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Effects of Tapering for Competition on MicroRNA and Performance and Physical Skills for Female Handball Players.

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Abstract

Some of the physiological changes associated with the taper and their relationship with athletic performance are known since 1960s. Since the 1980s numerous studies have examined various physiological responses associated with the cardiorespiratory, metabolic, hormonal, neuromuscular and immunological systems during the pre-event taper across a number of sports but there were not a clear results obtained. Recently, miRNAs have been shown to play an important role in the regulation of muscle development. miRNA-1 and miRNA-206 are expressed in cardiac and skeletal muscle and are transcriptionally regulated by the myogenic differentiation factors. Purpose of this study was to investigate miRNAs 1 & 206 in response to training handball program followed by tapering for 5 days. Results are compared to some physical and skills performances in fourteen handball female players constitute Helwan University handball team. Results showed significant increased levels in both miRNAs due to both of training program and tapering with improved performance in physical and skills properties. It is concluded that tapering enhance miRNAs 1 & 206 transcription that enhance muscle differentiations and proliferation leading to improved performance.

Introduction:

It is well established that sports players should follow a tapering to increase performance, which is a period of progressively reduced training load before an important competition **(Bosquet et al., 2007).** Positive changes of psychometric, physiological, and performance-related parameters have been observed during the tapering period (**Hooper et al., 1999; Mujika et al., 1996; Papoti et al., 2007).** Performance improvement is probably achieved by an appropriately planned maintenance of intensity and reduction of the training load **(Mujika and Padilla, 2003).**

The tapering is a reduction in the training load of athletes in the final days before important competition, with the aim of optimizing performance. This reduction of the training load can be achieved through the alteration of several components, including the training volume, intensity, and frequency **(Wenger and Bell, 1986),** as well as the pattern of the tapering (i.e., progressive or step tapering) and its duration **(Mujika and Padilla, 2003).** The tapering is widely used by athletes participating in a wide range of sports differing in their biomechanical and physiological demands to gain a performance edge over competitors. In fact, significant improvements have been reported after tapering for runners **(Houmard et al., 1994),** swimmers **(Mujika et al., 2002),** cyclists **(Neary et al., 2003)**, rowers **(Jurimae et al., 2003)**, and triathletes **(Margaritis et al., 2003).** Many strategies to decrease the training load have been reported in the tapering literature, most of them leading to an improvement in performance and/or its physiological correlates **(Mujika, 1998 & Luden et al., 2010).**

Tapering is a strategy typically implemented by athletes in the days and weeks leading up to a competition of emphasis. It is defined by a systematic reduction of training load **(Margaritis et al., 2003).** Tapering yields physiological and psychological benefits that together improve performance by approximately 2–8%. Evidence of tapering practice prior to competitive sports was eradicated since mid-1960s, but it was not until the 1980s that scientists began to examine the underlying physiology. Since then, a considerable amount of studies has been undergone on swimmers, cyclists and runners. These studies have predominantly targeted cardiovascular, metabolic, and neuromuscular parameters in an attempt to identify the physiological factors supporting the ergogenic effect of tapering **(Luden et al., 2010).**

However, the reduction of total training volume was notably accompanied by an increase in the amount of high-intensity training, which appears to have provided a unique training stimulus rather than the desired recovery. In general, no obvious physiological, biochemical or psychological changes have been discovered to upgrade athletes' performance after tapering.

Recently, microRNA molecules (miRNAs) have been identified as essential intracellular mediators of processes inherent in exercise adaptation including angiogenesis **(Zhang, 2010),** inflammation **(Davidson-Moncada *et al.* 2010),** mitochondrial metabolism **(Chan *et al.* 2009; Dang, 2010),** cardiac/skeletal muscle contractile force generation, and tissue hypertrophy **(Davidsen *et al.* 2011).**

These short single stranded miRNAs are incorporated into the RNA-induced silencing complex (RISC) which regulates protein expression (**Wessner et al., 2011)**.

The discovery of microRNAs (miRNAs) provides a new avenue that will extend our knowledge of factors controlling skeletal muscle function during tapering periods. miRNAs may also improve our understanding and application of current tapering approaches as well as enable the identification of new identified strategies and targets aimed at maintaining and/or improving skeletal muscle health before competitions

In response to increased use through regular exercise training, skeletal muscle can increase its size and capacity to produce force **(Fry, 2004),** improve its resistance to fatigue and enhance its oxidation of carbohydrates and fats **(Coyle, 2000).**

A suite of miRNAs, highly enriched in cardiac and/or skeletal muscle (referred to as myomiRs), has recently been identified and include miR-1, miR-133, miR-133a, miR-133b, miR-206, miR-208, miR-208b, miR-486 and miR-499 **(McCarthy & Esser, 2007; Callis *et al.* 2008 and van Rooij *et al.* 2009).** miR-1 stimulates differentiation through its direct inhibition of HDAC4, an inhibitor of differentiation, while miR-206 indirectly inhibits helix–loop–helix, the latter, a repressor of Myo-development.

Up to now it is unproven whether miRNAs are involved in tissues repair processes within skeletal muscle tissues after acute bouts of training sessions but a small number of striated muscle-specific miRNAs so called MyomiRs have been identified and shown to have an important role in myogenesis, muscle growth and cardiac function and hypertrophy **(Zhang et al., 2010).** miR-206, a muscle-specific miRNA that is up-regulated by exercise in the intracellular space **(Nielsen *et al.*2010)** may be involved in tissue repair and adaptation.

Data defining circulating micro-RNA (c-miRNA) behavior in the settings of acute exercise bouts and sustained exercise training in healthy humans are lacking. Identification of miRNAs specifically regulated by exercise could reveal unique biomarkers of exercise physiology and would lend significant insight into the molecular control of exercise adaptation.

The purpose of this study was to assess miR-1 and miRNA-206 concentrations in handball athletes at rest and after a proposed handball game after 8 weeks period of training program followed by five days of tapering. Results will be correlated to some handball performance skills before and after tapering.

Material and Methods

Subjects:

Fourteen female handball players from the Helwan University team constituted subjects of this study. Written informed consent was obtained from all participants according to the ethical approval for this study conformed to the standards of the Declaration of Helsinki. The Helwan University review board approved the protocol before study initiation. Height, weight and age were recorded for all investigated subjects. All participants were required to abstain from non-steroidal anti-inflammatory use for at least 7 days prior the experimental study day.

Training protocol:

The subjects trained for 8 weeks as 3 days/week in a handball training program. In the first four weeks, training session were performed once daily but raised to be twice daily in the second four weeks. Each training session consisted of 20 min. warm up followed by 60 – 80 min. of specific handball skills training program under specialized coach supervision, then positive cool down for another 10 min. Proposed handball game was played at the days of sampling. Then tapering sessions were applied to the players as decreasing in training intensity by about 20% and sessions performed as once daily again for five days. Then, an official game was played for the second sampling collection.

Plasma sampling:

Five milliliters of blood was collected under aseptic conditions in standard anticoagulant (EDTA)-treated vacutainer tubes at baseline (prior to exercise testing) and immediately post-exercise testing. All blood samples were centrifuged at 2000 *g* for 10 min to pellet cellular elements immediately after each blood draw. The supernatant plasma was then separated and immediately frozen at –80◦C till PCR assay.

miRNA extraction:

Total RNA extraction was performed using a MicroRNA Extraction Kit (Shanghai ShineGene Molecular Biotech, Inc., Shanghai, China). Quantitative efficacy and reproducibility of c-miRNA extraction was confirmed by extraction via serial dilutions.

Quantification of microRNA expression:

To quantify levels of select c-miRNA, standard reverse transcription-quantitative (real time) polymerase chain reaction (RT-PCR) was utilized. Specifically, reverse transcription was performed to generate cDNA representing levels of mature c-miRNA molecules.

Fold-change of both miRNA 1 and miRNA 206 species were calculated using the formula specified by the manufacturing company. Specifically, in this formula, *C*t represents the 'real time' cycle number at which the increase in miRNA probe fluorescence is exponential. As reference control for miRNA quantification, *C*t values were then subtracted from *C*t values obtained from exogenously added miR-1 or miR-206, as described above. *Ct values were then compared (ΔC*t) with each athlete's own resting baseline at the pre-tapering time point (normalized to fold-change of 1).

Handball skills Measurements:

Handball skills were assessed before and after the program and after tapering period as follow:

Statistical analysis:

A Computer program (SPSS version 17) was used to analyze obtained results. Student's Mean, standard deviation of players and ANOVA test were used to examine the difference between baseline, after program and after tapering measurements before and after the training sessions. Probability was assigned significant at ≤ 0.05.

Results

There were no apparent differences between all athletes in the anthropometric variables including weight, height, age and training age (Table 1) since both skewness and kurtosis ranged between ± 3.

Table 1
Anthropometric parameters homogeneity

	Age	Height	T age	Weight
N	14	14	14	14
Mean	19.29	166.21	12.07	66.18
Std. Deviation	.91	2.58	1.00	1.87
Skewness	.04	.17	-.16	.03
Kurtosis	-.65	-1.13	.75	-.83

Handball training program lead to increased skills with remarkable significant difference in results ($p < 01$) of all skills. Results before program compared to that obtained after program and tapering effects are represented in table (2) and (2-b).

Table 2
Skills data parameters (Mean ± SD)

	Before Program		After Program		After Tapering	
	Mean	± SD	Mean	± SD	Mean	± SD
dripping	17.17	1.29	16.52	1.10	18.48	1.46
high jump pointing	7.50	1.09	8.21	.70	6.07	1.14
long jump pointing	3.02	.46	3.48	.38	2.18	.44
passing	30.00	1.75	31.07	1.69	24.21	2.52
side defense steps	6.67	.69	6.27	.76	7.86	.83

Of course tapering – as expected- improved all skills more than that of program ending that of high intensity training sessions (Table 2-B).

(Table 2-B)
Skills ANOVA test

		Sum of Squares	df	Mean Square	F	p
Dripping	Between Groups	28.009	2	14.004	8.372	.001
	Within Groups	65.234	39	1.673		
	Total	93.243	41			
high jump pointing	Between Groups	33.333	2	16.667	16.759	.000
	Within Groups	38.786	39	.995		
	Total	72.119	41			
long jump pointing	Between Groups	12.208	2	6.104	33.513	.000
	Within Groups	7.104	39	.182		
	Total	19.312	41			
Passing	Between Groups	381.000	2	190.500	46.643	.000
	Within Groups	159.286	39	4.084		
	Total	540.286	41			
side defense steps	Between Groups	19.068	2	9.534	16.426	.000
	Within Groups	22.637	39	.580		
	Total	41.705	41			

More over, all physical parameters showed highly significant differences after program compared to that before programs. Also, tapering more increased physical efficiency for all players with significant differences compared to that obtained after program (Table 3 & 3-B).

(Table 3) Physical data parameters (Mean ± SD)

	Before Program	After Program	After Tapering
Accuracy	21.1 ± 2.38	28.28 ± 2.33	29.42 ± 2.10
Agility	18.28 ± 0-68	19.27 ± 0.54	21.77 ± 0.47
Compatibility	16.34 ± 1.23	15.06 ± 0.78	14.27 ± 0.57
Elasticity	45.89 ± 5.56	42.30 ± 4.41	40.10 ± 4.39
velocity	6.44 ± 038	5.70 ± 0.47	5.39 ± 0.42
Right arm power	25.85 ± 2.14	29.07 ± 1.94	30.42 ± 1.65
Left arm power	21.21 ± 1.93	23.33 ± 1.29	24.67 ± 1.20
Legs power	66.53 ± 3.38	69.21 ± 3.04	70.42 ± 2.93
arms muscle capacity	17.38 ± 1.48	25.21 ± 2.15	26.71 ± 2.16
legs muscle capacity (A)	24.07 ± 1.90	28.46 ± 5.06	29.75 ± 4.39
legs muscle capacity (B)	2.12 ± 0.26	2.79 ± 2.04	3.35 ± 1.95
muscle endurance (Abdomen)	22.42 ± 2.46	29.5 ± 3.90	30.92 ± 3.77
muscle endurance (arms)	23.71 ± 2.52	30.92 ± 3.22	33.07 ± 3.27
muscle endurance (legs)	21 ± 2.66	26.64 ± 3.75	27.42 ± 4.07
psychological endurance	4.22 ± 0.49	3.52 ± 0.44	3.32 ± 0.43

(Table 3-B)
Physical ANOVA test (Before program, after program & after tapering)

		Sum of Squares	df	Mean Square	F	p
accuracy	Between Groups	233.029	2	116.515	23.349	.000
	Within Groups	194.614	39	4.990		
	Total	427.643	41			
agility	Between Groups	24.368	2	12.184	21.010	.000
	Within Groups	22.617	39	.580		
	Total	46.985	41			
arms muscle capacity	Between Groups	108.535	2	54.267	26.996	.000
	Within Groups	78.397	39	2.010		

		Sum of Squares	df	Mean Square	F	p
	Total	186.931	41			
compatibility	Between Groups	12.984	2	6.492	3.787	.031
	Within Groups	66.860	39	1.714		
	Total	79.844	41			
elasticity	Between Groups	142.000	2	71.000	2.676	.081
	Within Groups	1034.696	39	26.531		
	Total	1176.696	41			
Left arm power	Between Groups	76.264	2	38.132	10.150	.000
	Within Groups	146.521	39	3.757		
	Total	222.786	41			
legs muscle capacity A	Between Groups	190.452	2	95.226	17.883	.000
	Within Groups	207.667	39	5.325		
	Total	398.119	41			
legs muscle capacity B	Between Groups	6.938	2	3.469	25.115	.000
	Within Groups	5.387	39	.138		
	Total	12.324	41			
Legs power	Between Groups	131.936	2	65.968	7.153	.002
	Within Groups	359.665	39	9.222		
	Total	491.601	41			
muscle endurance (Abdomen)	Between Groups	594.302	2	297.151	22.133	.000
	Within Groups	523.603	39	13.426		
	Total	1117.905	41			
muscle endurance (arms)	Between Groups	683.460	2	341.730	38.501	.000
	Within Groups	346.159	39	8.876		
	Total	1029.619	41			
muscle endurance (legs)	Between Groups	375.136	2	187.568	15.908	.000
	Within Groups	459.840	39	11.791		
	Total	834.976	41			
psychological endurance	Between Groups	5.717	2	2.859	13.035	.000
	Within Groups	8.553	39	.219		
	Total	14.270	41			
Right arm power	Between Groups	147.312	2	73.656	19.012	.000
	Within Groups	151.093	39	3.874		
	Total	298.405	41			
velocity	Between Groups	7.153	2	3.576	17.225	.000
	Within Groups	8.097	39	.208		
	Total	15.250	41			

Although miRNA 206 levels increased numerically after the training program in resting, there were no significant differences in results. Reverse to miRNA 206 results, miRNA 1 results were significantly increased in all stages of the training sessions including tapering. Effort results are increased after program with significant increase in ratio of miRNA levels indicating adaptation of skeletal muscles to the training program with remarkable increase after tapering too (Table 4 & 4-B).

(Table 4 & 4-B)
Difference between microRNA- 1 & 206 levels for investigated subjects
(before training program compared to after program & after tapering)

	Mean ± SD		
miRNA 1 (U/ml)	Before program	After program	After tapering
rest	433.1 ± 88.41	599.9 ± 62.96	622.2 ± 112.83
effort	478.2 ± 68.09	750.7 ± 59.25	852.8 ± 116.73
ratio change due to effort	1.12 ± 0.08	1.39 ± 0.15	1.26 ± 0.14
miRNA 206 (U/ml)	Before program	After program	After tapering
rest	654.8 ± 64.98	684 ± 70.41	684.2 ± 74.86
effort	685.5 ± 72.55	783.2 ± 95.35	761.5 ± 49.51
ratio change due to effort	1.047 ± 0.06	1.148 ± 0.11	1.12 ±0.07

(Table 4-B)
ANOVA test for miRNA levels investigated in handball players

		Sum of Squares	df	Mean Square	F	Sig.
miRNA 1 rest	Between Groups	213594.467	2	106797.233	13.072	.000
	Within Groups	220595.400	27	8170.200		
	Total	434189.867	29			
miRNA 1 effort	Between Groups	750019.400	2	375009.700	51.670	.000
	Within Groups	195959.300	27	7257.752		
	Total	945978.700	29			
miRNA 1 ratio	Between Groups	.365	2	.183	11.479	.000
	Within Groups	.430	27	.016		
	Total	.795	29			
miRNA 206 rest	Between Groups	5723.467	2	2861.733	.581	.566
	Within Groups	133053.200	27	4927.896		
	Total	138776.667	29			
miRNA 206 effort	Between Groups	52640.600	2	26320.300	4.698	.018
	Within Groups	151266.600	27	5602.467		
	Total	203907.200	29			
miRNA 206 ratio	Between Groups	.054	2	.027	3.910	.032
	Within Groups	.188	27	.007		
	Total	.242	29			

Discussion

The present study was proposed to study effects of both handball training program followed by tapering period on plasma miRNA-1 & 206 and on progress of handball skills after eight successive weeks followed by five days of tapering.

Discussing the purpose of this study was to assess the effects of the alterations of taper components on performance in handball athletes. In accordance with previous suggestions **(Bosquet et al., 2007),** it is found that maximal gains are obtained with a tapering intervention of five days duration, where the training volume is exponentially decreased by about 20%, without any modification of training regularity. In agreement with previous suggestions **(Houmard and Johns 1994 & Mujika and Padilla 2003),** this study has confirmed that performance improvement was sensitive to the reduction in training volume. Maximal performance gains are obtained with a total reduction in training volume of 41–60% of pre-taper value. Training volume can be altered through the decrease of the duration of each training session and/or the decrease of training frequency.

(Mujika et al., 2004) From a neuromuscular perspective, the taper usually results in markedly increased muscular strength and power, often associated with performance gains at the muscular and whole body level. Oxidative enzyme activities can increase, along with positive changes in single muscle fiber size, metabolic properties and contractile properties. Limited research on the influence of the taper on athletes' immune status indicates that small changes in immune cells, immunoglobulins and cytokines are unlikely to compromise overall immunological protection.

The pre-event taper may also be characterized by psychological changes in the athlete, including a reduction in total mood disturbance and somatic complaints, improved somatic relaxation and self-assessed physical conditioning scores, reduced perception of effort and improved quality of sleep. These changes are often associated with improved post-taper performances expressed in this study.

Mujika , 2010 suggested that training at high intensities before the taper plays a key role in inducing maximal physiological and performance adaptations in both moderately trained subjects and highly trained athletes. High-intensity training can also maintain or further enhance training-induced adaptations while athletes reduce their training before a major competition. On the other hand, training volume can be markedly reduced without a negative impact on athletes' performance. Therefore, the training load should not be reduced at the expense of intensity during the taper. Intense exercise is often a performance-determining factor during match play in team sports, and high-intensity training can also elicit major fitness gains in team sport athletes. A tapering and peaking program before the start of a league format championship or a major tournament should be characterized by high-intensity activities.

Results of this study revealed significant effect of tapering on plasma miRNA 1 & 206 which is the first to be obtained in athletes. Hence, it is a pioneer study to prove why tapering increase athlete performance empirically on the basis of molecular biology.

Following an acute bout of resistance exercise, performed by both young and older men, pri-miR-1 and pri-miR-133a were reduced in muscle biopsy samples taken from the young subjects 6 h post exercise **(Drummond *et al.* 2008).** In contrast, pri-miR-206 was increased at 3 and 6 h post exercise in the older and young subjects, respectively **(Drummond *et al.* 2008).** Of the mature miRNAs, only miR-1 was reduced at 3 and 6 h post exercise in the older and young subjects, respectively; no changes in miR-206 were observed.

Following functional overload-induced hypertrophy, primary miR-1, and -206 are increased. In contrast, the mature forms of miR-1 are decreased while miR-206 is unchanged. There is also an increase in several growth-related genes that are predicted targets of these miRNAs **(Guller and Russell, 2010).** An injection of a cocktail of miRNAs including miR-1, -133 and -206 into injured muscle site enhanced muscle regeneration and prevented fibrosis **(Nakasa *et al.* 2009).** These results suggested a role of miR-1, and -206 in tissue repair after acute exercise training sessions and probably participate in tissue enhanced performance at tapering periods.

RNA interference (RNAi) is a natural process that cells use to turn down the activity of specific genes. In conjunction with this, MiRNAs, or microRNAs, are endogenous triggers of RNAi which have been shown to have essential roles in developmental processes including in skeletal muscle **(Sibley and Wood, 2011; Mishra and Bertino, 2009).** A key feature of the RNAi and miRNA mechanism is sequence specificity.

These studies show that exercise is capable of regulating miRNA levels. It will be important to identify which exercise-induced pathways alter miRNA expression and how miRNA regulation contributes to the physiological adaptations to exercise. Investigations that identify the miRNAs responsible for exercise-induced adaptations, or which can mimic some exercise-induced adaptations, will significantly advance the miRNA–muscle field.

At the end, some questions are arising from this study although they were not included in the intellectual consideration at the beginning. First, miRNAs are small, non-coding RNAs that they can be synthesized in vitro so easy. Hence, they can be used as doping before further investigation required for safety. Do athletes will wait? Second, they are highly metabolized, and then if they are used as doping, they will be discovered? Also, Are Scientists will administrate miRNAs to embryos to get genetically assessed babies? This means many studies are required in this field to answer such questions.

Conclusion

The purpose of this investigation was to assess the effects of taper components on performance in handball athletes, through assessment of miRNAs 1 & 206 plasma levels. A 5 days taper during which training volume is exponentially reduced by 20% without altering training intensity appears to be efficient strategy to maximize performance gains. This study provides a recent data about tapering effect on miRNAs 1 & 206 that can be useful for athletes, coaches and sport scientists to optimize their tapering strategy. Future investigations should evaluate alternative and innovative tapering strategies exclusive to those included

in this study, which could prove to provide further performance benefits to athletes.

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