

The effects of ketogenic diet on oxidative stress and antioxidative capacity markers of Taekwondo athletes

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The purpose of this study was to investigate the effects of the ketogenic diet through 3 weeks on oxidative stress and antioxidative capacity markers in Taekwondo athletes. The participants selected for this research were 18 high school taekwondo contestants aged 15-18 who had at least 5 yr of career as contestant. The subjects were randomly assigned to the ketogenic diet (KD) group and the Non ketogenic diet (NDK) group. Body composition and oxidative stress and antioxidative capacity markers (LDH, MDA, ROS, HDL, and SOD) were analysed before and after 3 weeks of ketogenic diet. No significant difference was found between the groups in body composition, ROS and SOD level.

The KD group showed an elevated HDL level and NDK group showed an elevated LDH and MDA level after ketogenic diet by 3 weeks. This result suggests that weight loss by 3 weeks of calorie restriction and exercise can cause oxidative stress, and that ketogenic diet can be effective for preventing it. It could also be inferred that ketogenic diet can be effective for increasing blood antioxidative capacity.

Keywords: Taekwondo, Ketogenic diet, Oxidative stress, Antioxidative capacity

INTRODUCTION

Contestants participating in a weight class contest must meet a certain weight limit. Since a contestant with a heavier weight usually has relatively stronger muscle power than one with a lighter weight, most contestants with heavier weights attempt to lose weight in abnormal ways so as to take advantage of their stronger muscle power by participating in contests of lower weight classes than their normal weight classes. Most of the weight loss methods used by weight class contestants involve artificially induced short-term dehydration, restricted diet, and severe exercise inducing body fluid loss. It has been reported that such methods might cause increased reactive oxygen species (ROS) production (Anuradha and Balakrishnan, 1998).

ROS, produced by physiological metabolism or external stimuli, are receiving growing recognition as a major factor that induces oxidative stress and thereby causes not only cell and tissue damage

but also accelerated aging and various diseases (including cardiovascular diseases, cancers, Alzheimer's disease and Parkinson's disease) (Fruehauf and Meyskens, 2007; Weinberg and Chandel, 2009). In addition, it was reported that accelerated lipid peroxidation caused by ROS had a positive correlation with skeletal muscle damage (Daekeun et al., 2012).

For this reason, an effective weight loss method has continuously been demanded that does not deteriorate the performance and health of the weight class contestants who definitely need weight loss (Paoli et al., 2012). Meanwhile, Nazarewicz et al. (2007) reported that 14 days of ketogenic diet with dietary restriction involving healthy adult women resulted in weight loss and significant improvement in total antioxidative status, without causing blood oxidative stress. Ketogenic diet is a low carbohydrate and high fat diet that causes the concentration of ketone bodies, which are by-products of fat burning, to become higher than that of glucose in the blood. When such a physiological state, called ketosis,

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is reached, fat begins to be burned fast, fat accumulation stops, and fat becomes the main source of energy metabolism (Pethick and Lindsay, 1982). In the research of Nazarewicz et al. (2007), however, ketogenic diet was conducted without a control group, and therefore it is not clear whether the antioxidant effect was due to ketogenic diet or due to weight loss caused by dietary restriction. There have been some other researches reporting the oxidative stress and antioxidant effect by ketogenic diet, but most of their research subjects were patients with diseases; moreover, their results disagree with each other (Gasior et al., 2006; Jain et al., 1999; Leonardi et al., 2010; Nazarewicz et al., 2007).

Therefore, the present research involved male high school taekwondo contestants to verify the efficacy of ketogenic diet for weight loss by examining the effect of 3 weeks of diet on oxidative stress and antioxidative capacity.

MATERIALS AND METHODS

Participants

The participants selected for this research were 18 high school taekwondo contestants aged 15-18 who had at least 5 yr of career as contestant and who neither had particular medical diseases (such as muscular skeletal diseases, genetic diseases and heart diseases) nor was taking dietary supplements (such as vitamins).

The participants were randomly assigned to 2 groups: 9 of them to the ketogenic diet (KD) group, and the remaining 9 to the non ketogenic diet (NND) group. Their physical characteristics are presented in Table 1.

Experimental procedures

Evaluation of body mass and body composition

Before and after the 3 weeks of experimental diet period, the subjects fasted 12 h. Then, wearing a simple cloth in the morning, they were subjected to the measurement of body composition. In order to evaluate changes in body composition caused by the 3 weeks of KD and NND, respectively, measurements were taken of height, weight and BMI, as well as of %body fat and lean body mass using the electric resistance method.

Table 1. Physical characteristics of the subjects

Group (n=20)	Age (yr)	Height (cm)	Weight (kg)
KD (n=10)	16.22±0.79	174.41±5.30	64.37±5.21
NND (n=10)	16.67±0.82	172.48±6.19	62.51±6.684

Values are means ± SD. KD, ketogenic diet; NND, non-ketogenic diet.

Method and procedure for diet

Before the experimental diet period, every research subject prepared a document stating calorie intake for 3 days, and their daily mean calorie intake was calculated. Then each of the KD and NND groups was provided with a different menu containing 75% of the calculated daily mean calories.

Based on the menu used by Paoli et al. (2012), the menu for the KD group consisted of high fat foods (such as beef, pork, fish, bean, egg and cheese) whose lipid, protein and carbohydrate contents were 55.0%, 40.7%, and 4.3%, respectively; while alcohol, bread, rice, noodles, coffee and tea were restricted. Whereas the NND group was provided with a menu whose lipid, protein and carbohydrate contents were 30%, 30%, and 40%, respectively.

Exercise program

Six days a week for 3 weeks during the weight loss period, both groups participated in a training program that emphasized physical strength improvement. The daily plan of the program consisted of 1 h of low intensity dawn exercise; 2 h of morning exercise, mostly for physical strength improvement; and 2 h of afternoon exercise, mostly for taekwondo skills training.

Blood collection and analysis

Blood was collected from the antecubital vein using 22 gage needles, in the morning of the day before the beginning of the study, and in the morning after the 3 weeks of the study. The collected blood was subjected to analysis of lactatedehydrogenase (LDH), malondialdehyde (MDA), ROS, superoxide dismutase (SOD), high density lipoprotein (HDL). Serum LDH and HDL concentrations were detected by biochemical analyzer (Kodak, USA). Serum ROS levels were determined using an OxiSelect™ *In Vitro* ROS/RNS Assay Kit (#STA-347, CELL BIOLABS, USA). The MDA concentrations in the sera were determined by the Colorimetric Assay, using commercially available BIOXYTECH LPO-586 kit (#21012, Oxis, USA). The SOD concentrations in the sera were determined by the Colorimetric Assay, using commercially available Superoxide Dismutase Assay Kit(#CM706002, IBL-Internationall, Germany). All reagents, dilutions, and calculations were applied according to the manufacturer's instructions. The absorbance was measured at 450 nm with a microplate reader (GENios, TECAN, Austria).

Statistical analyses

Data are presented as means ± standard deviation (SD). The significance of differences among mean values between pre and post-

diet, as well as between KD and NKD groups, were determined by two-way analysis of variance (ANOVA) using SPSS 18.0 for Windows. Statistical significance was set at $\alpha = 0.05$.

RESULTS

Body composition

Changes in body mass and body composition after diet, compared to before diet, by the diet groups are presented in Table 2.

Compared to before the diet period, there was significant de-

crease in weight after the period, as well as in %body fat, fat free mass and BMI ($P < 0.05$). However, no difference was found between the KD and NKD groups, and there was also no effect of interaction between the independent variables.

Oxidative stress and antioxidative capacity markers

Changes in oxidative stress and antioxidative capacity markers after diet during 3 weeks, compared to before diet, by the diet groups are presented in Table 3.

In the cases of ROS and SOD, no significant difference was found

Table 2. Changes in body composition

Variables	KD (n=10)		NKD (n=10)		F-value	
	Pre	Post	Pre	Post		
Weight (kg)	64.37 ± 5.52	60.99 ± 6.65	62.51 ± 7.08	60.11 ± 7.54	G	0.188
					T	50.971*
					G×T	1.460
%Body fat (%)	13.04 ± 3.88	11.90 ± 3.19	10.93 ± 1.36	10.21 ± 2.03	G	2.186
					T	10.420*
					G×T	0.533
Lean body mass (kg)	55.01 ± 3.99	52.72 ± 4.88	54.97 ± 6.90	52.78 ± 8.26	G	0.000
					T	28.782*
					G×T	0.014
BMI (kg/m ²)	21.13 ± 0.98	19.97 ± 1.15	20.94 ± 1.30	20.13 ± 1.44	G	0.000
					T	53.656*
					G×T	1.734

Values are means ± SD. KD, ketogenic diet; NKD, non-ketogenic diet. * $P < 0.05$.

Table 3. Oxidative stress and antioxidative capacity markers

	KD (n=10)		NKD (n=10)		F-value	
	Pre	Post	Pre	Post		
LDH (U/L)	404.89 ± 55.94	399.78 ± 76.39	395.56 ± 32.12	422.00 ± 33.47	G	0.074
					T	2.125
					G×T	4.650*
MDA (pmol/mg)	0.134 ± 0.080	0.131 ± 0.064	0.131 ± 0.07	0.184 ± 0.048	G	0.745
					T	4.171
					G×T	5.316*
ROS (pg/mL)	87.16 ± 30.31	88.84 ± 21.57	77.73 ± 15.89	82.63 ± 30.23	G	0.638
					T	0.241
					G×T	0.057
HDL (mg/dL)	59.21 ± 8.41	73.32 ± 13.15	61.14 ± 6.67	63.81 ± 7.86	G	0.863
					T	25.029*
					G×T	11.646*
SOD (%SOD activity)	50.34 ± 5.32	46.70 ± 3.52	47.98 ± 2.55	47.25 ± 3.1	G	0.518
					T	3.75
					G×T	1.661

Values are means ± SD. KD, ketogenic diet; NKD, non-ketogenic diet. * $P < 0.05$.

between the independent variables. In contrast, in the case of LDH, MDA and HDL interaction effect was found when comparison was made between before and after diet and between diet groups ($P < 0.05$). According to result of the main effect analysis, the KD group showed an elevated HDL level and NKD group showed an elevated both LDH and MDA level after diet compared to before diet ($P < 0.05$).

DISCUSSION

During the last several yr, there have been many research reports that proved the effects of ketogenic diet on short-term weight loss, as well as on metabolic profile with regard to insulin sensitivity, glycemic control and serum lipid values (Nazarewicz et al., 2007; Stafford et al., 2010; Veech, 2004). In addition, Paoli et al. (2012) reported that 30 days of ketogenic diet could decrease the weight and body fat mass of elite artistic gymnasts without affecting their performance. Such research results show that ketogenic diet can be very effective for weight class contestants who need rapid short-term weight loss without negative effect on performance. However, in spite of many reports on the effect of ketogenic diet, there have been rare researches that involved athletic contestants to investigate the effect of short-term ketogenic diet on body mass and body composition.

Therefore the present research involved taekwondo contestants to examine the weight loss effect of 3 weeks of ketogenic diet vs common diet, with calorie restriction. The result showed that 3 weeks of Ketogenic diet and Non ketogenic diet both caused decreases of weight, body fat mass and BMI, and that there was no difference between the 2 groups in the extent of such decreases. In addition, although Paoli et al. (2012) reported that ketogenic diet could increase muscle mass, the result of the present research showed decrease of lean body mass in both groups. Such a difference is considered due to the effect of the 25% calorie restriction and extensive aerobic exercise program that were used for both groups in this research, because preceding researches on ketogenic diet did not involve calorie restriction.

Ketogenic diet leads to the production of ketone bodies, such as β -hydroxybutyrate and acetoacetate, which can be used as an alternative energy source. There is a recent report that ketone bodies potentially decreased ROS production (Bough and Rho, 2007; Gredilla and Barja, 2005). In the present research, however, both of the 2 groups showed no change in ROS after the 3 weeks of diet.

Instead, increases in LDH and MDA were shown by the NKD group after the 3 weeks of diet, but not by the KD group. Oxida-

tive stress caused by exercise can result in damage to skeletal muscle cells, which in turn leads to increase of LDH (Brancaccio et al., 2010). MDA is produced as the result of the degradation of polyunsaturated fatty acid peroxidation products, and it has been used as a traditional oxidative stress index (Rachmilewitz et al., 1976). In addition, increase of HDL was also shown after the 3 weeks of KD diet in the present research. HDL has been known to be a potential antioxidant suppressing the accumulation of oxidized lipids (Vohl et al., 1999). In a research involving healthy women, Nazarewicz et al. (2007) showed that 14 days of ketogenic diet resulted in improved total antioxidative status as well as increased uric acid and HDL levels. They interpreted this result as demonstrating the effect of ketogenic diet on antioxidative capacity. Accordingly, the result of the present research indicates that weight loss by 3 weeks of calorie restriction and exercise can cause oxidative stress, and that ketogenic diet can be effective for preventing it. It could also be inferred that ketogenic diet can be effective for increasing blood antioxidative capacity.

Researches concerning the effects of ketogenic diet on oxidative stress and antioxidative capacity have reported opposite results according to the kinds of the diseases of research subjects, while research involving healthy individuals has been very insufficient. Especially, there has been no research report involving weight class contestants, and additional research would have to be carried out in this regard.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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