

Blood parameters as a measure for controlling physical performance of young Algerian cyclists (U23 category)

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Abstract

Purpose: The use of blood parameters in monitoring athletes is an essential but an unstandardized component of managing athletic preparation. This study aims to describe and evaluate typical measurements and responses observed while monitoring elite cyclist during a training camp. The reported observations might contribute in constituting a scientific support for other practitioners to employ.

Material: 35 elite cyclists from the Algerian National team aged 16 – 23 years participated in this study. Peripheral fasting blood samples were collected in resting after 24 hrs of physical inactivity and outside competitions. Complete blood count (CBC) and hormonal index values (Cortisol, Testosterone, ProBNP and TnT) were tested twice before and after the training camp. The statistical data were analysed by the SPSS software version 22.0.

Results: The observed rates of change were significant ($p < 0,01$, $p < 0,05$) for most erythrocyte variables, except for leukocyte and platelet distribution levels. Hormonal values recorded for Troponin ($\downarrow 92,78\%$, $p = 0,000$) and Cortisol ($\downarrow 11,85\%$, $p = 0,000$) remained significantly as an anticipatory response to competition. The responses of the ProBNP and testosterone were not statistically significant and experienced a different response with regards to their kinetics.

Conclusions: This study is further support suggesting a viable approach to monitoring physical performance index in elite athletes. The results imply that reducing volume while increasing intensity of training just before competition can enhance performance during short preparation periods.

Keywords: physiological follow-up, physical performance, hematologic indices, hormonal profile, young cyclists.

Introduction

To bring a cyclist at his highest level of performance requires a rational management of the training process. This consists of organizing, controlling, monitoring certain parameters that are decisive for the establishment of high-level performance [1].

The management and organization of training periods and cycles is based on the competition schedule. Short-term “intensified training” (IT) are often used during training cycles over the course of a sporting season to elicit performance gains [2]. These typically short-term IT periods occur in the forms of both training (training camp) and during busy competition schedules (stage races, tournaments) [3].

The process of training camp is commonly used in several endurance sports [4] to enhance training adaptation at specific times in the season [5]. In sport such cycling, the main goal of training camp is to prepare cyclists for the upcoming competition period [6].

For the preparation of the Arab Championships, the Algerian Cycling Federation had, among others, resorted to a short-term training course. The nature of training camp was manipulated to align with certain goals:

improving aerobic capacities, perform high intensity work and preparing for a specific competition. To date, little is known about the hematological and hormonal effects of this kind of training.

Many athletes, coaches, and support staff are taking an increasingly scientific approach to both designing and monitoring training programs [7]. However, highly congested competition calendars in combination with inadequate athletic preparation management could negatively impact athlete performance.

Given the complexity of athletic preparation, some blood markers are typically employed for physiological profiling and monitoring purposes in athletes. During training and competition, hematologic [8], hormonal and immunological [9, 10] markers are routinely used in evaluating the health and performance of professional athletes.

In fact, training induced changes observed in various biochemical variables can be attributed to appropriate load dynamics [11]. Monitoring athletes using blood biomarkers seems to be appropriate tool for making statistical inferences in several key biological systems affected by training.

With the purpose that results can be correctly interpreted and useful in the sport practice, it is crucial to have reference values specific for athletes. So, it is important to establish baseline indices for the main

variables (cortisol, testosterone, hematocrit) involved in the biological monitoring of cyclists [6]. Physiological baseline values would allow coaches to improve proxies of physical performance.

Although several descriptive and cross-sectional studies have well documented the physiological and / or physical responses of high-level cyclists [12, 13]. The use of blood parameters, as indices of physical performance during short-term IT camp, are not yet justified. Despite the extent of monitoring in elite and professional sport, most of blood tests data remain protected and unpublished.

Given the current literature, this study was undertaken to assess hematologic profile and hormonal indices in younger elite cyclists during a training camp, in anticipation of participation in the Arab Championships in 2018.

Purpose: The objectives of this study are first, a definition of the hematological and hormonal profiles of elite cyclists. Secondly, evaluating the rate of change of these parameters as well as determining the physiological status of cyclists after 4 wks. of training camp.

Materials and Methods

Participants.

35 cyclists from the national team playing in Algeria, France, and Spain, participated in the study. The participants were 15 cadets (15.6 ± 0.6 yrs.), 10 juniors (17.7 ± 0.4 yrs.) and 10 seniors (21.07 ± 1.6 yrs.). In order to be included, they had to meet the criteria of high performance. According to the criteria of Jeukendrup et al. [14], “high-level” or “well-trained” cyclists must answer for their training habits and level of practice. All cyclists had, on average: a seniority of practice (6.6 ± 1.9 yrs.); a training frequency (5 ± 2 times / week); a duration of training (4.2 ± 1.07 h / day); a frequency and level of competitions in the same category (44.5 ± 14.7 race days / yr.) and a UCI Africa- Tour, for U23 only, among the top 200 for at least the last 2 years.

During the training camp, athletes undertook high-intensity intermittent training (3.4 ± 0.5 h / day), with

recovery days. The average weekly training volume was (87.4 ± 11.5 km), mainly at an aerobic rate. The study was conducted in accordance with the declaration of Helsinki and approved by the local ethic committee.

Procedure.

The experiment took place over a period of 4 wks. during the training camp for participation in the Arab championships 2018. Anthropometric measurements were performed at the CNMS (National Center for Medicine and Sport) in Algiers a week advance (Table.1). Blood sample were drawn 24h before and after the training camp in the laboratory of UCH (University Hospital Center) in Mascara. The subjects were asked to refrain from training the day before the sampling.

Diet and Training.

Diet was strictly controlled by team physicians. To maintain circadian rhythms, nutrition, hydration, timing of food intake, and sleep were held constant on the days prior the blood sampling [15]. In addition, the subjects did not administer any supplement or medication during the course of the experiment.

Anthropometric Measurements.

The body surface area was calculated by the method of Izakson [16]. Muscle and bone mass were determined by the method of Mateigka [17]. The fat mass was determined by the equation of Kozlov and Gladisheva [18], taking into account the skin folds tricipital, sub-scapular, supra-iliac and bicipital. Subjects’ anthropometric characteristics are listed in Table 1.

Blood Samples.

Resting blood was drawn from cubital vein in the morning and after a fast of ($9h \pm 1$ hour). For the hormonal status, whole blood was centrifuged to separate the serum. Hematological and Hormonal serum were stored in a sealed box at $4-12$ °C. Controlled temperature was assured during transportation (portable electric refrigerator). Samples were transported by car to the laboratory of the Military University Hospital of Oran (MUHO) for immediate analysis or storage at -20° until analysis. The hematological parameters were analysed

Table 1. Basic descriptive statistics for Anthropometric parameters (N=35).

Variables	Mean	Min	Max	Range	SD	Skew	Kurt
Age (year)	17.58	14	23	9	2.42	0.90	-0.22
W (kg)	64.11	45.0	82.6	37.6	7.20	0.14	0.35
H (cm)	174.53	165	189	24	5.11	0.41	0.27
BSA (m2)	1.77	1.16	2.04	0.88	0.14	-1.50	6.49
FM (kg)	7.52	5.74	17.22	11.47	1.67	4.02	22.04
MM (kg)	28.28	19.88	39.60	19.72	3.85	0.681	1.007
BM (kg)	14.76	11.17	18.18	7.01	1.67	-0.281	-4.15
BI (cm)	80.10	68.26	102.27	34.003	7.85	0.88	0.527
LL (cm)	86.93	79	117	38	6.45	2.68	10.37
LT (cm)	49.37	43	58	15	4.11	0.123	-0.98

W - Weight; SH - Height; BSA - body surface area; FM - Fat mass; MM - Muscular mass; BM - Bone mass; BI - breath index; LL - leg length; LT - length thigh.

approximately after 2 hours from blood drawing. To avoid the effects of the circadian rhythm on hormonal secretion, blood samples were taken at the same time of the day (7h ± 1 hour). The percentage variation in plasma volume ($\Delta PV\%$) was calculated according to Dill and Costill [19] using the equation:

$$\Delta PV\% = 100 \times [(Hbpre/Hbpost) \times (1 - Htpost/100) / (1 - Htpost/100)] - 100.$$

Extraction of Reference Values.

The hematological or hormonal values belonging to a cyclist will be referred to as reference value, according to the terminology of the International Federation of Clinical Chemistry [20]. The participants underwent several samples. Subjects, therefore, have more than one reference value for a given parameter. In this case, we selected for each subject the median for the statistical analysis. The hematological profiles are shown in Table 2.

To gain further insight in the physiological adaptation involved, hormonal variables were included in the analysis. The Hormonal profiles studied in our sample (Table 3), relate only to the hormones that were examined as part of endurance training in young cyclists of high level.

Statistical Analysis.

Statistical analyses were performed by using the SPSS software version 22.0. Descriptive statistics comprised: the mean value (mean) with the corresponding standard deviation (SD). Numerical results minimum (Min) and maximum (Max), and range (Range). Rate of change (RoC)

is the increase (↑) or the decrease (↓) as a percentage of a variable. Discriminant measurements were performed by two procedures: Skewness coefficient (SKEW) pointing to the symmetry of the distribution around the arithmetic mean. The Kurtosis coefficient (KURT) denoting the peakedness or flatness of the distribution. Correlational analysis was performed between variables by using Pearson's correlation coefficient (*r*). Paired Student *t* tests were used to determine the differences between the pre- and post-test mean values of the various blood parameters.

Results

Hematological Variables.

We studied in young cyclists the variations of hematological markers spread over two tests: before and after the training camp. The hematological parameters varied differently. The performance of cyclists after the short-term IT task brought about significant changes in the total red line (Table 4). RBC rose by ↑1,75% along with Hb by ↑4.59% and Hct by ↑4,08%. At this time point plasma volume changes (ΔPV) mean was about -6,77%. Regarding the RBC indices, MCV increase by a mean ~ 2,25 fl and MCH by 0.73 pg. These pre-post changes were significant ($P < 0.001$, $P < 0.01$, $P < 0.05$). The MCHC remained unchanged.

Considering the level of performance, analysis revealed no significant difference in the total white line. WBC increase by ↑11,38%, $P = 0,062$. With reference to WBCs subsets, Neutrophil decrease by ↓8,49% and

Table 2. Basic descriptive statistics for hematological parameters (N=35).

Variables	Mean	Min	Max	Range	SD	Skew	Kurt
WBC ($10^6/mm^3$)	6.43	3.80	9.60	5.80	1.38	0.30	-0.36
NEUT (%)	46.36	25.9	63.8	37.9	9.0	0.07	-0.20
LYM (%)	40.37	23.4	55.2	31.8	7.32	-0.03	-0.38
RBC ($10^3/mm^3$)	4.92	4.36	6.09	1.73	0.34	0.55	1.44
Hb (g/dL)	14.37	12.6	16.2	3.6	0.84	0.21	-0.79
Hct (%)	41.12	36.6	47.6	11.0	2.47	0.44	-0.39
MCV (fl)	83.79	68.1	93.7	25.6	5.53	-0.83	-0.45
MCH (pg)	29.26	22.9	32.4	9.5	1.97	-1.13	1.32
MCHC (%)	35.84	33.0	65.8	32.8	5.05	5.18	27.92
Plt ($10^3/mm^3$)	216.16	110	343	233	47.47	0.16	0.35

WBC - white blood cells; NEUT - neutrophil, LYM - lymphocyte; RBC - Red blood cells; Hct - hematocrit, Hb - Hemoglobin; MCV - mean corpuscular volume; MCH -Mean corpuscular haemoglobin; MCHC - Mean corpuscular hemoglobin concentration; Plt - Platelets.

Table 3. Basic descriptive statistics for hormonal parameters (N=35).

Variables	Mean	Min	Max	Range	SD	Skew	Kurt
CORT (nmol/l)	397.90	169.00	510.00	341.00	83.73	-1.176	1.331
TESTO (nmol/l)	4.77	1.41	8.11	6.70	1.51	0.279	-0.067
TNT (ng/ml)	6.56	3.27	12.00	8.73	2.23	0.526	-0.518
ProBnp (pg/ml)	320.37	140.00	549.00	409.00	96.46	0.231	-0.529

CORT - steroid hormone; TESTO - Testosterone; TNT - Troponin; ProBnp - prohormone Brain natriuretic peptide.

Lymphocyte by ↓3,80%. The same pattern was observed in platelets count (↑5,20%; $P = 0,167$). From the pre to post-test, the average values of all the haematological parameters were within the reference range.

Hormonal Variables.

Descriptive analysis of stress hormone in well trained cyclist are presented in Table.5 before. For Cortisol, significant differences were observed between the pre and post-test (11,85%; $p < 0.05$). The Testosterone concentration increased at post-test by ↑16.49%, and this change was statistically non-significant ($p = 0,241$).

According to this study, the measured values of cardiac markers of myocardial stress varied differently. The analysis of pre and post-test hormones revealed a significant ($p < 0.001$) decrease in Troponin level by ↓92,78%. In contrast to Troponine, proBnp was decreased by ↓19,56% without statistical significance ($p = 0.051$).

Pre-test proBnp-positive (percentage of subjects exceeding the URL= upper reference level: 350 pg/ml) was 31.41% compared with 22,85% at post-test. When age was considered, 17.14% of cyclist aged >19 yrs. had

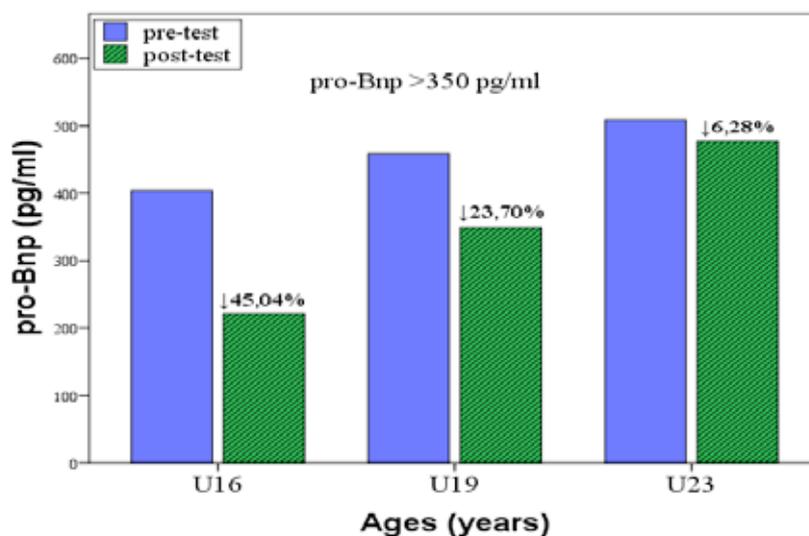


Figure.1. Reduction in proBnp as a function of age and age-adjusted cut-off values in well trained cyclist. $P > 0.05$, no significant different between pre and post- test for proBnp value >350pg/ml.

Table 4. Descriptive and analytical Statistics of hematological parameters under study (N=35).

Variables	Pre-test	Post-test	P values	Usual Values
WBC ($10^6/mm^3$)	6.46 ± 1.47	7.20 ± 1.89	0.062 NS	4.0 – 10.0
NEUT (%)	49.01 ± 8.37	51.85 ± 8.96	0.090 NS	40 - 75
LYM (%)	39.29 ± 6.93	37.80 ± 8.17	0.301 NS	20 - 45
RBC ($10^3/mm^3$)	4.91 ± 0.34	5.00 ± 0.30	0.042*	4.2 – 5.7
Hb (g/dL)	14.38 ± 0.86	15.04 ± 0.92	0.000**	14 - 17
Hct (%)	41.48 ± 2.56	43.18 ± 2.21	0.000**	40 - 52
MCV (fl)	84.46 ± 5.41	86.71 ± 3.64	0.001**	80 - 95
MCH (pg)	29.37 ± 2.04	30.10 ± 1.81	0.012*	28 - 32
MCHC (%)	35.67 ± 5.45	34.48 ± 0.92	0.224 NS	30 - 35
Plt ($10^3/mm^3$)	215.52 ± 50.33	226.73 ± 37.32	0.167 NS	150 - 350

Values are expressed as Mean ± SD; significant difference: $p < 0.05^*$, $p < 0.01^{**}$, NS: not significant.

Table 5. Descriptive and analytical Statistics of hormonal parameters (N=35).

Variables	Pre-test	Post-test	P values	Usual Values
CORT (nmol/l)	404.82 ± 120.85	356.48 ± 98.97	0.021*	171 - 536
TESTO (nmol/l)	4.50 ± 2.15	5.24 ± 2.40	0.241 NS	2.8 – 8.0
TNT (ng/ml)	8.33 ± 4.00	4.32 ± 2.58	0.000**	00 - 14
ProBnp (pg/ml)	418.48 ± 188.54	349.98 ± 188.17	0.051NS	≤ 350

Values are expressed as Mean ± SD; significant difference: $p < 0.05^*$, $p < 0.01^{**}$, NS: not significant.

proBnp values >350 pg/ml at post-test; this was reduced to only 6.28% (Fig.1). In contrast, we found a marked reduction in proBnp after short-term IT in the U16 and U19 compared to their pre-test level. The elevation in proBnp values was associated with increasing age ($r=0.594$, $r=0.598$; $p<0.01$).

Discussion

In this paper, the authors attempt to provide an update about the most significant changes of selected blood parameters in response to 4 wks. of training camp. To our knowledge, no previous studies have evaluated both haematological and hormonal response to short-term IT in elite road cyclists of different age (U23).

Hematological Variables.

In endurance sports; the transport of oxygen (O₂) through hemoglobin (Hb) blood is an essential factor for the athlete’s good muscle performance [21]. Parameters required to evaluate O₂ carrying capacity are: Hb, red blood cells (RBC) and Hematocrit (Hct) in the blood [22].

A highly increased RBC and Hb improve performance by facilitating O₂ transport and delivery to metabolically active tissue [23]. Thus, it is a clear advantage for aerobic athletic performance to have a high O₂ carrying capacity [24], particularly in sports such as running and cycling

[21].

Previous cross-sectional and longitudinal studies have investigated the effects of different types of exercise on RBCs variables in various sports. The effects found have been a decrease or increase in RBC, Hb and Hct concentration [25–27]. Nonetheless, regular screening of hematological variables was desirable for the control and the establishment of normal range.

In this study well trained cyclist show, with increasing intensity and decreasing duration of training, the same pattern of elevation in most of RBCs profile. We observed a significant increase ($p<0.05$) in the order of 1.75 to 4.59% in resting RBC, Hct and Hb at post-test. Other investigations also reported similar findings in this regard although employing different exercise protocols [28–30].

The results of this study are not really unexpected. Several mechanisms are discussed as contributing to these adaptations. The primary cause is addressed to a pronounced plasma volume (PV) variation observed after IT [31]. $\Delta PV\%$ changes were calculated from Hct + Hb [19], and gave a mean decrease of 6,77 at rest.

Because during plasma shifts, no blood cells nor Hb leave the vascular system, a part of the increase in Hct value and Hb concentration is due to the 6,77% decrease in PV. Pointing to the same conclusion is a significant

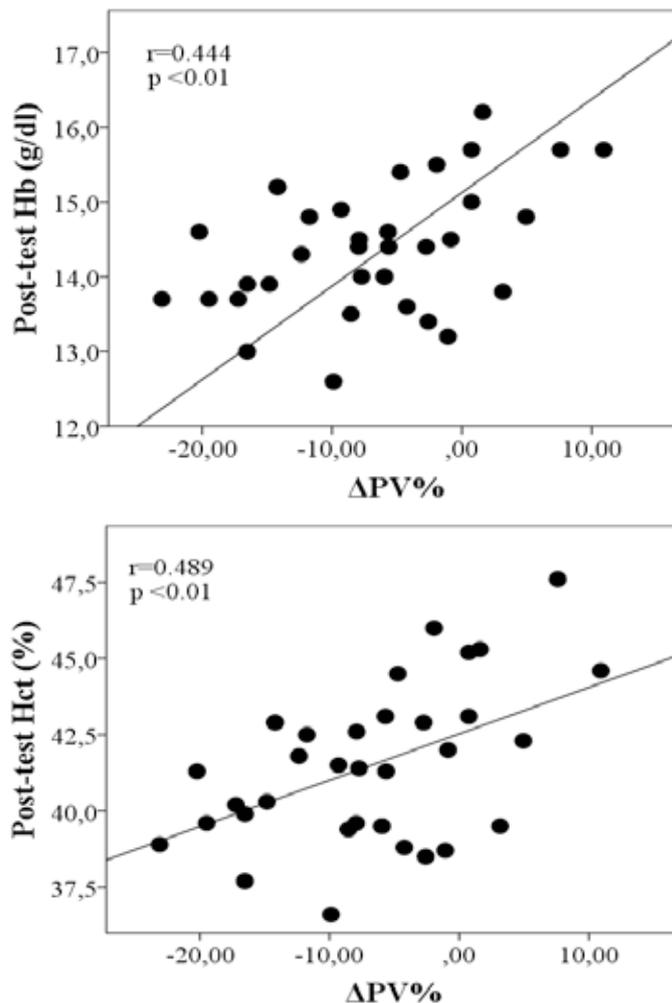


Figure 2. Correlation between plasma volume ($\Delta PV\%$) and individual values combined from post-test Hematocrit (Hct) + Hemoglobin (Hb) in 35 well trained cyclists.

change in Hct and Hb associated with the decrease of PV. Figure.2 gives the association between $\% \Delta PV$ and individual values of Hct + Hb after short-term IT.

Our results add to the evidence that a repeated brief and intensified exercises decreased PV [32, 33], leading to an increase in RBC, Hb, and Hct counts [34]. Thus, short-term IT exercise can induce a 5%–10% decrease in PV of young cyclists.

Furthermore, professional road cyclists are known for performing very high training volumes, up to 30 to 35,000 km/yr. [35]. Nevertheless, training seems to influence the blood system on a seasonal base, as most erythrocyte variable show significant change during IT period [36].

Finally, as cited before [28], 99% of O_2 is carried in combination with Hb in the blood. Elite cyclists are known to possess a height O_2 -uptake capability [37]. Thus, if the need for O_2 in tissues and blood portable O_2 content are not balanced, the body will respond to it through increasing the blood O_2 -carrying capacity, i.e., Hb and RBC [38].

Taken together reports and our results, revealed that IT exercise has no adverse effect on RBCs variables in the short-term. Our finding appears to be consistent with the cyclists' physiological response to maintain and improve highly demanding performances day-after-day [39]. The competition runners (cyclists) reported personal records at the Arab Championships in 2018.

White blood cells (WBCs) or leukocytes are a heterogeneous group of immune cells made up of various subsets with specific function. To date, a vast amount of research has aimed to explain how physical exercise alters the immune system. Increasing attention is being directed to the relationship between exercise (in term of duration and intensity) and immune function [40].

Some researcher addressed decreased or unchanged levels of WBC both during and after exercise [41,42]. Others have suggested that intense exercise can rapidly decrease Lymphocyte counts and that short intense exercise has no effect on Neutrophil function [43, 44]. Evidence is now accumulating that immune response depends on exercise intensity [45], however there is a paucity of data to support these finding.

Anyway, intense exercise may induce perturbations to immune system [46] and homeostasis might not be restored for several hours. Gleeson et al [47] suggest that exercise can cause anti-inflammatory reactions triggered by muscle injury, resulting up to 24 h after exercise. This trend has not been observed in our athletes. The major finding of this study was in the white series which varied inversely to erythrocyte one.

The WBC profile showed non-significant change ($p > 0,05$), despite a rise by $\uparrow 11,38\%$ after 24h of recovery. With reference to WBCs subsets, resting Neutrophil count were 5,81% lower than pre-training values ($49,01 \pm 8,37$ vs $51,85 \pm 8,96$ / %, $p = 0,090$). Short-term IT does not appear to alter significantly WBC counts, including Neutrophils, because the Neutrophils account for 50% to 70% of all circulating leukocytes.

Lymphocytes are involved in the specific immune

response including antigen-antibody reactions [48], and make up 20% to 25% of the peripheral leukocytes. Thus, correct functioning of the lymphatic system identifies the proper physiological state of the immune system. Similarly, to WBC, Lymphocytes remain unchanged ($p > 0,05$), decreasing the possibility that 4-wks of short-term IT acts in an anti-inflammatory fashion that hinder microcirculation.

In general, it was observed that most of the WBCs measures are not altered by short-term IT periods such as 4wks in athletes already training in average ~ 28 h/wk. It is noteworthy; however, that, cyclists habitually high training loads may be associated with some immune regulation to preserve performance and immunity during IT.

Further, possibly the lower number of hours (< 3 h) of exercise training per days may have resulted in less fluctuation in WBCs variables. Post-exercise immune function dysfunction is most pronounced when the exercise is continuous, prolonged (> 1.5 h), of moderate to high intensity [53]. Other studies marked also no significant difference in WBC count [49, 50], Neutrophils number [51] and circulating Lymphocytes [52] after short periods (2–4 wk.) of IT.

Based on our finding, our data strongly suggest that resting immune status was unrelated to exercise intensity, but may depends on training level and exercise duration. The authors concluded that, short-term IT exercises does not affect (or might even enhance) immune function in well trained cyclists.

Platelet (Plt) serves an important role in the physiological process of hemostasis. Exercise and training are known to have multiple effect on blood hemostasis [54]; involving platelet activity [55]. Thus, maintaining platelet homeostasis is important to avoid spontaneous bleeding and organ damage [56].

Like other blood cells, Plt count may not remain constant and vary to different physiological conditions. From the available studies, intensified exercise was found to increase Plt index during recovery [57–59]; although this could not be confirmed in all studies [60, 61].

In our cohort, Plts numbers showed no significant difference ($\uparrow 5,20\%$, $p = 0,167$) at rest, but within the reference range. No coagulopathy was detected in all subjects. Similar observations have been reported by Drygas et al [62] and Scalzi et al [63] on sports participants other than cyclists.

Studies on effect of short-term IT exercise on Plts count in elite cyclists are lacking. Many factors can explain the diverging between our results and the mentioned researches. There is no standardised protocol for defining intensity or duration exercise, uniformity of sampling, and populations studied.

Initially, the sample of these studies used healthy or sedentary subjects, so it is difficult to extrapolate their results to elite athletes. At last, different methods were used to measure Plts function. Studies have varied from laboratory tests based on ergometer or treadmill protocols at a given $VO_{2max}\%$, to field-testing protocols during

competition events.

Thus, a definitive conclusion about effect of short-term IT on Plt counts in competitive cyclists should not be established. The contrasting data, reported in the few published papers, deserve further studies to clarify the importance of our observation.

In conclusion, hematological parameters and its knowledge can be used to assess the performance as well as the physiological status of elite cyclists. In accordance with our hypothesis, the present findings indicate that short-term IT induced significant effects in the erythrocyte measures than leucocytes one. This means a larger significant gain in O₂ allocation / transport capacity with maintaining and / or mild elevation of immune function. Our study documents that reducing volume while increasing intensity of training just before competition was favorable to performance.

Hormonal Variables.

Looking for an effective index to monitor the physical performance in elite athletes has great physiological significance. Many researches have been carried out to identify physiological markers that might be used to monitor training program and to prevent or even to diagnose overtraining.

Two very important hormonal markers of chronic stress and fatigue are Testosterone (as anabolic hormone) and Cortisol (as catabolic hormone). The relationships between these two hormones are thought to reflect the balance between anabolic and catabolic processes [64].

Cortisol the primary stress hormone was used in many studies to evaluate exercise induce-stress. According to Michailidis [65], the release of Cortisol is related to stress and depends positively on the intensity and duration of exercise. In literature, resting levels of Cortisol are generally seen to increase with volume [66,67] and intensity [68] of training.

In the present research, high-intensity, short duration of exercise training showed a significant decrease by 11,85% in resting Cortisol. However, all values of our subject were within the reference limits. Our results align perfectly with other studies including competitive cyclists [69, 70], and the direction of changes was related to the external training load.

An interesting aspect of this study was that the training camp occurred 3 days before the Arabian championships. The training duration, intensity as applied by coaches in this study appears to play an important role. In such instance, there are some considerations when interpreting Cortisol decrease in this study.

First, post-test Cortisol decrease may be due partly to a marked reduction in the training load in the days before the Arab championships. This procedure was undertaken by coaches with the implicit goals of maximizing gained performance and minimizing risk of negative training outcomes. In most case, such reduction in training intensity and/or volume is referred to as tapering.

As defined by Mujika and Padilla [71], tapering is the reduction in training before a major competition. But in contrast to athletes from other sports, the road cyclist

doesn't practise tapering. It was taper-like, training at the usual exercise intensities but with a progressive reduction in duration for the 7-10 days before a race [72].

Interestingly, reduced training periods (2 to 4 wks.) has been observed to improve several physiological and performance measures in cyclists [73, 74]. This possibility is partly supported by our finding and in the study of Chenaoui et al. [69].

Apart from reduction in the training load, other factors may have also influenced the Cortisol response seen in this investigation. Subjects in this study have typically been athletes-exercisers (see methods). In this regard, scientists have shown that Cortisol tend to decrease in response to exercise [75], likely, because of the trained individual being better at preserving blood glucose [76].

Clearly, post-training Cortisol raised in this study was prevented [77] to allow muscle glycogen to become supercompensated in post-exercise [78]. For our cyclists to be prepared for subsequent competition, restoration of muscle glycogen stores is required to sustain the capacity for continued high-intensity activity.

However, the high levels of Cortisol observed at pre-test, may be associated to the stressful condition accumulated during the season [79]. It should be emphasised that the training camp was at the end of the season. Furthermore, for our younger cyclists, it is not uncommon to complete about 25 000 km and 70 competition days after 8 month of cycling season (see methods).

Likewise, in younger age, Skoluda et al [80] found that a higher training volume (measured in kilometers run per wk) was associated with rising Cortisol levels. It appears likely as well trained cyclists exhibit a chronic mild hypercortisolism at pre-test that may be an adaptive response to chronic exercise [81]. But the level of response highly depends on a previous level of training.

In conclusion, our results imply that short-term IT would decrease cortisol in well trained cyclists in post-recovery. Collectively, post-training reduction in cortisol has been reported to be ideal for an athlete to achieve positive adaptations [67]. Finally, our findings lead us to assume that 4 wks. of short-term IT exercise provide the adaptation needed for optimal cycling performance.

Regarding Testosterone the other dependent variable of this investigation, conflicting results have been reported. Some studies involving well trained cyclists reported that, endurance training often reduce serum Testosterone [82, 83], while others have described an increase [84, 85].

In this study as an anabolic profile, Testosterone plasma increases by 16.49% after 4 wks. of short-term IT. But this alteration was no significant ($P>0.05$). Our results are somewhat unexpected in view of previously published papers. Explanations for our observations are lacking, so we will examine a relatively unexplored factor.

The key theme emerging from our findings is that anabolic processes were affected negatively, as indicated by the significant decrease in Cortisol (see Table.5). First evidence for this phenomenon was provided by Cumming et al. [86], discussing the ability of Cortisol to inhibit the

synthesis of testosterone. However, the prevalence of these finding in the case of sports is not yet quite determined.

Whatever the cause, the observed increased Testosterone and decreased Cortisol may have a role in the bioenergetics and metabolic function of skeletal muscles. Physiologically, besides anabolic effects, testosterone may possess anti-catabolic properties [87]. In this regard, Testosterone and Cortisol are through to have opposing effects on muscle metabolism and protein synthesis [75].

Given the latter, it was tempting to conclude that decreased plasma level of Cortisol may result in increased flux of Testosterone in post-recovery. Physiologically, increased Testosterone corresponds to the activation of the anabolic process necessary for the restoration of the body's homeostasis during recovery. In these states, an anabolic internal environment would be present in this study. While the level and direction of activation (i.e., anabolic process) depend on many factors are not easily interpreted (see ref [88, 89] for extensive discussions).

Therefore, despite the Testosterone response did not result in significant increase in this study, but a 16.49% increase may be considered practically significant. In this issue, the anabolic deficit is assumed as an increasing Testosterone counter-regulatory works against the proteolysis effect (i.e., muscle proteins degradation).

Clearly, since Testosterone increases muscle protein synthesis [90], increased plasma Testosterone could also act against the catabolic properties of protein. Consequently, an anabolic response might be initiated while muscle protein synthesis is higher than proteolysis.

Consecutively, because Testosterone increases muscle glycogen synthesis [91], increased plasma Testosterone might reduce the glycogenolysis induced by Cortisol. For our cyclists, this saving of glycogen stores is critical for complete recovery [67], mostly when limited time is between training sessions and competition.

Taken as a whole, there are some take-home messages from this research. First, our findings demonstrate that in well-trained cyclists, a modest increase in Testosterone levels does not need to predict decreased performance. Bermon et al [92] found no correlation between serum Testosterone and performance in elite's athletes.

Second, the observed increased Testosterone and decreased Cortisol would be indicative of enhanced recovery [71], which may express itself in increased performance. Furthermore, short-term IT exercise is desirable for the development of body anabolic processes (i.e. the restoration of the body's homeostasis).

In summary, we could state that short-term IT increases the expression of anabolic process that in turn keep decreasing Cortisol levels. Our findings demonstrate a physiological validation of coach's objective. Therefore, tracking resting Testosterone and Cortisol concentration could be used as stress and recovery state indicators to reflect the training response. However, we are the first to demonstrate that, short-term IT induced Cortisol decreases would perhaps result in subsequent increases in circulating Testosterone.

The study of cardiac-specific biomarkers is crucial

to understanding the physiology of endurance training. Since, the mechanism underlying the release of cardiac biomarkers has become an area of intense scientific investigation. Here, we describe and evaluate change in Troponin and Brain Natriuretic Peptide hormone.

As a cardiac biomarker, Troponin (TnT) is a contractile protein specific to cardiac muscle. In sport medicine, TnT assay is currently widely used in the assessment of myocardial and skeletal muscle injuries. Regarding competitive endurance sports, post-exercise release of TnT have been suggested to be elevated.

The extant literature is replete with observation of significant rise in cTnT after prolonged intense endurance exercise [93–95]. Nevertheless, elevated TnT are considered as indexes of myocardial damage. This theory has been challenged by numerous studies [96, 97], though no definitive explanation has been provided so far [98].

Conversely, other authors demonstrated marked increase in TnT after intense exercise, in absence of clinical symptoms of a myocardial dysfunction [99,100]. Despite many publications, there is, however controversy on the phenomenon of post-exertional TnT elevation, possible explanations have been published [101].

Relatively little is known, however, whether high exercise intensity (with shortened durations) may mediate TnT release [94]. Accurate interpretation of TnT concentrations in this context is challenging. The foremost question being is whether exercise-induced TnT release of physiological or pathological finding.

The key novel finding from this study is that short duration, high intensity cycling training elicit a significant decrease ($p<0,001$) in TnT post-recovery. Since, TnT is the main protein that regulate muscle contraction and relaxation [102], our findings indicate an interesting observation.

Injured skeletal muscle may release proteins that are detected by cTnT assays [101, 103]. Subsequently, the asymptomatic decreased TnT observed in all cyclists may reflect physiologic rather than pathologic substrate [104]. Together, ours finding provides novel insight into the physiologic substrate responsible for the post-exercise release of TnT after short-term IT. The implications of this observation are straightforward.

Based on our observations and the post-exercise TnT observed in other work [105], it is likely that TnT decreased as the training duration decreased. To this end, it is unlikely that a decrease by $\downarrow 92,78\%$ in post-exercise TnT reflect irreversible damage. Actually, the beneficial effect of moderate duration exercise on cardiovascular health is well recognized [106].

Previous identified factors; age and training experience [95, 100, 106] have also been reported to regulate post-exercise TnT but may not be the dominant ones. In this study, subjects were younger ($17,58\pm 2,42$) and athletes-exercisers (see methods). Further, a correlation was found between age and pre-test level of TnT ($r=0,023$, $p<0,05$). Consequently, we cannot rule out a specific role of age and training status in the current study. At present, these possibilities have no substantive evidence to support it but

is worthy of an ongoing investigation.

In conclusion, short-term IT led to significantly low post-recovery levels of TnT in elite cyclist. We propose that exercise duration may influence TnT levels, but the mechanism of release is physiologic. In the absence of cardiac symptoms, we conclude that the myocardium of elite cyclists is more adapted to intensified exercise.

Since the discovery of natriuretic peptide, our knowledge on cardiovascular response to exercise has improved considerably. Cardiac-derived natriuretic peptides, mostly plasma B-type natriuretic peptide (BNP) have become useful markers in diverse aspects of cardiology [107]. Now, BNP and its N-terminal fragment (NT-proBNP) are widely used to appreciate cardiac tolerance of high level athletes undergoing intense training [108].

Previous studies reported that intensified endurance exercise induces significant elevation of NT-proBNP levels [96, 109]. Theoretically, elevated NT-proBNP is often argued as transitory myocardial damage in professional and recreational athletes [110].

Recently, a pro-peptide of BNP (proBnp) has been proposed as potential novel “white count” for earlier cardiac dysfunction [111, 112]. Because proBnp concentration has a longer biological half-life [113], is more stable and has less biological variability [111]. Given the molecular nature of proBnp and its importance as a diagnostic analyte, we have used proBnp assay.

Although the clinical significance of proBnp has been extensively investigated (for review see ref [114]), the biosynthesis of proBnp in the case of sport is strikingly scarce. Until now, no study has assessed concentrations of circulating proBnp on sport performance.

For the first time, this study indicates that, younger cyclists (<23 yr) exhibit both elevated pre and post-test proBnp levels. We found that, post-test proBnp decrease by $\downarrow 19,36\%$ ($P>0.05$). As NT-proBNP represents proBnp [115] and have the same clinical significance, our data coincided with previous results [116, 117].

However, we do not fully understand the cardiac effects of such physiological elevation in proBnp levels. To avoid misinterpretation of elevated values, it is of interest to determine the importance and role of factors that mediate proBnp release.

According to recent research, exercise-induced increase in proBnp depends primarily on exercise duration [118–120]. In the current study exercise duration (<3 hr) may have been insufficient to stimulate a significant proBnp response in spite of intensity.

Exercise duration and intensity alone do not appear to explain clearly the observed proBnp values. The patho-physiology of proBnp modifications is still poorly explained but could be multifactorial. Age [121]; training history [122] and inter-individual variability [123], are factors that can markedly influence proBnp release.

At bivariate correlation, peak pre and post-test plasma of proBnp was evident only with participant age ($r=0.594$, $r=0.598$; $p<0.01$). When age was considered, it is the U16 and U19 who exhibit the largest exercise-induced

increases in proBnp (Fig.3). We suggest that proBnp levels tend to increase with increasing age.

Although only as a trend, similar correlation was also found in the study of König et al [121]. We therefore conclude that the relationship between exercise duration and intensity on the kinetic of proBnp could be influenced by age.

However, analysis between categories found a difference among cyclists over 19 yrs. (U23). 17.14% of cyclist aged >19 yrs. had proBnp values >350 pg/ml (i.e. exceeding the URL) at post-test; this was reduced to only 6.28% (Fig.1). Our findings do not provide a clear explanation for the discrepancy between adolescent and adult.

Anyway, the natriuretic peptides are neurohormones that reflect a condition of increased cardiac stress. As highlighted by Hamazaki [124], even though exercise is performed at high-intensity, if the duration was short, excessive cardiac stress could be preventive.

In turn, it is irrelevant to conclude that an athlete’s cardiac functional state deteriorates from elevated proBnp. Even if proBnp levels exceed the cutoff values, elevated proBnp does not represent myocardial damage [124], resulting in lower athletic performance [163].

Therefore, the observed response here, defined as a decrease in proBnp of >19%, would be suggestive of a physiological reparative or adaptive process [96, 125]. In agreement with earlier investigation [126], we confirm that 4 wks. of short-term exercise decrease proBnp by 20% to 30% and improved myocardial function.

All in all, exercise intensity and age have a regulatory effect on proBnp concentrations, whereas exercise duration significantly influences TnT. The current findings demonstrate that asymptomatic elevated level of proBnp can frequently be found in younger cyclists including adolescents. The lack of in-exercise blood sample in current study is a limitation and warrants further investigation.

Conclusion

Although there were changes in hematologic indices in terms of increase or decrease, it was found that 4wks of short-term IT improved significantly all erythrocyte indices except leukocytes cells. Significant RBCs increases may improve microcirculation and increase the supply of O_2 and essential energy substrates during intensified training exercise. While WBC counts at rest might be lower in elite cyclists, decreasing the possibility that 4-wks of short-term IT acts in an anti-inflammatory fashion.

Moreover, the main findings of this study on the parameters representing the cardiac stress. TnT and Cortisol show that these have remained too significantly diminished as an anticipatory response to the competition. Given the anabolic effects of Testosterone, this can therefore, together with the stable differences in post-test measures of proBnp, render effective the quality of the recovery of the exercise. Our study documents that reducing volume while increasing intensity of training

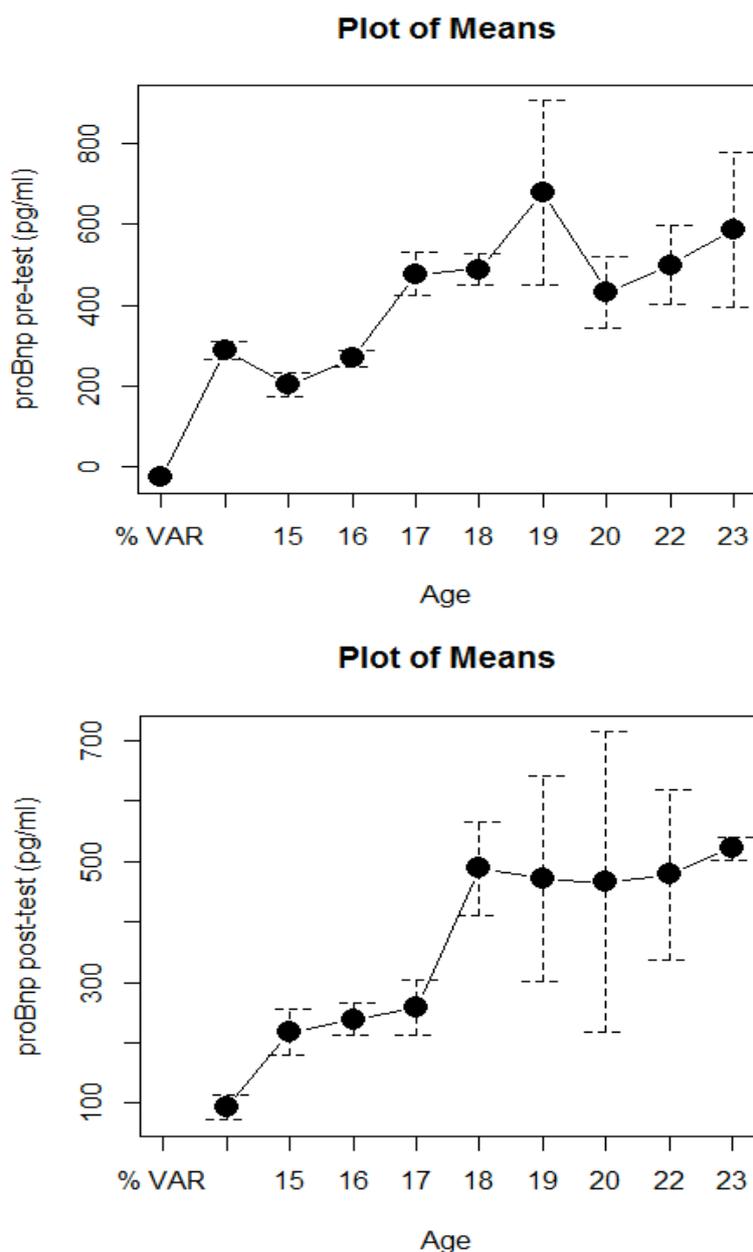


Figure 3. Association between pre and post-test (means \pm SD) plasma concentrations of proBnp and age in professional cyclists (N=35). The correlation coefficient is statistically significant ($p < 0.01$)

just before competition was favorable to performance during short preparation periods.

In contrast, there is great variation in the cardiac hormone as observed between ages. These variations have further underlined the need to establish appropriate reference values for elite athletes. In this context, hematological indices and hormonal profiles and its knowledge can be used to assess as well as monitoring training status and performance of cyclists. With reference to the findings of this study, short-term IT exercise should be applied to specifically induce gains in cycling performance in younger elite athletes.

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Conflict of interests

The author declares no conflict of interests.

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