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RESEARCH ARTICLE

Using circuit training and mini-game based sessions to assess the circulating leukocytes' responsiveness to exercise among College soccer players in the subtropical environment.

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Abstract

This experimental study aimed at comparing the variations of the circulating leukocytes within the 24 hours following an anaerobic circuit training session (CT), to those of an aerobic mini-game based session (AE) in the hot climate of Benin. In a random order, sixteen College soccer players (22.8 ± 0.5 years) took part in CT and AE training sessions, and a 5 hours rest (R5) was used as control. Blood samples were taken in the end, 6 and 24 hours after each session, 15 days apart. The circulating leukocyte counts were assessed by flow cytometry. Twenty-four hours after the training session, the subsequent effect ($p < 0.05$) was noticed for CD4 lymphocytes alone. After CT, no time measurement effect was seen for any sub-type of circulating leukocyte ($p > 0.05$). On the contrary, AE induced a significant increase ($p < 0.05$) in eosinophil and monocyte counts at the end of the session, followed 24 hours later by a return to the primary values. There was a significant decrease ($p < 0.05$) in neutrophil and CD4 lymphocyte counts during and 24 hours after the AE session.
Considering the conditions under which this study was carried out, the AE session can be used for assessing the quantitative immune response to exercise in soccer players. However, the immune system requires more than 24 hours for recovering. The use of a CT session, i.e. resistance training, requires another experimental study for determining the suitable training design.

Introduction

The sporting environment in the Republic of Benin is currently marked by a strong ambition to attain high performances, particularly in soccer, with a tendency in athletes to intensify training. Soccer training is organized, taking into account the physiological match requirements such as the distance covered by the players, i.e. approximately 10 -11 kms including 28 to 36% of sprint and high intensity races (Cazorla&Farhi, 1998) during a 90 min period, whatever the playing position may be (Stolen et al., 2005; Bangsbo, 1994). The planning of training sessions is thus based on an adequate combination of aerobic or anaerobic exercises, the impact of which on the players' bodies has to be known by the trainers. This need is particularly felt as the elite soccer players currently do train at least daily, arising the problem of the cardiovascular, immune and energy recovery delays. As far as energy is concerned, recovery delays according to exercise patterns are known, i.e. eight to 24 hours and 48 to 72

hours, respectively for anaerobic and aerobic metabolisms (Matveiev, 1980). The immune response to various training designs carried out in laboratories in European countries and North America is also largely reported in the literature (Petridou et al., 2007; Calle& Fernandez, 2010). This responsiveness depends on several factors including exercise intensity, duration and mode, hydration status and body temperature (Smith et al, 1989; Nieman, 1997). Thus, a prolonged intense training session is known to induce a temporary immunosuppression known as O*pen Windows*, within the 72 following hours (Kakanis et al., 2010; Scharhag et al., 2005; Pedersen et al., 1998). On the one hand, frequent intensive training weakens the immune system and exposes athletes to respiratory tract infections, because of leukopenia and the associated inflammatory processes (Nieman et al., 1995). On the other hand, specific data related to field exercise are scarce, particularly those carried out in a specific environment like that of the Republic of Benin, i.e. a hot (temperature from 32 to 35 °C), and humid (60 to 75% of relative humidity) environment marked by a high prevalence of communicable diseases (MPH-Benin, 2004). However, we know for example that exercise in a hot environment induces a more accentuated immune response than in a temperate climate (Mitchel et al., 2002). So trainers in the Republic of Benin need to know the necessary delays for the players' immune systems to recover between matches, in order to preserve their bodies' integrity, while increasing their performance capacities. Unfortunately, it is not yet the case. In this context, it appears hazardous to go on using the current training standards and designs, without a preliminary assessment of the consequences of the association of thermal and physical stress (known as training-induced stress) on the soccer players' immune systems in the Republic of Benin. This study was thus, carried out in order to test the hypothesis that recovery of the immune system from a mini game-based session takes longer than recovery from a circuit training in student soccer players.

Material and Methods

Settings and experimental design

This study was carried out in Porto-Novo, in the south-eastern region of the Republic of Benin (West Africa), using a 3 x 4 experimental design. Fifteen days apart, the same players were randomly subjected to three training sessions: a circuit training (CT) and a mini game based (AE) sessions with a 5 hours rest which served as control. Measures were taken before, at the end, 24 and 48 hours after each of the three training sessions.

Study sample

The study sample was constituted of 16 apparently healthy students aged 18 to 27, who were all members of the soccer team of the National Institute of Youth, Physical Education and Sports (INJEPS) of the Republic of Benin. Apart from the regular hours of physical and sports practice that were scheduled for curricular reasons (8 to 16 hours weekly during 26 to 32 weeks during the academic year), these students have two soccer training sessions i.e. four hours each week as optional course, during the academic year. None of the players was under drug treatment during the study period. Exercise was to be deferred for any sick participant until the symptoms disappear or he was no more under drug treatment for at least five days. The players gave their written informed consent to participate in the study, which was carried out in respect with the recommendations of Helsinki (1964) andapproved by the Committee of Sports Sciences of the University of Abomey-Calavi, seating as an Ethical Committee.

Measurements

The dependent variables were the circulating leukocytes subtype counts, as the white blood cell count (WBC), neutrophil (NEU), basophil (BA), eosinophil (EO), monocyte (MO), lymphocyte (LYM) and CD4 lymphocyte (CD4) cell counts. The independent variable is the *type of training session*, comprising the three following modalities: anaerobic circuit training (CT), aerobic game-based session (AE) and a 5 hours rest (R5) which was used as control.

Blood samples were drawn by venepuncture into EDTA tubes and preserved at room temperature for no more than two hours before assessment of the immune cell counts in laboratory. All the analyses were made by a single blind fashion, using a KX21 automat (Sysmex, GmbH Europe) for WBC and its subtype populations. The CD4 count was assessed by flow-cytometry with a CYFLOW® COUNTER (Partec, Germany), using the *No lyse No wash* method and the *True Volumetric Counting* System. This system which associates the ALIGN FREE^{TM}technology (CyTecs, Germany) with the use of monoclonal antibody CD4-PE recommended by the manufacturer of the Cyflow counter and thesoftwareFlowMax® (Quantum Analysis), makes it possible to obtain the printed results in 60 seconds.

Procedures

Two weeks before the beginning of the study, the players had to sit for the 20 meters shuttle run test (Cazorla&Léger, 1993) for their VO_2max assessment. They were asked to abstain from

involving into an intense exercise or activity within the 72 hours preceding each experimental session, just as after the training sessions, and until the last blood sampling. The first blood sampling was made at rest, i.e. at a quarter past 8 a.m. for CT and AE sessions, then at 5 hours for the R5 one. The second blood sampling was undertaken at the end of each session, i.e. at 10 hours. The third was carried out 6 hours after the second one, i.e. at 16 hours and finally the fourth and last blood sampling was done, 24 hours after the end of each training session, i.e. the following day at 10 a.m.

All the players had spent the night before and after each experimental session at INJEPS, but they were not allowed to sleep during the day. They could listen to music while resting throughout the day, without sleeping. The time spent to watch television was reduced to three hours maximum between 7 and 10 p.m.

During the week before and throughout the period of the study, the players were allowed to consume neither dietary supplements nor drugs likely to influence the immune response. They were subjected to the same boarding school diet and ate the same meals within the 24 hours prior to and after each of the three experimental sessions. Standard meals with high potency of carbohydrates and proteins (rice, beans ragout or yams for lunch; pasta products or corn paste for dinner) were given to them at 10 a.m., half past 1 p.m. and half past 8 p.m., the days before and after the training. They could drink water *ad libitum* during training sessions and resting periods.

Training sessions

The players participated randomly in the training sessions as follows: circuit training (CT), 5 hours rest (R5) and aerobic game-based session (AE).

The circuit training session (CT): after 10 min of warm up, the session was organized in 3 series with rotation on 9 workshops for 65 min. Each player executed in 1 min, the maximum of repetitions of the prescribed exercise at each workshop, with a 30 second-recovery between the workshops and an active 5 min-rest (rehydration, footing and stretching) between the series. Exercises on workshops (WS) consisted in carrying out: a 5 m x 10 shuttle run (WS1 and WS5); flexion-extensions of the bust on legs or *crunches* (WS2); side multi-jumps above a bench (WS3); flexion–extensions of the arms in facial support or *push ups* (WS4 and WS8); in abdominal decubitus, upper limbs in the prolongation of the body, rising-going down simultaneous and repeated by the four limbs to the top (WS6); crunch flexion-extensions of the lower limbs with the bust right (WS7); multi-jumps (WS9).

The 5 hours of rest session (R5) was spent lying in bed but without sleeping. The players were allowed to read by keeping a semi-lengthened position, except during the 15 min preceding blood sampling during which they sat down, as it has been recommended (Ronsen et al., 2001).

The aerobic mini game-based session (AE) which lasted 65 min, comprising 10 min of warm up and three 15 min sequences of mini games with 4 students playing against 4. There were 5 min of active recovery (rehydration and stretching) after each sequence. During the session, the average heart rate of the players was 179 ± 4 bpm [176 – 195 bpm], which corresponds to 92% of their maximal heart rate. For each sequence, the rules are as follows: two teams of 4 players each, are alternately opposed on a handball ground (40 m x 20 m) with removable goals (120 cm x 60 cm). There was a referee, assisted by four ball boys for each side of the ground.

The playing instructions are as follows: **a)** the ball passed should not go up above the knee of the receiver; **b)** any receiver is allowed to touch the ball twice maximum before he passes it to a partner; **c)** after he passes the ball, any player can follow it, by running towards the adverse goals, with the intention to receive the ball once more or he runs back to support the new ball receiver (in any case, he should not stop after a ball pass); **d)** the pass is done to a player who runs and asks for the ball; **e)** there is a goal, when: the shoot does not go up above the knee, the shoot is made from the right specified area, all attackers are in the adverse camp (attacked camp) at the time of the shoot, the ball crosses the goal line; **f)** it does not an off-side and all the other rules regarding the behaviour towards the adverse players during a soccer game are observed.

The rules designed **a)**and **b)** must be respected scrupulously. The referee must whistle a fault against the team whose player does not respect any of these two rules and dispossess the aforementioned team of the ball, for the benefit of the opposing team.

Statistical analysis

The data were processed with the software STATISTICA (Stat Soft Inc., Version 7). For each variable, the mean value (M) and the standard error of the mean (SEM) were calculated. A two-way repeated analysis of variance (Anova) (time of measurement x type of training session) was used to compare leukocyte counts. The post hoc test HSD of Tukey was used for multiple comparisons, whenever Anova was significant. The statistical significance of the tests was settled at $p < 0.05$.

Results

Biometric characteristics of the players

The players' mean resting heart rate (HRr) was 58 ± 1 bpm, and their maximum heart rate was on average 193 ± 1 bpm (Table 1). The estimated VO_2max, using the shuttle test of Cazorla& Leger (1993) was on average, 52.3 ± 0.8 $mL.min^{-1}.kg^{-1}$.

Interaction between type of training session and time of measurement

The interaction (type of training session x time of measurement) was significant only for NEU ($p = 0.006$), MO ($p = 0.048$) and CD4 ($p = 0.0007$).

Variation of the leukocyte counts

A constant basophilopenia appeared in the group of soccer players implied in this study, whatever the type of training session and the time measurement ($p > 0.05$).

A training effect was highlighted ($p < 0.05$) after the twenty four hour-resting following the training session, for CD4 alone (Table 1). The significant differences are between CT and R5 as well before the training sessions ($p = 0.02$) as within the following 24 hours ($p < 0.05$). Significant differences were recorded between AE and R5 ($p = 0.001$) at the end of the sessions, then between AE and CT ($p = 0.006$) 24 hours later.

At the end of the sessions, a training effect ($p = 0.003$) was observed for NEU count (Table 2), and significant differences were found between AE and R5 ($p = 0.02$) sessions and between AE and CT ones ($p = 0.003$).

For the other leukocyte counts (WBC, EO, MO, LYM), there was no significant difference ($p > 0.05$) between training sessions at any time of measurement (Table 2).

Concerning the CT session, there was no time of measurement effect ($p > 0.05$) for any type of leukocyte count. However, a non-significant decrease ($p > 0.05$) in WBC, NEU, EO and LYM counts was recorded at the end of the CT session, followed by a progressive and non-significant increase ($p > 0.05$) within 24 hours. MO and CD4 counts showed a constant but non-significant decrease ($p > 0.05$) within the following 24 hours.

The EO and MO counts rose to a significant degree, respectively by 193.35% and 126.42% ($p < 0.05$) at the end of the AE session (Tables 1 and 2), and then tended gradually towards the initial values, within 24 hours. The CD4 count decreased non-significantly ($p > 0.05$) from rest (1.15 ± 0.06 to 1.07 ± 0.05 x 10^9/L cells) to six hours after the AE session, and reached the lowest value (0.80 ± 0.06 x 10^9/L cells), i.e. significantly lower than each of the other values ($p < 0.01$) (Table 2), 24 hours later.

Regarding the R5 session, a significant decrease in the WBC (- 40%, $p < 0.05$) and NEU counts was found from resting to six hours after the training session, followed within 24 hours, by a progressive increase towards the initial values (Table 1). The variations were non-significant ($p > 0.05$) for all the other leukocyte subtype counts.

Table 1 : Biometriccharacteristics of the studied soccer players (n = 16).

	M ± SEM
Age (years)	22.8 ± 0.5
Weight (kg)	65.8 ± 1.3
Height (cm)	171.8 ± 1.0
Estimated VO_2max ($mL.kg^{-1}.min^{-1}$)	52.3 ± 0.8
HRr (bpm)	58 ± 1
HRmax (bpm)	193 ± 1

n: sample size ; HRr : resting heart rate ; HRmax : maximal heart rate, assessed during the shuttle race test; estimated VO_2max : maximal oxygen consumption assessedwith the 20 m shuttle race test (Cazorla and Léger, 1993) ; M : mean value ; SEM : standarderror of the mean.

Table 2 : Modifications in white blood cell, eosinophil and neutrophil cell counts ($10^9.L^{-1}$) by training session and according to time of measurement, in soccer players of Benin (n = 16).

	AT REST	IN THE END	6 HOURS LATER	24 HOURS LATER
WBC				
Circuit training	11.35± 1.66	7.98 ± 1.08	9.32 ± 0.92	10.65 ± 1.50
AE	10.06 ± 1.32	9.63 ± 1.36	8.70 ± 1.78	8.46 ± 1.36
5 hours of rest	11.41 ± 0.58	7.95 ± 0.66 **(-30.3%)†**	6.79 ± 0.48 **(-40.5%)†**	10.35 ± 1.05 **(-9.3%)**
EOSINOPHILS (EO)				
Circuit training	0.23 ± 0.07	0.19 ± 0.08	0.13 ± 0.04	0.14 ± 0.04
AE	0.15 ± 0.05	0.45 ± 0.10 **(+193.3%)†**	0.22 ± 0.06 **(+49.0%)**	8.46 ± 1.36 **(+35.8%)**
5 hours of rest	0.42 ± 0.12	0.26 ± 0.09	0.26 ± 0.06	0.25 ± 0.09
NEUTROPHILS (NEU)				
Circuit training	5.91 ± 0.74	3.80 ± 0.63	4.72 ± 0.43	5.47 ± 0.65
AE	5.77 ± 0.86	6.75 ± 0.75	4.19 ± 0.73	4.38 ± 1.15
5 hours of rest	6.40 ± 0.44	4.47 ± 0.34	3.83 ± 0.36	6.65 ± 0.65

In the end: that is at the end of the training session; WBC: white blood cell count; values in the cases are mean values ± standard errors of the mean; those in (%) are percentages of variation related to values prior to the training session; † : difference between cell count at a moment and that recorded prior to the training session, significant at $p < 0,05$; AE: aerobic game-based session.

Table 3 : Modifications in monocyte and CD3 lymphocyte counts ($10^9.L^{-1}$) by training session and according to time of measurement, in soccer players of Benin (n = 16).

	AT REST	IN THE END	6 HOURS LATER	24 HOURS LATER
MONOCYTES				
Circuit training	0.48 ± 0.13	0.44 ± 0.10	0.28 ± 0.08	0.24 ± 0.05
AE	0.30 ± 0.09	0.69 ± 0.12 (+126.4%)†	0.39 ± 0.11 (+28.5%)	0.15 ± 0.04 (-48.7%)
5 hours of rest	0.60 ± 0.10	0.37 ± 0.09	0.32 ± 0.08	0.39 ± 0.12
CD3 LYMPHOCYTES				
Circuit training	4.69 ± 1.05	3.50 ± 0.54	4.01 ± 0.76	4.74 ± 1.04
AE	3.20 ± 0.51	3.61 ± 0.45 (+12.7%)	3.87 ± 0.93 (+20.9%)	2.49 ± 0.37 (-22.0%)
5 hours of rest	4.00 ± 0.52	2.84 ± 0.44	2.34 ± 0.44	3.04 ± 0.40
CD4 LYMPHOCYTES				
Circuit training	1.26 ± 0.09	1.20 ± 0.08	4.72 ± 0.43	1.10 ± 0.07
AE	1.15 ± 0.06	1.12 ± 0.04	4.19 ± 0.73	0.80 ± 0.06
5 hours of rest	0.96 ± 0.07	4.47 ± 0.34	3.83 ± 0.36	0.88 ± 0.05

In the end: that is at the end of the training session; values in the cases are mean values ± standard error of the mean; those in (%) are percentages of variation related to values prior to the training session; † : difference between cell count at a moment and that recorded prior to the training session, significant at $p < 0,05$; AE: aerobic game-based session.

Discussion

Characteristics of the players and methodological choices

The mean HRr was 58 bpm in the observed group of soccer players. These players had on average a resting bradycardia which characterizes well trained athletes, but the 52.3 $mL.kg^{-1}$. min^{-1}mean value recorded for VO_2max, seems just higher than that recorded in non-practitioners of comparable ages, i.e 48.9 ± 7.2 $mL.kg^{-1}.min^{-1}$ (Vehrs et al., 1998). The mean height, weight and VO_2max of these players show well that their profile does not match with that of international level players studied by Cazorla&Farhi (1998), whose data are respectively higher than 178 cm, 72 kg and 58 $mL.kg^{-1}.min^{-1}$. It is however necessary to be careful while interpreting the data of VO_2max, since we used a field test which might underestimate individual results (Poortmans et al., 1986). We could also have used laboratory training designs, as it was done in many studies (Gray et al., 1993; Natale et al., 2003). It was not the case in the current study, because the choice of carrying out the training sessions on the playing ground, matches with the desire to solve problems related to the use of field-specific training designs. We recognise that the use of field training designs may raise the problem of reproducibility and standardization. However, one of the interests of the current study is the effort we made to have reproducible training sessions, reducing to the maximum, the limits associated with the use of this kind of training designs in the research field. As it is shown in table 1, the mean heart rate recorded during the AE session based on mini games was 179 bpm, corresponding to 92% of the players' maximum heart rate (HRmax). This heart rate value accounts for the high intensity of the players' activity during the 15 min 4 against 4 games. These 92% of HRmax appear higher than the 80 – 90%, corresponding to 70 - 75% of VO_2max generally reached during a soccer game (Stolen et al., 2005).

Circulating leukocyte count response

The R5 session induced leukocytes counts significantly different from those of CT session (CT) only for CD4, in the end and 24 hours after. It is reasonable to think that only CD4 cells were sensitive to the workload associated with the CT session. The significant differences between the R5 session design and the AE one appeared for neutrophils and CD4 cells, only at the end of the training sessions. Thus, these increases testify a neutrophilia and a CD4 lymphocytosis associated with the intravascular mobilization of these leukocyte cells during the aerobic session (Walsh et al., 2011; Steensberg et al., 2001).

A non-significant decrease in WBC was however noticed during and within the 24 hours following the CT session. This result could be associated with the reduced study sample size. It could also indicate that the workload associated with this training session was insufficient for stimulating the immune system in the studied players. Since we ensured that each subject worked at his optimal capacity and rythm, the question is to know whether the total amount of exercises was insufficient or rather the recovery times were too long. This result related to the CT session is in conformity neither with that of Gray et al. (1992) which observed a significant increase in WBC and LYM count following one minute of maximum exercise, nor with that of Boas et al. (1996). Boas et al. reported that a three minute-anaerobic exercise caused a significant increase in WBC count, by 28%, and by 43% in LYM count.

In the current study, an immediate transitory eosinophilosis and monocytosis occurred at the end of the AE session. This result which expresses a well-known phenomenon (Okutsu et al., 2008) is in conformity with those of Rhind et al. (1996) and Mitchell et al. (2002) with regard to MO count. These authors found that a submaximal exercise induced an increase in WBC, NEU and MO counts. In the series of Mitchell et al. (2002), the maximum counts of the different leukocyte subsets were reached two hours after the end of exercise.

The constant decrease in CD4 count found within the 24 hours following the AE session, as well as the non-significant reduction recorded after the CT one confirm the data of Ramel et al. (2003) and suggest that the recovery time needed after an aerobic session by the lymphocytes, particularly the CD4 ones, is longer than 24 hours.

The CD4 count assessed prior to the R5 period was significantly weaker than that found before the CT session. There is no obvious explanation to this result, since the provisions were taken for carrying out the three sessions under the same conditions. It is however possible that the physical workloads imposed to these student players during the 15 days separating two protocols were so demanding that, they induce a significant decrease in CD4 cells count. If such was the case, the 72 hour-recovery was not probably enough to neutralize the impact of the last training session and allow the increase in CD4 count.

Practical implications of the results

We observed a significant decrease in CD4 and neutrophil counts during and 24 hours after the AE session. So, the schedule of a second high intensity training session during the same day in the afternoon,

for our players and in the beninese environment would be particularly stress increasing, a situation which should be avoided. In the afternoon (4 p.m.), the ambient temperature rises above 32 °C, even in the south of the country where this study has been undertaken. This can increase the secretion of hormones like cortisol and catecholamine which cause demargination of leukocytes from the lymphoid tissue (Brenner et al., 1998). The cumulated effects of two training sessions are likely to cause a leukopenia and an inflection in immune cells' function, in the event of repetition of this situation (Ronsen et al., 2001). Under our study conditions, this result could be considered as a scientific argument in favour of the theory of Matveiev (1980) related to diachronic post-effort recovery. According to this author, one needs a 24 to 48 hour recovery following a resistance session and a 48 to 72 hours recovery period, i.e. a longer time, after an aerobic one. The current work did not permit to know the qualitative immune response to the training session designs used, particularly regarding cytokines secretions, pro and anti-inflammatory substances. This aspect will be approached shortly in an integral study design for example in addition to the numeration of NK, NTF $\alpha 1$ and $\alpha 2$ cells, the proportioning of IL2, IL6, and C reactive protein (CRP), etc.

Conclusion

The physical load associated with the circuit training (CT) session is probably not sufficient to induce the quantitative immune response in the studied soccer players. Reducing recovery times and increasing the total number of repetitions during exercises might lead to a wider reaction. Since exercise causes a metabolic imbalance which induces adaptation in the immune system, the current results do not inform whether one can schedule another training session within the 6 hours following a CT training session. However, it appears that training 24 hours after such a session, may be suitable regarding immune response, since the numbers of circulating leukocytes at that time neared that counted prior to the session. Considering the conditions under which this study was carried out, the results obtained with the aerobic mini game-based session (AE) allow to use it for assessing the quantitative immune response in the soccer players. The use of a circuit training session, i.e. resistance training for this purpose, requires another experimental study, aiming at determining the suitable training design.

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