in blood values during and after intensive exercise caused by differences such as the state of individual training, environmental factors and nutrition [57–62]. As the only change in the routine of athletes between the two races was the pequi oil supplementation, the results of the before-after comparisons suggest that the increase in MCH and MCHC after pequi supplementation was probably due to its protective action on RBCs against the exercise-induced oxidative damage. The general trend of reduction in RDW values, with a return to baseline after using the capsules, corroborates this suggestion, since this hematimetric index assesses the degree of anisocytosis in the population of erythrocytes [56], and exhaustive exercise can compromise our ability to detoxify ROS inside the blood cells, where the RBCs are more susceptible to oxidative damage [63, 64].

The increase in oxidative damage associated with aging has been well documented, and aged muscles exhibit a lower antioxidant capacity of adaptation than young muscles. These facts, along with the knowledge that strenuous exercise increases ROS generation [44, 45, 65–68], are consistent with our results for CK and AST, which also indicated that the protection provided by the pequi capsules was more efficient for younger groups and the distance of 6–7 km. This suggests that long-distance running can be harmful for subjects, especially older athletes, due to increased oxidative stress above the adaptability of the organism, even with the use of antioxidant supplements.

Outcomes also showed a positive correlation between age and distance covered, where greater distances were covered by young adults and middle-aged athletes, with the participation of only 1 woman in the distance of 16–21 km. This difference between men and women can be explained by differences in anthropometry and physiology: men have a higher muscle mass than women, besides larger hearts, and therefore they have a higher cardiac output [69]. Such differences also serve to at least partially explain the differences between the sexes observed in the results of biochemical dosages and hemogram for the same parameters described in the literature, with different reference values for females and males.

However, physical performance is a physiological state that is highly dependent on different and complex factors. It involves a set of characteristics such as agility, muscle power, speed, equilibrium and coordination, flexibility, force and muscular resistance, cardiorespiratory resistance, corporal composition among others, which lead to better physical aptitude [70]. As a multifactorial phenotype, it is influenced by physique, and by biomechanical, physiological, metabolic, behavioral, psychological, social and genetic characteristics [12, 71]. Thus, considering what has previously been discussed, and since, despite the significant differences between sexes, age groups and distances, no correlation was found between these parameters and the analyzed polymorphisms, it is more likely that the significant differences presented in this study are related to dietary intervention affecting genes/alleles and genetic factors affecting the response to supplementation.

It is estimated that approximately 30,000 genes and approximately 10 million SNPs may be related in some way to physical performance, with heritability estimates varying between 20 and 75% [70]. Although several candidate genes have been proposed to explain individual differences in human endurance phenotypic traits, particularly those involved in energy

metabolism or cardiovascular function [10, 13], muscle efficiency has been less studied in the scientific literature than these traits [10], and it is also a critical factor determining endurance performance. In this context, although cardiorespiratory fitness was not tested in our work by the conventional methods ($V_{O_{2max}}$, $V_{O_2}R$, $HR_{max}$, HRR and LT, among others), the fitness of the athletes in terms of muscle performance was established here through biochemical parameters and complies with previous reports [3, 72]. Moreover, since the aerobic capacity is directly dependent on the oxygen transport by hemoglobin contained within RBCs, and levels of RBCs are tightly controlled according to the demand for oxygen [73], evaluations of the erythrogram may be a good parameter to help assess cardiorespiratory fitness, given that the availability of oxygen plays a critical role in athletic performance [73].

Although some of the analyzed biochemical and hematological parameters showed significant differences among sexes, age groups and distance covered, these have already been thoroughly discussed above and in our previous reports [3, 4, 9]. Additionally, the significant differences between sexes were expected, since they had already been observed for the same parameters reported in the literature for females and males with different reference values [9, 24, 30–33]. Since there was no correlation among sexes, age groups or distance covered with EPO and ACTN3 polymorphisms, our data indicate a significant influence from the EPO polymorphism on the results of the erythrogram and plateletgram. They also suggest an aerobic advantage for the TG genotype, which significantly increased MCHC values compared to the TT genotype before pequi supplementation, indicating a higher oxygen-carrying capacity. Although the TT genotype has been associated with increased EPO concentration in human vitreous body and microvascular complications of diabetes, including proliferative diabetic retinopathy and diabetic end-stage renal disease [36], as mentioned earlier, the T allele is referred to as the ancestral one by the official website [17], which means that the T allele is the normal allele. Seen in this way, the variant G allele of SNP rs1617640 in the EPO gene would reduce EPO expression, and our results are not in complete agreement with these findings.

However, EPO is the main endogenous hormone regulator of erythropoiesis, which promotes erythroid differentiation and initiates hemoglobin synthesis, and its expression provides protection against apoptosis of erythroid progenitors and is upregulated in the liver and kidney by hypoxia [74–76]. As our study was conducted with athletes and not with diabetic patients, the results of the erythrogram suggest that the presence of the G allele in the heterozygous individual could favor a better response to the ischemia/perfusion process that can occur in these organs due to the cyclical process of contraction-relaxation established during the race [1]. Moreover, from our results and given the fact that during endurance exercise hemolysis can also occur, as a result of mechanical trauma in the capillaries of runners' feet [77] or erythrocyte rupture by mechanical, osmotic and oxidative events [78], it is more likely that the low frequency of the GG genotype found in our study may be related to possible vascular complications due to platelet activation. This is because MPV is an indicator of platelet activation [4], and this genotype presented significantly higher MPV than the TT and TG genotypes, besides showing significantly higher PDW values than the TT genotype. As PDW is an indicator of variation in platelet size, which has been reported as a sign of active platelet release and as a more specific marker of platelet activation, being increased in vaso-occlusive events [79, 80], our results of the erythrogram and plateletgram suggest a possibly higher association of the G allele with possible microvascular complication events in endurance athletes compared with the T allele. Moreover, because diet can interact with the human genome to influence health and disease, and genetic variability can influence response to diet [4], results also suggest that pequi oil could reduce this risk, since the significant differences among the genotypes disappeared after pequi oil supplementation. Furthermore, because erythrocytes are prone to suffer from exercise-induced lipid oxidative damage [64], mechanical

stress and cytosolic and extracellular pH changes [63], the significant reduction in RDW values for TT and TG genotypes and nonsignificant fall for the GG genotype indicates that pequi oil exerted protective effects on RBCs.

Regarding the ACTN3 R577X polymorphism, although the R allele has been found to be associated with power-oriented performance, and the XX genotype with endurance ability [14, 15, 81], our study did not find the latter association, corroborating another study [82]. Perhaps this occurred because skeletal muscles of athletes involved in endurance sports are characterized by a high proportion of type I (slow-twitch) fibers, as well as by high activity levels of marker enzymes of aerobic oxidative metabolism [83], and the ACTN3 gene (locus 11q13-q14) encodes α-actinin-3, a structural protein of the sarcomeric Z line of type II (fast-twitch) muscle fibers, related to power and muscular strength, and with predominance in the anaerobic energy metabolism type [15, 84, 85]. In skeletal muscle, there are two closely related α-actinins, the α-actinin-2 and α-actinin-3 isoforms, which are the main structural component of the sarcomeric Z lines that anchor the actin-containing thin filaments and maintain the spatial relationship between myofilaments [14]. Since the ACTN3 gene is expressed only in type II muscle fibers, the ACTN2 gene expresses the α-actinin-2 in all skeletal muscle fibers, and the 577XX genotype results in complete deficiency of protein α-actinin-3, the high frequency of α-actinin-3 deficiency in the general population and the absence of an obviously associated disease phenotype suggests that α-actinin-3 could be functionally redundant in humans [14]. Moreover, the high proportion of type I fibers in endurance athletes expressing α-actinin-2, added to a lower proportion of type II fibers expressing the same protein, could compensate for the deficiency of α-actinin-3 in type II fibers of individuals carrying the 577XX genotype. In fact, it has been demonstrated in an ACTN3 knockout mouse that there is a compensatory upregulation of the related isoform α-actinin-2, which is expressed in all muscle fibers in postnatal knockout muscles, mimicking the pattern of expression in humans deficient for α-actinin-3 [86]. All these aspects may explain our finding no significant difference among the ACTN3 genotypes for the biochemical parameters analyzed in this study. Additionally, they also indicate that ACTN3 may not be an appropriate candidate gene to assess athletic performance, at least in endurance athletes. On the other hand, muscle damage can compromise performance, and the cytosolic proteins CK and AST increase in the circulation after exercise, reflecting cellular injury, and can thus be used as exercise-induced damage markers [70]. Therefore, the significant responses for the ACTN3 XX genotype in the results of CK and for the RX genotype in the results of AST after pequi oil supplementation emphasize the importance of studying nutrigenomic effects on athletes' performance.

## Conclusions

In summary, before pequi supplementation, the EPO polymorphism influenced the results of the erythrogram and plateletgram, suggesting an aerobic advantage for the TG genotype and a disadvantage for the GG genotype with regard to possible microvascular complication events, while no association was found for the ACTN3 polymorphism with endurance performance. Both polymorphisms influenced runners' response to pequi oil, where significant responses were observed for the EPO wild-type genotype (TT) in the RBCs, HCT, MCH and MCHC values, and for the TT and TG genotypes in the RDW values. Significant differences were also observed in the plateletgram, but only for the TT and TG genotypes. The ACTN3 polymorphism mainly influenced AST and CK values, where RX heterozygotes had a significant reduction in AST values and homozygous XX individuals in CK values after pequi oil supplementation, emphasizing the importance of studying nutrigenomic effects on athletes' performance.

## Acknowledgments

The authors gratefully acknowledge the subjects who participated in this research, Sabin Institute/Sabin Laboratories and Farmacotécnica for technical support. They also thank the National Council for Technological and Scientific Development (CNPq), the Coordination for Further Training of Graduate Staff (CAPES) and the Scientific and Technological Enterprises Foundation (FINATEC) for financial support.

## Disclosure Statement

The authors declare no conflict of interest.

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