Effects of annual training cycle on the metabolic response to supra-maximal exercise test in beach volleyball players

by
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The aim of this study was to analyze the effects of sports training on the physiological response to supra-maximal exercise during consecutive phases of the annual training cycle. The study was carried out in volleyball players at the onset of each training phase. VO_2 max was determined by an indirect method using the Ästand-Rhyming nomogram and biochemical analyses were performed before and after the Wingate test. Concentrations of lactate in capillary blood were measured and levels of glucose, insulin, visfatin, resistin, thiobarbituric acid reactive substances (TBARS) of serum and the total antioxidative status of plasma were determined using venous blood.

Most significant differences with respect to physiological and biochemical variables centered around the pre-competitive phase when compared to other phases of the annual training cycle. Blood visfatin concentration in highly trained volleyball players is reduced by supra-maximal exercise, whereas levels of resistin remain relatively constant at rest. With the exception of the competitive phase, values of the insulin resistance index fit within the reference range. Levels of lipid peroxidation products were inversely correlated with the insulin resistance index and resistin concentrations.

The physical training during the annual cycle does not affect resistin levels, but influences insulin, glucose and visfatin concentrations, along with markers of pro-oxidant/antioxidant balance in beach volleyball players.

Key words: resistin, visfatin, $HOMA_{IR}$, supra-maximal exercise, beach volleyball

Introduction

Beach volleyball is a sport that is demanding both anaerobically and aerobically. Since teams of only two players take part in beach volleyball matches, players have to be active at all times, usually while exposed to high temperatures and sunlight, often playing barefoot on soft ground (Lejeune et al, 1988). Moreover, tournaments are organized in such a way that participants play

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three matches a day (with only short breaks in between), during three consecutive days. During the competition, the heart rate often reaches 180.66±8.60 beats per minute (Davies, 2000).

Available literature contains very few papers studying the physiological and biochemical changes induced by heavy physical exercise performed by players during matches (Zamparo et al, 1992; Zetou et al, 2008).

According to literature, both single exercise stimulation and sport training may positively influence exercise metabolism and insulin resistance (Kirwan et al.1992). However, excessive synthesis of reactive oxygen species has been noted during the competition period (characterized by significant physical and psychological loads) and along with mechanical injuries of the skeletal muscles, affects both insulin resistance and transmembrane transport (Del Aguila et al., 2000). Therefore, the aim of this study was to analyze the effects of the annual training cycle on carbohydrate metabolism parameters and oxidative stress measures after supra-maximal exercise in members of the national beach volleyball team. Their anaerobic power was determined by means of the Wingate test, while aerobic capacity was measured by maximum oxygen uptake.

Methods

This study included 8 beach volleyball players at the mean age of 18±1.0 years (mean training duration of 4±1.5 years). The study

procedures, upon their acceptance by the Ethics Committee of the Karol Marcinkowski Medical University in Poznan, were carried out during 5 phases of the annual training cycle (2008/2009), i.e. at the onset of the first preparatory, pre-competitive, competitive, transitional and second preparatory phases. Training loads of the annual cycle is presented in Table 1.

On the first day of each phase studied, somatic measurements were taken, including body height and weight, as well as waist and hip circumferences. Body composition was measured by means of bioelectrical impedance analysis (BIA) with the Bodystat® 1500 analyzer (Bodystat Ltd., UK), while peak oxygen uptake was determined according to Ästrand (1954).

On the second day, players were exposed to an anaerobic test, which is a supra-maximal exercise in the form of a 30-second Wingate test.

Prior to the test and after its completion, heart rate (HR) was measured with the aid of a POLAR Accureex-Plus sport tester. The systolic (SBP) and diastolic (DBP) blood pressures were also determined in all subjects. Moreover, blood for biochemical analyses was obtained from the basilic vein and digital pulp.

Biochemical analyses

Lactate concentration was determined in capillary blood using the Boehringer-Manheim kit. The remaining biochemical parameters were determined from venous blood.

Table 1

Training load during the annual cycle

Phases of an annual training cycle for beach volleyball players

Phase and duration	Transitional 10 weeks			General preparatory 20 weeks			Pre-competitive preparatory 4 weeks			Competitive 18 weeks		
Type of exercise	aerobic	mixed	anaerobic	aerobic	mixed	anaerobic lactic **+** non lactic	aerobic	mixed	anaerobic	aerobic	mixed	anaerobic
Exercise duration [hours]	60	0	0	100	0	0	40	12	0	72	60	0
				44	0	5 + 1						
				35	12	4 + 1						
				35	12	4 + 1						
				30	12	0						
%	100	0	0	82.5	12	5.5	77	23	0	55	45	
Total [hours]		60			296			52			132	

Table 2

Basic characteristics of study participants (n=8)

Parameter	Phase I $\bar{x} \pm SD$	Phase II $\bar{x} \pm SD$	Phase III $\bar{x} \pm SD$	Phase IV $\bar{x} \pm SD$	PhaseV $\bar{x} \pm SD$
Body height (cm)	187.9 ± 4.15	188.0 ± 3.51	188.2 ± 4.90	188.6 ± 4.47	189.1 ± 4.39
Body mass (kg)	78.2 ± 5.06 [**vs.II]	77.3 ± 9.51	78.0 ± 3.93	78.6 ± 3.68	80.2 ± 4.14
BMI (kg/m²)	22.2 ± 1.30	21.9 ± 1.59	22.2 ± 0.94	22.1 ± 1.16	22.3 ± 1.08
WHR	0.84 ± 0.02	0.85 ± 0.22	0.86 ± 0.10	0.84 ± 0.04	0.85 ± 0.02
Total body fat (%)	12.4 ± 2.09 [**vs.II]	9.5 ± 2.23 [**vs.III, IV, V]	12.6 ± 2.66	11.9 ± 2.54	12.8 ± 2.81
Total water (%)	57.7 ± 2.43 [*vs.II]	61.2 ± 2.46 [**vs.III, V]	57.8 ± 3.01	58.5 ± 2.69	57.5 ± 3.06

Phase I – preparatory phase (2008), Phase II – pre-competitive phase,

Phase III – competitive phase, Phase IV – transitional phase,

Phase V – preparative phase (2009); significantly different between phases:

[**]p≤0.01, [*]p<0.05

Glucose concentration was measured with the Cormay-Poland kit. Insulin concentration was determined by means of radio-immune assay (Bio-Source Europe S.A., Belgium). Resistin and visfatin concentrations were measured by means of ELISA immuno-enzymatic assays (R&D Systems, USA, and ALPCO Diagnostic, USA, respectively). The concentration of thiobarbituric acid reactive substances (TBARS) was determined with spectrophotometry (with chromogene extracted with n-butanol) according to Beuge and Aust (1991). Total antioxidative status (TAS) of

plasma was measured with a test by Randox Laboratories Ltd., Drumlin, Co. (Antrim, UK).

The homeostatic insulin resistance index value was calculated (for resting values only) using the following formula proposed by Matthews et al., (1985):

HOMA$_{IR}$ = C$_{INS}$ (µIU/ml) x C$_{GLUC}$ (mmol/l)/22.5.

Exercise protocol

In all five phases of the study, peak oxygen uptake (VO$_2$ max) was determined during morning hours (8 a.m.-11 a.m.). On the following day, a maximum anaerobic power test was performed on a Monark 824 E (Sweden) cycloergometer.

Peak oxygen uptake was determined by indirect method with the Ästand-Rhyming nomogram (1954). This nomogram is based on the relationship between workload performed on a cycloergometer and heart rate, with subjects achieving a state of functional balance at a certain workload. Oxygen uptake in liters per minute was determined from this nomogram.

During the maximum anaerobic power test (Wingate test), workload in the form of mechanically set pedal force was adjusted depending on the subject's weight. The workload was calculated based on the following formula:

workload=0.075 x body weight (kg).

The exercise was preceded by a 5-minute warm up at 50 W, followed by 5-minutes of rest. During the actual test (30 seconds), players were encouraged verbally to achieve maximal pedaling frequency in the shortest possible time, and to maintain that frequency for as long as possible until the cessation of the test. The test was started with the pedals in an established position – the arm of the right pedal was positioned to the ground at an angle of 35°. During the test, the cycloergometer was connected to a computer with Multi Cyklo Ergometr MCE V 2.3 software that calculated: overall work (KJ), peak power (W), time to peak power (s), time at peak power (s) and anaerobic fatigue (%). Overall work performed by the subjects during the 30-second exercise period was proportional to the number of pedal rotations and the predefined load.

Statistical analysis

Statistical analysis of results was carried out using the Statistica 8.0 package. Normal distributions of all parameters studied were verified with the Shapiro-Wilk test. Continuous variables were presented as arithmetic means ($\overline{x}$) and standard deviations (SD). Differences were considered significant with $p<0.05$. Assumption on sphericity was tested by Mauchley's test (verifying if variances of certain variables are identical and equal to respective co-variances). The arithmetic means of normally-distributed independent parameters determined in the training phases analyzed, were compared with one-way analysis of variance with repeated measures (ANOVA). Arithmetic means of normally-distributed non-spherical parameters were compared with multi-way analysis of variance with repeated measures.

Table 3

Mean values of physiological and physical parameters obtained during the Wingate test and peak oxygen uptake test in beach volleyball players during their annual training cycle (n=8)

Parameter	Phase I $\bar{x} \pm SD$	Phase II $\bar{x} \pm SD$	Phase III $\bar{x} \pm SD$	Phase IV $\bar{x} \pm SD$	Phase V $\bar{x} \pm SD$
HR$_S$ (bpm)	74.2 ± 11.51	65.7 ± 9.08	64.7 ± 10.98	72.1 ± 13.08	76.1 ± 8.97
HR$_W$ (bpm)	181.7 ± 7.94	176.5 ± 4.78	179.5 ± 8.47	177.5 ± 7.91	180.9 ± 7.53
SBP$_S$ (mmHg)	114.7 ± 6.56 *vs.II, ** vs.III, V	124.1 ± 5.46	129.6 ± 7.31	120.4 ± 9.81	126.2 ± 9.16
SBP$_W$ (mmHg)	168.6 ± 9.02 * vs.IV	184.6 ± 15.90	166.5 ± 17.27	165.1 ± 13.99	179.7 ± 15.13
DBP$_S$ (mmHg)	69.4 ± 7.27	69.0 ± 5.71	73.9 ± 7.32	73.0 ± 8.62	70.0 ± 8.86
DBP$_W$ (mmHg)	92.5 ± 28.09	77.9 ± 4.39	75.4 ± 9.44	81.5 ± 11.06	71.0 ± 13.18
Peak power (W)	1251.4 ± 161.21	1217.8 ± 170.64	1425.4 ± 183.31	1648.0 ± 254.90	1516.0 ± 203.85
Average power (W)	565.1 ± 44.22 **vs.III, IV, V	542.6 ± 43.31 **vs.III, IV, V	746.3 ± 68.37	742.3 ± 55.77	729.5 ± 52.47
Relative peak power (W/kg body weight)	15.9 ± 1.38 *vs.III, **vs. IV, V	15.7 ± 1.54 *vs.III, **vs. IV, V	18.3 ± 1.93 *vs.IV	20.9 ± 2.67	19.0 ± 2.71
Anaerobic fatigue (%)	16.4 ± 3.24 *vs.IV	13.1 ± 2.77 **vs.III, IV, V	19.8 ± 5.04	21.3 ± 1.90	20.5 ± 3.80
Time at peak power (s)	3.3 ± 0.58 *vs.IV	3.4 ± 1.02 *vs.IV	4.6 ± 1.35	5.3 ± 1.19 **vs.V	3.2 ± 1.05
Overall work (KJ)	18.8 ± 1.47	18.1 ± 1.44	24.9 ± 2.28	24.7 ± 1.86	24.3 ± 1.75
LA$_S$ (mmol/L)	1.5 ± 0.21 **vs.IV	1.2 ± 0.19 **vs.IV	1.3 ± 0.21 *vs.IV	2.0 ± 0.87 **vs.V	1.2 ± 0.28
LA$_W$ (mmol/L)	11.1 ± 1.10 **vs.III, *vs.IV	11.3 ± 1.33 *vs.III, **vs.IV	9.6 ± 0.60 **vs.IV, *vs.V	13.3 ± 0.57 **vs.V	11.1 ± 1.14
VO$_2$max (ml·kg^{-1}·min^{-1})	33.2 ± 4.90 **vs.II, III, IV, V	37.5 ± 5.46 *vs.III, **vs. IV, V	34.8 ± 6.06 *vs.IV	37.3 ± 8.61	54.0 ± 7.59

S – at rest, W – post-exercise; significantly different between phases:
*** p≤0.01, *p<0.05*

Table 4

Biochemical characteristics of beach volleyball players (n=8) during their annual training cycle

Parametr	Phase I $\bar{X} \pm SD$	Phase II $\bar{X} \pm SD$	Phase III $\bar{X} \pm SD$	Phase IV $\bar{X} \pm SD$	Phase V $\bar{X} \pm SD$
Glucose s (mg/dl)	81.2 ± 10.94	72.9 ± 6.54 **vs.V	75.2 ± 7.64 *vs.V	74.9 ± 7.72 *vs.V	89.8 ± 8.55
Glucose w (mg/dl)	86.5 ± 6.01	87.2 ± 15.88	86.2 ± 6.38	89.3 ± 11.13	97.4 ± 12.11
Insulin s (μIU/ml)	4.7 ± 1.74	7.5 ± 1.49	9.5 ± 2.09 **vs.IV, V	6.4 ± 1.54	5.8 ± 0.84
Insulin w (μIU/ml)	7.7 ± 3.64 **vs.IV	15.7 ± 3.85 **vs.IV	21.5 ± 8.20	21.2 ± 8.92	18.1 ± 7.51
HOMA IR	1.6 ± 0.49	1.5 ± 0.42	1.8 ± 0.50 *vs.IV	1.1 ± 0.19	1.3 ± 0.22
Resistin s (ng/ml)	8.4 ± 2.60	7.9 ± 2.13	10.1 ± 2.76	8.7 ± 2.31	7.4 ± 1.64
Resistin w (ng/ml)	8.8 ± 2.53	8.8 ± 3.08	9.6 ± 3.06	8.5 ± 1.84	8.1 ± 1.55
Visfatin s (ng/ml)	7.0 ± 3.12 **vs.II	4.5 ± 1.09 **vs.III, IV, V	4.4 ± 2.27 *vs.V	4.1 ± 1.66 *vs.V	7.7 ± 2.47
Visfatin w (ng/ml)	5.6 ± 3.79 **vs.II	3.3 ± 3.88 **vs.III, IV, V	2.5 ± 1.03	2.6 ± 0.80	3.0 ± 1.05
TAS s (mmol/L)	1.4 ± 0.21	1.2 ± 0.41 *vs.V	1.4 ± 0.14	1.1 ± 0.83**vs.V	1.6 ± 0.28
TAS w (mmol/L)	1.8 ± 0.22 *vs.II, **vs.IV	1.3 ± 0.24 **vs.V	1.6 ± 0.09	1.2 ± 0.17 **vs.V	2.0 ± 0.16
TBARS s (mmol/L)	7.1 ± 2.28 **vs.II, III, IV, V	4.1 ± 1.33	2.3 ± 0.53	3.0 ± 1.49	3.8 ± 0.55
TBARS w (mmol/L)	7.8 ± 2.11**vs.II, III, IV, V	5.0 ± 1.03 **vs.III	2.5 ± 0.71	3.5 ± 1.57	4.1 ± 0.70

*S – at rest, W – post exercise; significantly different between phases: ** $p \leq 0.01$, * $p < 0.05$*

Table 5

Linear coefficients of correlation between pairs of variables tested

Parameter	Preparatory phase (I)		Pre-competitive phase (II)		Transitional phase (IV)	
	r	P	r	P	r	P
Glucose/TBARS (s)	-0.76	0.0287*				
Glucose/TBARS (w)	-0.72	0.0442*				
Insulin/TBARS (s)	-0.81	0.0154*				
HOMA IR /TBARS (s)	-0.89	0.0024**				
HOMA IR/TBARS (w)	-0.90	0.0022**				
LA/TBARS (w)			0.72	0.0438*		
Visfatin/Insulin (s)	-0.70	0.0251*				
Visfatin/ Resistin (s)	-0.68	0.0289*				
Visfatin/ BMI (s)					-0.89	0.0006**
Resistin/HOMA IR (s)					-0.66	0.0368*

*s - at rest, w - post-exercise; ** $p \leq 0.01$, * $p < 0.05$*

Tukey post-hoc test for equal samples was further used to compare the individual pairs of means.Arithmetic means of abnormally-distributed parameters for training phases analyzed were compared with non-parametric Friedman's analysis of variance with a respective post-hoc test.

Associations between pairs of normally-distributed variables were tested with Pearson's coefficient of linear correlation, whereas Spearman's coefficients of correlation were calculated for those pairs where at least one variable distribution was abnormal.

Results

The results of this study are summarized in 5 tables. Mean values of anthropometric characteristics are presented in Table 2. It should be noted that no significant differences were observed between the mean values of BMI and WHR analyzed throughout the annual training cycle. During the pre-competitive phase, the body fat content of participants was significantly decreased compared to other time periods analyzed. Moreover, there were significant differences in body water content when comparing phase I and II to phases III and V.

Table 3 presents mean values of physiological and physical parameters obtained during the Wingate test and peak oxygen uptake in beach volleyball players during their annual training cycle. Analysis of variance did not reveal significant differences in the mean values of such

parameters as post-exercise heart rate, resting and post-exercise diastolic pressure, and peak oxygen uptake. In case of the remaining parameters, significant differences were observed between values determined throughout the annual training cycle. The most significant differences in the analyzed parameters centered around phase I and II (preparatory and pre-competitive phases) when compared to other phases of the annual training cycle. Moreover, there were no significant differences observed between most parameters determined in phases I and V (preparatory phases). Significant variability was observed regarding heart rate at rest, while no changes were noted in post exercise pulse values. Similar findings pertained to resting and post exercise values of blood pressure. The highest values of maximal power were registered during the transitional phase (1648.0±254.90W), whereas average power values were highest in the competitive phase (746.3±68.37 W). The time at peak power, along with the anaerobic fatigue index, were highest during the transitional phase. The overall work performed by the volleyball players was greatest (24.9±2.28 KJ) during the competitive phase of their training cycle. It should be noted that no significant differences in peak oxygen uptake were observed amongst consecutive phases of the annual training cycle. The values of this parameter were highest during the pre-competitive phase (56.3±8.20 ml·kg^{-1}·min^{-1}) and lowest during the first preparatory phase (49.7±7.37 ml·kg^{-1}·min^{-1}). During the competitive

phase, the Wingate test resulted in the lowest blood lactate concentration (8.3 mmol/L).

Mean values of the tested biochemical parameters along with their standard deviations and analysis of variance are summarized in Table 4. According to data presented in this table, post-exercise resistin concentrations were the only parameter whose significant differences were not noted during the annual training cycle. For some parameters, mainly post-exercise glucose, resting resistin concentrations and HOMA$_{IR}$ values, the differences were observed at a confidence level of 5%. In case of the remaining parameters, analysis of variance revealed significant differences at $p \leq 0.01$.

Significant differences in glucose concentrations at rest were observed between phase V when compared to phases II, III, and IV. Significant differences in resting insulin levels were noted between phases IV and V when compared to phase III. In case of post-exercise insulin values, significant differences were observed between phases I and IV, and between phase II and phase IV.

Resting visfatin concentrations differed significantly between phases I and II, II and III, IV and V, as well as between phases III and V and IV and V.

Both resting and post-exercise concentrations of lipid peroxidation products (TBARS) differed significantly when comparing phase I to other phases of the training cycle. The resting value of total plasma antioxidative status in phase V was significantly different compared to phases II and IV, whereas post-exercise values of this parameter differed significantly between phase I and II or IV, and between phase V and II or IV.

As shown in Table 5, significant inverse correlations were noted during phase I of the training cycle between levels of lipid peroxidation and carbohydrate metabolism parameters or of the insulin resistance index. Significant inverse correlations were also noted between visfatin and insulin concentrations, and between visfatin and resistin concentrations. During phase IV (transitional phase), significant inverse correlations were observed between visfatin concentrations and BMI, as well as between resistin concentrations and the insulin resistance index. Moreover, positive correlations were found between post-exercise concentrations of lactate and lipid peroxidation products during the pre-competitive phase. Besides the aforementioned ones, no significant correlations were observed during the remaining phases of the annual training cycle.

Discussion

The purpose of sports training is to induce morphologic and physiologic changes in order to increase physical efficiency, thereby improving exercise ability and reducing fatigue. These aforementioned changes refer in particular to aerobic and anaerobic efficiency and are illustrated by the results summarized in Table 3. The training loads during preparatory and pre-

competitive phases (Table 1) were characterized by the predominance of aerobic work which could be reflected by changes in body composition (Table 2). In reference to anaerobic capacity (which was evaluated using the Wingate test), the most favorable results were observed during the competitive phase (Table 3), as confirmed by the highest average power and highest total work. However, the time at peak power was highest during the transitional phase, similar to value of peak power. The resting heart rate of the studied athletes was lowest during the competitive phase when compared to the other periods, being another indicator of the cardiovascular system's growing adaptation to exercise. It should be noted that the analyzed volleyball players were characterized by a relatively high maximal oxygen uptake (Table 3).

Although exercise metabolism of muscle cells plays a significant role in the muscle's ability to perform work, recent studies have concentrated on other factors limiting exercise ability. One group of such factors are reactive oxygen species generated during physical exercise (Cazzola et al, 2003; Franzoni et al, 2005), and another – adipokines that participate in carbohydrate metabolism (O'Reilly et al, 2010) and in inflammatory reactions.

Regular physical exercise increases insulin sensitivity since it stimulates the muscular uptake of glucose related to muscular contraction (Santos et al, 2008). It is also associated with an increased expression and translocation of GLUT-4

glucotransporters and their coding mRNA, and with the enhancement of signal cascade element expression such as substrate-1 of the insulin receptor and the receptor itself (Holten et al, 2004; McGee and Hargreaves, 2006). Our previous studies revealed that regular physical exercise increases the affinity of insulin receptor to its agonists (Szcześniak et al, 1998). During each phase of this study (with the exception of the third, i.e. competitive phase), the values of $HOMA_{IR}$, fit within the range proposed by Matthews (1985). During the third (competitive) phase, however, the insulin resistance index was above this reference threshold (Table 4). This increase in the insulin resistance index resulted from muscle injuries which took place during competition. Volleyball matches are characterized by numerous myofibrillar injuries, which induce weakened insulin signaling and decrease transmembrane transport of glucose. Since skeletal muscles are responsible for about 85% of post-exercise glucose uptake, their injuries induce a decrease in insulin sensitivity (Baron et al, 1988). Increased insulin resistance in volleyball players during the competitive phase compared to the training period was described by Kuo et al, (2006). In this paper, the authors compared the curve of glucose tolerance after one week of matches with the respective curve after one week of volleyball training. An increase in insulin resistance was observed after the competitive phase and accompanied by higher activities of creatinine

kinase – an enzyme which is an indicator of muscle injury.

In our study, resting blood insulin concentrations during the competitive phase were highest when compared to other phases (Table 4), confirming the findings of Kuo et al, (2006). It should be noted that in our study, however, both glucose and insulin blood concentrations fit within respective reference limits in each studied phase (Table 4).

Studies on resistin and visfatin dealt mainly with their roles in the reduction of obesity-related insulin resistance. Visfatin has been shown to have insulin-mimetic activity, since binding to insulin receptor causes phosphorylation of protein tyrosine kinases Akt (protein kinase B) and MAPK (mitogen-activated protein kinase), with no influence on insulin binding itself (Fukuhara et al, 2005). In this study, resting visfatin concentrations determined during the pre-competitive phase were twice as high as during the first and second preparatory phases and nearly three times higher when compared to the competitive and transitional phases (Table 4). The test of maximal power was reflected by a decrease in the blood concentration of this compound in each of the study periods (Table 4). This data is consistent with the results obtained by Jürimäe et al, (2009), who analyzed the effects of 2-hour low intensity exercise on visfatin concentrations in rowers. They observed a significant decrease in visfatin concentrations compared to baseline values at rest and to

controls, 30 minutes after exercise testing was completed. Further research is needed to explain these findings.

The role of resistin in modulating insulin resistance is still controversial (Flier, 2001; Smith and Ravussin, 2002). It has been suggested that resistin plays a role in inflammatory reactions since its over-expression has been observed in human macrophages (Patel et al, 2003). Additionally, a study by Reilly et al, (2005) revealed a relationship between resistin concentration and levels of inflammatory factors: IL-6 and C-reactive protein (CRP).

Resistin concentrations at rest ranged from 7.4±1.64 to 10.1±2.76 ng/ml. After exercise they ranged from 8.1±1.55 to 9.6±3.06 ng/ml (Table 5). The highest values were measured during the competitive phase of the training cycle, i.e. at the highest exercise overload. However, exercise test was shown to have no significant effects on resistin concentrations, irrespective of training cycle phase. The increase in resistin concentrations observed during the competitive phase may be related to excessive overload, resulting in the expression of proinflammatory cytokines. Ostrowski et al (1999), showed that intense physical exercise induces a 2 to 3 fold increase in TNF-α and IL-1β levels, while Pedersen and Hoffman-Goetz (2000) revealed that it may modulate dramatic increases in IL-6 levels.

Statistical analysis showed a significant inverse relationship between concentrations of visfatin and insulin (p<0.05) and between visfatin

and resistin (p<0.05) during the preparatory phase of the training cycle. During the transitional phase, significant inverse associations were noted between resistin concentrations and $HOMA_{IR}$ (p<0.05) and between visfatin concentrations and BMI (p≤0.01) (Table 5). These findings are in opposition to the results obtained by Perseghin et al (2006) who did not find any correlations in runners with a high sensitivity to insulin. Discrepancies between our results and those published by Perseghin et al (2006) also include differences in blood resistin concentrations and values of the insulin resistance index (WBISI). We used $HOMA_{IR}$ to determine the levels of insulin resistance in our study, while the aforementioned authors calculated WBISI using formula proposed by Matsuda and DeFronzo (1999). Resistin concentrations observed across the annual training cycle ranged from 7.4±1.64 to 10.1 ±2.76 ng/ml in our study, whereas Perseghin et al (2006) claimed a mean value of 4.5±1.6 ng/ml in elite runners. Although one original article claimed that there was an association between resistin concentration and insulin resistance in obese rodents (Steppan et al, 2001), further studies by Perseghin et al (2006) and Utzschneider et al (2005) did not find similar relationships in humans. Our study showed an inverse correlation between resistin concentrations and glucose tolerance, which may result from the fact that our study group was comprised of healthy, lean players. We also observed an inverse correlation between resistin and visfatin concentrations,

along with correlations between visfatin concentrations and insulin levels or BMI. Other authors, such as Rubin et al, (2008), showed that a significant correlation exists between peak oxygen uptake and resistin concentration (r=-0.37, p<0.05) and between resistin concentration and $HOMA_{IR}$ (r=0.29, p<0.05) in boys 10 to 14 years of age with elevated body weight. Similar associations were found by Tokuyama et al (2007) in their study on Japanese women with type 2 diabetes.

Finaud et al (2006) and Margonis et al (2007) proved that preparatory, pre-competitive and competitive training phases are characterized by increased levels of oxidative stress and decreased efficiency in antioxidative protection. These phenomena were proven by our results (Table 4). During the preparatory and pre-competitive phases of the training cycle, the highest increase in lipid peroxidation products was observed after the exercise test, while resting concentration of TBARS was highest compared to all other periods in the annual training cycle. It is worth noting that resting concentrations of this product were lowest during the competitive phase, differing significantly when compared to the other phases of the annual training cycle. This phenomenon is in accordance with a previously described training-related adaptation of antioxidative protection mechanisms in the athletes body. Marzatico et al (1999) suggested that contrary to prolonged exercise, lower levels of NADH and NADPH synthesis are responsible for the accumulation of ROS generating substrates

during anaerobic exercise. Increases in pro-oxidative changes in response to higher intensities of exercise were proven by other authors (Aslan et al, 1998; Goto et al, 2003). In the current study, significant inverse correlations were observed during the preparatory phase of the training cycle between TBARS concentrations and glucose/insulin concentration pre- and post-exercise testing, and between TBARS and HOMA$_{IR}$ (Table 5). These findings are characteristic for insulin receptor kinase activation. This process results from the stimulation of NADPH oxidase due to ligand/receptor reaction and subsequent synthesis of hydrogen peroxide. The latter in turn causes auto-phosphorylation and activation of insulin receptors (Schmidt et al, 1999). Multiple linear regression analyses revealed that binding of radio-labeled ^{125}I-insulin to its receptor in highly trained volleyball players is determined by TBARS concentration (D=34.57%) (Szcześniak et al, 1998).

The supra-maximal test performed by our subjects caused changes in plasma antioxidant levels which may be interpreted as an adaptive response to high intensity exercise (Table 4). Schneider et al (2005) revealed that an increase in plasma antioxidant levels was associated with exercise only at the highest of intensities. Other authors have also noted similar relationships (Vider et al, 2001). Increases in plasma antioxidant levels following intensive exercise are probably related to translocation of these compounds from the tissues into the plasma. Duthie et al (1990) and Aslan et al (1998) showed that increases in plasma concentrations of uric acid were responsible for the highest increase in total anti-oxidative status.

In conclusion, this study revealed that:
- insulin resistance occurs during the period of highest training loads (i.e. competitive phase),
- levels of lipid peroxidation products are inversely correlated with the insulin resistance index and resistin concentrations,
- blood visfatin concentrations in highly trained volleyball players are reduced by supra-maximal exercise and inversely correlated with insulin and resistin concentrations and BMI
- resting and post exercise resistin concentrations remain relatively unchanged throughout consecutive phases of the annual training cycle.

Conclusion

The physical training that beach volleyball players are subjected to during the annual cycle does not affect resistin levels but influences insulin, glucose, and visfatin concentrations, along with the markers of pro-oxidant/antioxidant   balance.

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