Monitoring of Biochemical Changes during different Phases of Training in Indian Female Cyclists

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ABSTRACT

The objective of the present study was to monitor the biochemical changes, during different phases of the training cycle, in Indian female cyclists. Blood sample was collected from six female cyclists, at the end of each training phase, to find out the impact of training load on hematological, enzymatic and hormonal profile. Results showed that the mean values of ferritin (Ist -48.1, IInd -37.3, IIIrd-32.96, IVth-39.7), testosterone (Ist-1.03, IInd -0.93, IIIrd-0.85) and Testosterone/ cortisol (Ist -0.007, IInd -0.004, IIIrd-0.010) ratio decreases as training load increases whereas total iron binding capacity (Ist-302.4, IInd -314.1, IIIrd-388.0, IVth-330.7), lactate dehydrogenase (Ist -253.9, IInd -351.4, IIIrd-469.2, IVth-513.5) and cortisol (Ist-150.94, IInd -205.5, IIIrd-236.9, IV-227.2) level increases as volume/intensity of training increases during the four different phases (Ist , IInd , IIIrd & IVth) of the training cycle which may negatively affect the performance of the cyclist. Hence, it was concluded that the monitoring of these biochemical variables, during different phases of the training cycle, helps in detecting / preventing overtraining, anemia and thereby enhancing the performance.

INTRODUCTION

Cycling is a sport with a wide variety of events ranging from very short match sprints of about 10 seconds to road races that sometimes last more than 5 hours (Burke, 1986). Competitive cycling requires both aerobic and anaerobic power (Faria, Parker & Faria, 2005). The cyclist who wants to achieve their best performance, during competition, wholly depends on the well-planned training programme (volume, intensity & frequency of training). The volume of training refers to the total quantity of activity performed in training; and is the sum of all training session durations. In cycling, this is often referred as the total number of kilometers (Bompa, 1983). A general pattern of training programme would place many continuous slow riding periods, in the first micro cycles of the general preparatory training

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phase, and more intense activities would be gradually integrated as the cyclist progresses towards the racing season (Boulay, 1995).

Several studies have shown the physiological approach to training in the sport of cycling (Hawley, Myburgh, Noakes & Dennis, 1997; Hoogeveen, 2000; Hoogeveen, Schep & Hoogsteen, 1999) and our study is concerned about the biochemical changes during the training cycle. Numerous strategies have been presented for monitoring the training status of competitive cyclists, in order to precisely evaluate the training methods and their efficacy during a training and competitive season. The magnitude of dependent variable reaction, as subsequent alterations in response to training, is remarkably varied (Faria, Parker & Faria, 2005). The present research is to evaluate the magnitude of biochemical variation during different phases of the training cycle.

Biochemical tests are used widely to assess the health and fitness of the intensively training athlete. Biochemical methods play a key role in examination, control of the degree of training, and to improve the performance of players (Butova & Masalov, 2009).

Hematological profile of athletes plays a significant role as it is directly involved in the performance of a player. Iron is essential for cellular life and is required in numerous metabolic processes. In normal healthy individuals, there are about 35 mg of iron per kg body weight in adult women and 45 mg/kg in adult males (Andrews, 1999; Andrews, 1999). About 65% of body iron is carried in the hemoglobin of circulating red blood cells while another 10% incorporated in myoglobin, cytochromes, and iron containing enzymes. The remaining 20-30% is stored in ferritin and hemosiderin (Conrad, Umbreit & Moore, 1999). Distribution within these stores can change under various conditions, such as training load, inflammation and increased erythrocyte production, or when absorption is abnormally increased (Zotter, Robinson, Zorzoli, Schattenberg, Saugy & Mangin, 2004).

Serum urea has been frequently used to evaluate the load of training and the recovery process (Nikolaidis, Protosygellou, Petridou, Tsalis, Tsigilis, & Mougios, 2003). The increased urea concentration, in athletes, is suggestive of a catabolic state and due to the higher protein intake by athletes as part of their higher energy intake, acute exercise has been shown to provoke prolonged increases in urea (Raastad & Hallen, 2000). The serum level of urea and uric acid indicates the training load imposed on the athletes. In addition, the urea and uric acid accumulation is most frequently used as a measure of protein catabolism and degradation of adenonucleotides (Andersson, Raastad, Nilsson, Paulsen, Garthe & Kadi, 2008; Degoutte, Jouanel, Begue, Colombier, Lac, Pequignot & Filaire, 2006; Kargotich, Keast, Goodman, Bhagat, Joske, Dawson & Morton, 2007).

Creatine Phosphokinase activity, in serum, is an indirect index of muscle cell damage and is elevated following strenuous activity (Nikolaidis

et al, 2003).Lactate dehydrogenase (LDH) is an enzyme of carbohydrate metabolism, and the activity of LDH is an important biochemical index that is used to assess the muscle tissue performance (Butova & Masalov, 2009). Creatine phospho kinase and lactate dehydrogenase are used as a biomarker for training load (Majumdar, Mandal, Yadav & Gupta, 2008).

Hormone levels are influenced by the physical exercise, especially of testosterone and cortisol level (Galbo, 1983). Endogenous hormones are essential for physiological reactions, adaptations during physical work, and influence the recovery phase after exercise by modulating anabolic and catabolic processes (Fry, Morton & Keast, 1992). Hence, all the above mentioned biochemicals were influenced by the exercise training and these biochemical concentrations were quantified for this present study. As limited number of research studies have been carried out on female players and training load, this present study will be a stepping stone for judging the influence of training load on biochemicals, in female players.

The objective of the present research was :

- To find out the magnitude of variation of biochemical concentration.
- To quantify and to find out the impact of training load on various biochemical.
- To find out the iron status that is important for oxygen transport during exercise.
- To avoid overtraining.

METHODOLOGY

A total of six female cyclists volunteered to participate and they underwent training in Sports Authority of India, Bangalore. The training programme of Indian female cyclists is presented in Table 1. Blood samples were collected at the end of each training phase, to find out the influence of training intensity/volume on biochemical profile. The study was approved by the ethical committee of Sports Authority of India, India. The players provided their verbal informed consent before the commencement of this training study.

Five ml venous blood was collected from each subject after an overnight fast of 12-14 hours. Serum separated from the whole blood was used to analyze the biochemical profile hemoglobin, ferritin, iron, total iron binding capacity, urea, uric acid, creatine phosphokinase, lactate dehydrogenase, testosterone, cortisol and testosterone/cortisol ratio. Hemoglobin was estimated by Cyanmethaemoglobin Method; ferritin by Enzyme Immunoassay Method; iron and Total Iron Binding Capacity (TIBC) was estimated by Persjin Method; urea was estimated by Berthelot Method and uric acid by Uricase Enzymatic Method; lactate dehydrogenase and creatine phosphokinase were measured by Enzymatic Method; and hormones cortisol and testosterone were estimated quantitatively by Competitive Immunoenzymatic Method (ELISA). The testosterone/cortisol ratio was calculated from the value of testosterone and cortisol. The instruments used for these estimations were HITACHI UV-2000 Spectrophotometer (Japan), ELISA Reader-ELX800TM and Washing equipment (ERBA, smart wash).

The statistical software MINITAB14 and Statistical Package for Social Sciences (SPSS) MS Windows 9.0 was used for statistical analyses. Data are presented as Mean ± Standard deviation. One-way Analysis of Variance was used to determine the significant differences among the variables.

Table-1 : Training programme of Indian female cyclists

Training Days	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Phase I st 16 hours 40 minutes 460 km			E1/E2 80 km 2 hours 40 min Rollers E1/E2 20 min	E1/E2+2*E2S 14 min/8 km effort 90 km 3 hours Rollers E1/E2 20 min	E1/E2 90 km 2 hours 40 min Rollers E1/E2 20 min	E1/E2 + 2*E2S 14 min/8 km effort 90 km 3 hours Rollers E1/E2 20 min	E1/E2 110 km 4 hours
Phase II nd 19 hours 10 minutes 570 km		E1/E2+2*E2S14 min/8 km effort 110 km 3 hours Rollers E1/E2 20 min	E1/E2 90 km 2 hours 40 min	E1/E2 + 2*E2S 14 min/8 km effort 90 km 3 hours Rollers E1/E2 20 min	E1/E2 70 km 2 hours 15 min	E1/E2 + 3*E2S 14 min/8 km effort 90 km 3 hours Rollers E1/E2 20 min	E1/E2 120 km 4 hours 15 min
Phase III rd 17 hours 10 minutes 550 km		E1/E1b+E2/E2S 14 min/8 km effort 90 km 3 hours Rollers E1/E2 20 min	E1/E1 b+E2 90 km 2 hours 40 min	E1/E1 b+E2/E2S 14 min/8 km effort 90 km 3 hours Rollers E1/E2 20 min	E1/E1 b+E2 70 km 2 hours 15 min	E3+E4 90 km 1 hours Rollers E1/E2 20 min	E1/E1 b+E2 120 km 4 hours 15 min
Phase IV th 16 hours 55 minutes 460 km		E1/E2+1*E2S 14 min/8 km effort 70 km 2 hours 40 min Rollers E1/E2 20 min	E1/E2 90 km 2 hours 40 min	E1/E2 + 1* E2S 14 min/8 km effort 70 km 2 hours 40 min Rollers E1/E2 20 min	E1/E2 70 km 2 hours 15 min	E1/E2 70 km 2 hours 40 min Rollers E1/E2 20 min	E1/E2 90 km 3 hours

E1 → Up to 60% HR, **E1b** → 60–69% HR, **E2** → 69–80% HR (Threshold), **E2S** → Working at 80% HR (Threshold, S = Strength)
E3 → 81–90% HR, **E4** → 90–100% HR.

RESULT & DISCUSSION

Table-2 : Biochemical profile of Indian female cyclists in different phases of the training cycle.

Variables	Desirable Range	Phase Ist	Phase IInd	Phase IIIrd	Phase IVth	One-way ANOVA	
						F Ratio	Sig.
Hemoglobin (g %)	12-16 g%	13.29±0.86 (n=5)	13.70±0.46 (n=4)	14.08±0.94 (n=6)	14.62±0.85 (n=5)	2.347	0.111
Ferritin (ng/ml)	20-240 ng/ml	48.1±32.7 (n=5)	37.3±24.8 (n=4)	32.96±16.5 (n=6)	39.7±31.2 (n=5)	0.303	0.823
Iron (ng/ml)	36.87-145.25 ng/ml	161.3±60.7 (n=5)	101.0±36.1 (n=4)	153.35±19.64 (n=6)	110.96±11.47 (n=5)	3.278	0.048*
Total Iron Binding Capacity (ng/ml)	274-394 ng/ml	302.4±69.5 (n=5)	314.1±96.7 (n=4)	388.0±95.3 (n=6)	330.7±47.2 (n=5)	1.252	0.324
Urea (mg %)	13-45 mg%	30.79±5.79 (n=5)	29.48±3.47 (n=4)	25.70±3.41 (n=6)	26.42±4.89 (n=5)	1.514	0.249
Uric acid (mg %)	1.4 – 7.4 mg%	3.08±0.83 (n=5)	3.21±0.95 (n=4)	3.59±0.94 (n=6)	3.36±0.43 (n=5)	0.390	0.762
Creatine phospho kinase (IUL/L)	15-130 IU/L	99±47 (n=5)	78.2±29.3 (n=4)	101.5±46 (n=6)	76.1±39.7 (n=5)	0.515	0.678
Lactate dehydrogenase (IU/L)	103-227 IU/L	253.9±97.4 (n=5)	351.4±53.8 (n=4)	469.2±44.5 (n=6)	513.5±215.4 (n=5)	4.606	0.017*
Testosterone (ng/ml)	0.2 – 2.0 ng/ml	1.03±** (n=1)	0.93±0.87 (n=3)	0.85±0.65 (n=6)	**** (n=0)	0.031	0.970
Cortisol (ng/ml)	60-230 ng/ml	150.94±** (n=1)	205.5±121.2 (n=3)	236.9±177.5 (n=6)	227.2±188.9 (n=5)	0.081	0.969
Testosterone/Cortisol ratio	> 0.031	0.007±** (n=1)	0.004±0.001 (n=3)	0.010±0.014 (n=6)	***** **** (n=0)	0.220	0.808

*P<0.05, **p<0.01, ***p<0.001. n – Number of subjects

Table-2 shows the changes observed in serum biochemical profile, in four different phases of the training cycle. The mean hemoglobin level progressively increased, in phases. The mean ferritin level decreased till third phase, and thereafter increased during the recovery phase; whereas, TIBC level showed the opposite trend. The mean iron and transferrin saturation level decreased in IInd and IVth phase and increased in Ist and IIIrd phase. The level of serum urea decreased from Ist phase to IIIrd phase; whereas, uric acid level increases. The enzyme creatine phosphokinase level decreased in IInd and IVth phases; whereas, increased in the rest of the

phases; and the lactate dehydrogenase level increased, in phases. The hormone testosterone level decreased subsequently from Ist to IIIrd phases of the training cycle. The mean cortisol level decreased till IIIrd phase and increased in the IVth phase of training. The mean T/C ratio was high in the third phase when compared to the other phases of the training cycle.

For the achievement of best performance, the training has to be formulated according to the principles of periodization (Bompa, 1999). Player's performance, during competition, is the combined effect of biochemical, physiological, biomechanical, psychological and environmental factors (Kumar, Panda & Jeyasekharan, 1999). The training induced changes, observed in various biochemical variables, can be attributed to incremental training load. This can be used to assess the current status of an athlete and the degree of training adaptability and also provide a prospect to alter the training load accordingly to achieve the desired performance (Bompa, 1999). Biochemical parameters like iron profile, urea, uric acid, enzyme and hormonal profiles are of advantage in regulating the training load (Suhr, Porten, Hertrich, Brixius, Schmidt, Platen & Bloch, 2009).

Exercise is known to influence variables of iron metabolism to a great extent (Weight, Alexander & Jacobs, 1991). Assessing the hematological profile of a player is used to detect or prevent sports anemia. Several studies indicated that the female athletes are at a higher risk, than their male counterparts, of developing iron deficiency (Balaban, Cox, Snell, Vaughan & Frenkel, 1989). Hence, our study is focused on female cyclists. In the present study, the mean values of hematological profile, hemoglobin, ferritin, iron and total iron binding capacity were within the normal range. **Haemoglobin** is an iron containing protein that transports oxygen to muscle primarily (Dunford & Doyle, 2008). The hemoglobin level of individual cyclists is within the normal limit throughout the training cycle. The hemoglobin level increases subsequently during the training cycle. The hemoglobin level is high in the last phase i.e. recovery phase when compared to other phases of the training cycle. This shows that the level alters with the training and also revealed that the hemoglobin level is high during the lowest volume 460 km of training. Athlete needs to maintain normal haemoglobin level to optimize performance (Dunford & Doyle, 2008).

Ferritin is an indicator of stored iron in the body. Iron storage level decreases from first phase to third phase, increases in the last phase. This change in iron storage level is due to the increase in volume/intensity of training. During the recovery phase, the level increased 6.7%. The ferritin level of one cyclist is <20 ng/ml which indicates the iron depletion (Iron deficiency anemia) which may negatively affect performance. In addition, the minimum mean ferritin level is found in IIIrd phase which shows that the higher volume of 550km and highest intensity of E3 (81-90% HR), E4 (90-100% HR) of training depletes the iron storage, the most. Maximum mean ferritin

level, seen in I[st] phase, reveals that the use of iron from ferritin or iron depletion is less at the beginning of the training and is more in the subsequent periodized cycle. In many studies, it has been found that the low ferritin level affects the performance of a player (Lamanca & Haymes, 1992). It is very much needed to maintain the ferritin level within the normal limit. The best approach to eliminate the risk of a low ferritin, without anemia, in competitive athletes, is checking a serum ferritin, periodically.

Serum Iron is a measure of circulating iron bound to transferrin and reflects total body iron (Burns, Goldberg, Lawrence & Wenz, 1990; Finch & Huebers, 1986). Iron level decreased 60% in the second phase and increased 52% in third phase. There is again decrement of 38% in the recovery phase. The highest iron level is found in first phase which shows that the lowest volume of 460km and intensity of E1 (Upto 60% HR), E2 (69-80% HR), E2S (working at 80% HR) of training uses the iron the least.

Total Iron Binding Capacity (TIBC) is a medical laboratory test which measures the blood's capacity to bind iron with transferrin (26). TIBC level increased from first phase to third phase which reveals that increase in volume/intensity of training impacts the iron binding capacity. The increase in volume/intensity of training utilizes the circulating iron and the demand beyond that increases the TIBC level to use the maximum amount of iron. The TIBC level decreased from the third phase to the fourth phase, which is due to decrease in volume of 550 km to 460 km and intensity from E3,E4 (81-90%, 90-100% HR) to E1,E2 (Upto 60%, 60-69% HR) of training.

Urea and uric acid level of all the athletes were in the normal range during different phases of the training cycle.

The enzymes **creatine phosphokinase and lactate dehydrogenase** activities, during exercise, have been established in various studies. Adaptations of the body to physical load are associated with improvement of various mechanisms of regulation of enzyme activity (Butova & Masalov, 2009). Our study shows that the activity of enzyme differs in various phase of the training cycle. The creatine phospho kinase levels of the cyclists, in all the training phases, were within the normal range, throughout the training; whereas, the lactate dehydrogenase level of cyclists increased subsequently, in the training cycle. The increase in training load increases the lactate dehydrogenase level; this result supports the assumption that the changes in enzyme activity are a sensitive marker of muscle stress in response to training.

Hormones **testosterone and cortisol** concentrations are influenced by the exercise (Harber, Fry, Rubin, Smith & Weiss, 2004). Testosterone and T/C ratio levels decrease from first phase to third phase due to increase in training load. The higher volume of 550km and highest intensity of E3 (81-90% HR), E4 (90-100% HR) training, in third phase, impacts the testosterone and T/C ratio level, at most. Cortisol level increases from first phase to third; which is also due to increase in volume/intensity of

training. A number of studies have shown that the testosterone and cortisol are biochemical markers of training load; our findings also suggest that the level of hormones fluctuation depends on the volume/intensity of training imposed on cyclists.

The mean values of all the biochemical profile, except LDH, cortisol (in IIIrd phase), were within the normal range, which reveals that the higher volume of 550km and highest intensity of E3 (81-90% HR), E4 (90-100% HR) of training, in third phase, impacted the lactate dehydrogenase and cortisol level. Among the other biochemical variable, Lactate dehydrogenase and iron levels of cyclists show significant changes, during the different phases of training.

The findings of the present study suggest that the volume/intensity of training influences the hematological, enzymatic and hormonal status of the female cyclists. Adaptations to training are the main cause for the variations of hematological, enzymatic and hormonal profile of the cyclists. Our study on cyclists has revealed that the hormonal and enzymes stress marker profile increased with increase in volume/intensity of training. Hence, monitoring of these stress markers, during training, is very much essential in order to avoid over training and to enhance the performance. Although, some of the blood parameters (e.g. urea, uric acid) are not capable of detecting overtraining, they are helpful in providing information on the actual health status of the athlete. The hematological profile results show that the need/loss of iron increases with increase in training load. The ferritin (iron storage form) level also decreases with increase in training load. To avoid shortage of iron supply to body cells and to avoid sports anemia monitoring of these hematological profile, in each phase, is very much needed, as this profile is closely related to performance of the cyclists.

Our present study shows the impact of different intensity/volume of training on biochemical profile of cyclists, in different phases of the training. Identification of markers for over training and anemia helps in optimizing the training load thereby enhancing the performance.

CONCLUSION

Monitoring of stress markers and hematological profile, in different phases of the training, is necessary to avoid over training, to optimize training load, to avoid sports anemia and to enhance the performance of the cyclists. The main finding of our study is increase in stress markers level and increase in need/loss of iron with increasing training load. Several studies have shown that the stress markers and the hematological profile of players are correlated with the performance; hence, it is essential to monitor these variables, during different phases of the training cycle. Summing up, the observed changes in biochemical indices are suggestive of metabolic disturbances brought about by different intensity/volume of training.

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