

## CLINICAL STUDY

# Association between pentraxin 3 and growth differentiation factor-15 in adolescent male swimmers

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**ABSTRACT**

**BACKGROUND:** The purpose of this study was to evaluate plasma pentraxin 3 (PTX3) and growth differentiation factor-15 (GDF-15) concentrations in adolescent male swimmers and compare any possible interactions with canonical biochemical parameters.

**METHODS:** Twenty-six adolescent male swimmers and 29 gender- and age-matched sedentary controls participated in this study. Fasting blood samples were taken from the participants. Biochemical values and plasma PTX3 and GDF-15 levels were measured.

**RESULTS:** Plasma PTX-3 levels were markedly higher in the adolescent male swimmers than in the sedentary controls ( $378.44 \pm 173.93$  vs  $257.82 \pm 103.20$  pg mL<sup>-1</sup>,  $p = 0.002$ ). There was no significant difference in GDF-15 levels between the two groups ( $186.12 \pm 40.65$  vs  $203.60 \pm 36.77$  pg mL<sup>-1</sup> in the swimmers and the sedentary, respectively,  $P = 0.068$ ). Relationship between PTX3 and GDF-15 was linear.

**CONCLUSIONS:** This is the first study showing that adolescent male swimmers have higher PTX3 levels than sedentary controls and that there is a linear relationship between PTX3 and GDF-15 (Tab. 3, Fig. 2, Ref. 26). Text in PDF [www.elis.sk](http://www.elis.sk).

**KEY WORDS:** adolescent, exercise training, GDF-15, PTX3.

**Introduction**

Epidemiological studies have shown that regular exercise is associated with a decrease in all cause-mortality, especially from cardiovascular diseases (CVD) (1). Regular exercise training also decreases pro-inflammatory factors and increases anti-inflammatory factors (2). Standard cardiac injury biomarkers are useful in monitoring CVD patients, but in athletes they cannot be instructive enough about the cardiovascular risk, because their circulating concentrations do not increase over the pathological limit (3). Therefore, new cardiovascular biomarkers are needed in order to allow a better monitoring of sport performance, prognosis of overtraining and diagnosis of sport-related cardiac injuries.

Pentraxin 3 (PTX3) is a family of multimeric proteins produced by different cell types such as endothelial cells, monocytes/macrophages, skeletal muscle and vascular smooth muscle cells in response to tissue injury or inflammation (4, 5). In some previous studies (6, 7) it has been demonstrated that PTX3 has an anti-inflammatory and/or anti-atherosclerotic role by using

the PTX3-deficient or PTX3-over expressing mice. It has been also reported that a period of endurance or resistance exercise increases the plasma PTX3 levels in healthy young men (8). Miyaki et al (9) demonstrated that plasma PTX3 concentrations were higher in young endurance-trained men than sedentary controls and they also showed that there was a positive correlation between PTX3 and high density lipoprotein cholesterol (HDL-C). In another study (10) they showed that 8 weeks of exercise training induced an increase of plasma PTX3 concentrations in middle aged and elderly women. Additionally, it has been demonstrated that acute exercise induced an increase in plasma PTX3 concentrations, in both obese (11) and non-obese (12) young adults. These findings suggest that PTX3 may participate in the mechanism underlying exercise-induced cardioprotection and PTX3 deficiency may promote vascular inflammation, atherosclerosis and heart damage.

Growth differentiation factor-15 (GDF-15) belongs to the transforming growth factor-beta super family. GDF-15 is secreted at low concentrations from most of the tissues and studies have demonstrated that injury or inflammation upregulate GDF-15 expression in the heart (13). Also, in animal models, GDF-15 is released from the heart in response to ischemia-reperfusion injury, pressure overload and heart failure via pro-inflammatory cytokine and oxidative stress-dependent pathways (14, 15). GDF-15 seems to be a cardioprotective cytokine, possessing anti-proliferative and anti-apoptotic characteristic. To the best of our knowledge, no study to date has measured the GDF-15 levels in the athletes, especially in the adolescent swimmers at rest. In the previous

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studies (1, 16, 17) it has been demonstrated that both intense physical activity such as marathon running (17) or soccer match (16) and exercise training (3) caused an increase of GDF-15 levels in elite athletes.

In the present study, we hypothesized that adolescent swimmers have higher levels of PTX3 and GDF-15 levels than sedentary controls and this increase may partly be related to the canonical biochemical parameters such as insulin-like growth factor-1 (IGF-1), LDL-C, HDL-C. Since PTX3 and GDF-15 have cardioprotective effects, this elevation in these markers might be partly caused by the mechanism underlying exercise induced cardioprotection. Therefore, to the best of our knowledge, this is probably the first study evaluating resting PTX3 and GDF-15 concentrations in adolescent male swimmers rather than response to any type of exercise. Therefore, we aimed to investigate the circulating levels of PTX3 and GDF-15 and their relation with canonical biochemical parameters in adolescent male swimmers.

## Materials and methods

### Subjects

Twenty-six adolescent male swimmers (11–18 yr) and twenty-nine age and gender-matched sedentary controls (11–17 yr) participated in this study and all the subjects were Turkish. The study protocol was approved by the institutional review board of Medical Faculty of Selcuk University, Konya, Turkey. All procedures conformed to the principles outlined in the Helsinki Declaration. All the subjects and their parents were informed of the potential risks and the procedures involved in the study. Prior to their participation written informed consents were taken from the subjects or from their parents, eighteen years old or younger.

Participants were competitive swimmers for  $5.23 \pm 1.86$  years on average. They were swimming  $5.15 \pm 1.19$  d week<sup>-1</sup> on average, and performing 1.5–2 hr of swimming exercises with a rating of 15–17 on the Borg scale (i.e., hard to very hard). However, the sedentary adolescents have a sedentary lifestyle with a distinct class of behaviours (e.g., sitting, watching TV) characterized by little physical movement and low energy expenditure ( $\leq 1.5$  METs) for at least 2 yr (18).

All subjects were free of signs, symptoms and history of any chronic diseases. None of the participants had a history of smoking and none were currently taking any medications. In addition, none of the subjects were NSAID or vitamin supplements users.

### Blood sampling

Blood draws were performed by a physician-approved, licensed allied health care professional using a standard technique. Blood samples were collected between 8:30 a.m. to 10:00 a.m. Before all measurements, the subjects avoided intense physical activity for 48 h and fasted overnight (12 h) without water. A fifteen ml of blood sample was collected by standard venipuncture from the left median cubital vein after a resting period of at least 30 min at a constant room temperature (25 °C). Four milliliters of the sample were immediately transferred to non-additive tubes to obtain a serum sample and it was allowed to clot at room

temperature for 30 min. Six milliliters of the sample were immediately distributed and put into EDTA-coated tubes to obtain plasma samples. Serum and plasma samples were separated from the whole blood by centrifugation at 2000 g at 4 °C for 15 min. Samples were stored at –80 °C until biochemical analysis and thawed only once.

### Biochemical analysis

The lipid profile was done by fully auto analyzer (ERBA XL 1000; Transasia Bio-Medicals Ltd. Mumbai, India). Total testosterone was measured on a Beckman Coulter Unicel DXI 1600 analyzer by using an automated competitive binding immunoenzymatic assay (Beckman Coulter Inc., Fullerton, CA). Serum CRP levels were determined with an immunonephelometry system according to the methods described by the manufacturer (IMMAGE 800, Beckman Coulter, USA). Serum IGF-1 levels were assessed by Enzyme Amplified Sensitivity Immunoassay (EASIA) method, which was performed on polystyrene microtiterplates according to the manufacturer's instructions (DIAsource ImmunoAssays SA, Nivelles, Belgium).

Plasma PTX3 levels were analyzed using the sandwich ELISA detection system (Hycult Biotech, Uden, The Netherlands) as follows. Samples and standards are incubated in microplate wells coated with antibodies detecting PTX3. Biotinylated tracer antibody binds to captured PTX3. Streptavidin-peroxidase conjugate binds to the biotinylated tracer antibody and reacts with the substrate tetramethylbenzidine. The enzyme reaction is stopped by the addition of oxalic acid. The absorbance at 450 nm is measured with automatic ELISA reader (Powervawe XS; BioTek Instruments, USA). In this assay system, the correlation coefficient between the theoretical values and the actual values was 0.99 and the minimum detection level was about 0.01 ng ml<sup>-1</sup>. The intra-assay standard deviation was less than 10%. The ELISA assay did not cross-react with the CRP and SAA. PTX3 levels were expressed as pg ml<sup>-1</sup>.

Plasma GDF-15 levels were quantified by using the ELISA method (RayBiotech Inc., Norcross, GA, USA) according to the manufacturer's instructions. Briefly, 100 µl standard and sample were added into the wells. After incubating for 2.5 hours the solution was removed and washed 4 times with the wash solution. Then 100 µl of biotinylated antibody was added to the wells and incubated at room temperature for 1 hour. After 4 times of washing, 100 µl of streptavidin solution was added to the wells and incubated for 45 minutes. After 4 times of washing, 100 µl of TMB substrate reagent was added to the wells and incubated at room temperature for 30 minutes in the dark. Finally, 50 µl of stop solution was added. The absorbance at 450 nm is measured with automatic ELISA reader (Powervawe XS; BioTek Instruments, USA). The minimum detectable dose was 2 pg ml<sup>-1</sup> and inter- and intra-assay coefficients of variability were 10 % and 12 %, respectively. GDF-15 levels were expressed as pg ml<sup>-1</sup>.

### Statistical analysis

Statistical analysis was performed using SPSS v.18.0 for Windows (SPSS Inc., Chicago, IL, USA). All data are presented as

**Tab. 1. Characteristics of sedentary controls and adolescent male swimmers.**

	Sedentary (n=29)	Swimmer (n=26)	p
Age (yr)	13.76±1.98	13.96±2.40	0.979
Height (cm)	164.0±12.0	166.0±13.0	0.538
Weight (kg)	54.99±11.28	56.42±13.61	0.672
Body mass index (kg m <sup>-2</sup> )	20.26±2.46	20.11±2.61	0.825

Data are expressed as mean ± SD.

**Tab. 2. Biochemical markers of the sedentary controls and the adolescent male swimmers.**

	Sedentary (n=29)	Swimmer (n=26)	p
LDL-C (mg dL <sup>-1</sup> )	114.97±32.29	124.62±27.79	0.243
HDL-C (mg dL <sup>-1</sup> )	44.73±10.32	46.28±7.76	0.411
TG (mg dL <sup>-1</sup> )	81.07±41.77	79.85±36.62	0.816
TC (mg dL <sup>-1</sup> )	175.72±37.01	186.77±29.76	0.231
Testosterone (ng dL <sup>-1</sup> )	279.60±163.57	305.56±190.45	0.375
hs-CRP (mg dL <sup>-1</sup> )	4.18±5.12	2.68±1.60	0.953
IGF-1 (ng mL <sup>-1</sup> )	608.68±131.20	677.70±190.27	0.120

Data are expressed as mean ± SD. TC – total cholesterol; TG – triglyceride; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; hsCRP – high sensitivity C-reactive protein; IGF-1 – insulin-like growth factor 1

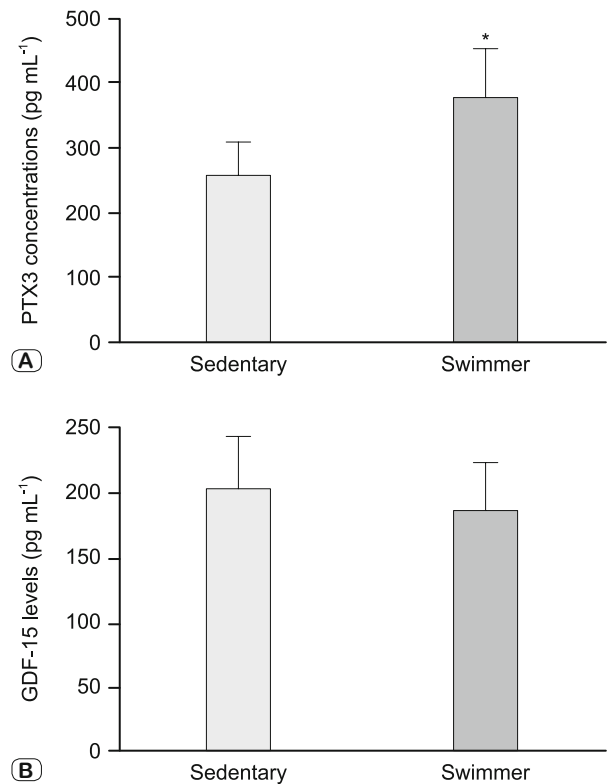
mean ± SD. The Shapiro–Wilk test was used to assess the normality of the distribution of the variables. In the case of variables that were not normally distributed a log transformation was performed. For normally distributed variables, Levene's test was used to control whether the variances among the groups were homogeneous. Student t-test for parametric data and Mann–Whitney U test for non-parametric data were used to evaluate statistical difference between the swimmers and the sedentary controls. Relationships among the variables were analyzed using Spearman's rank correlation coefficient. The level of statistical significance was set at  $p < 0.05$ .

## Results

Table 1 summarizes the characteristics of the swimmers and the sedentary controls. There were no significant differences in age, height, weight and body mass index between the adolescent male swimmers and the sedentary controls.

Table 2 shows the principal serum biochemical markers analyzed in the adolescent male swimmers and the sedentary adolescents. There were no significant difference in LDL-C, HDL-C, TG, TC, hs-CRP, testosterone and IGF-1 levels between the swimmers and the sedentary controls. However, serum LDL-C, HDL-C, TC, testosterone and IGF-1 levels tended to be higher and TG and hs-CRP levels tended to be lower in the adolescent swimmers than the sedentary controls.

Figures 1A and 1B demonstrate plasma PTX3 and GDF-15 concentrations in the two groups, respectively. Plasma PTX3 levels were higher in the adolescent male swimmers than that in the sedentary adolescents ( $378.44 \pm 173.93$  pg mL<sup>-1</sup> vs  $257.82 \pm 103.20$  pg mL<sup>-1</sup>, respectively) ( $p = 0.002$ ). There was no significant difference in GDF-15 levels between the two groups ( $186.12 \pm 40.65$  pg mL<sup>-1</sup> vs  $203.60 \pm 36.77$  pg mL<sup>-1</sup> in the swimmers and the sedentary, respectively) ( $p = 0.068$ ). However, it tended to be lower in the adolescent swimmers than the sedentary controls.

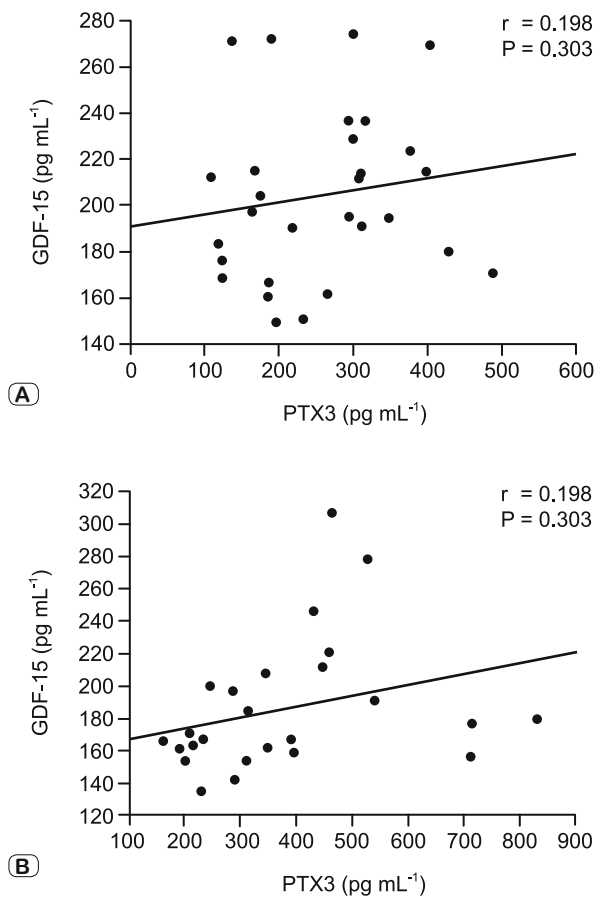


**Fig. 1. Plasma PTX3 (A) and GDF-15 (B) levels in sedentary controls and in adolescent male swimmers. Data are expressed as mean ± SD. \* $p < 0.05$  compared to sedentary.**

Statistical analysis of linear correlation was performed comparing PTX3 and GDF-15 with descriptive and biochemical parameters analyzed. In the sedentary controls, plasma PTX3 concentrations were not related to the GDF-15 (Fig. 2A). In the swimmer group, we found a positive correlation between plasma PTX3 and GDF-15 concentrations (Fig. 2B). Additionally, in the sedentary control group GDF-15 demonstrated a positive correlation with age and in the swimmers PTX3 showed a negative correlation with body mass index (Table 3). On the other hand, there was no significant correlation with the other parameters such as HDL-C, testosterone, hs-CRP and IGF-1 in both the sedentary controls and the swimmers (Tab. 3).

## Discussion

In the present study, we measured plasma PTX3 and GDF-15 levels in adolescent male swimmers. It was demonstrated firstly that plasma PTX3 levels were significantly higher in the adolescent male swimmers than that in the sedentary controls. Although plasma GDF-15 did not differ between the two groups, it was tended to be lower in the adolescent male swimmers. Furthermore, the relationship between the plasma concentrations of PTX3 and GDF-15 was linear. Although the effects of CVD were observed in adulthood, it is clearly understood that their develop-



**Fig. 2. Relationship between PTX3 and GDF-15 levels in sedentary controls (A) and in adolescent male swimmers (B).**

ment starts in childhood and adolescence (19). Habitual physical exercise using large muscle groups, such as swimming or running, induces cardiovascular adaptations that increase exercise capacity, endurance and skeletal muscle strength. It also prevents the development of coronary artery disease and decreases symptoms in patients with CVD (20).

In lipid profile (HDL-C, LDL-C, TG, TC), although all the values were within the normal ranges, we observed similar blood lipid profile between the groups. Results from cross-sectional

studies (21–23) suggest similar TC, lower TG and LDL-C and higher HDL-C levels in young athletes compared with controls. Age, sexual maturity status,  $VO_{max}$ , training status, dietary intake and body fat are indicatives of blood lipid profile. One possible explanation for the lack of an association between blood lipids and training volume in adolescent swimmers may be that the elevated training status is not influencing the lipoprotein metabolism when blood lipids are already at physiological concentrations during adolescence. One of the main effects of gonadal sex hormones on pubertal growth is improvement of growth hormone secretion and also IGF-1 production (24). Therefore, there is a close relationship between testosterone and IGF-1. In the present study we observed no significant difference in IGF-1 and testosterone levels between the two groups; though it tended to be higher in the adolescent male swimmers. This is probably due to the beneficial effects of regular physical exercise.

PTX3 and GDF-15 are recently used biomarkers to assess the cardiovascular status. In the present study, PTX3 levels were significantly higher in the adolescent male swimmers than in the sedentary controls. Our findings are consistent with the previously published studies (9, 10). Miyaki et al (9) showed that plasma PTX3 concentrations in young endurance-trained men were higher than in age-matched controls. In another study (10) they showed that 8 weeks of exercise training induced increase of PTX3 levels in middle aged and elderly women. Taken together, these data support the cardioprotective effects of PTX3. In the present study, no correlations were found between the PTX3 and serum CRP levels. Our findings are consistent with the previous studies (4, 5, 8) proposing that CRP and PTX3 are independent markers of inflammation. We also observed no correlation between PTX3 and lipid profile, especially HDL-C. These findings are inconsistent with a previous study (10) demonstrating positive correlation between PTX3 and HDL-C. The difference between the results might be depending on the age of the groups.

GDF-15 is accepted as an independent prognostic factor in predicting CVD and adverse outcome, over and above clinical and biochemical markers including troponins and natriuretic peptides (3). However, to the best of our knowledge no study to date has investigated the resting plasma GDF-15 levels in athletes, especially in the adolescents. In the present study we observed that GDF-15 levels were not different between the two groups, but it tended to be lower in the adolescent male swimmers than in the

**Tab. 3. Correlation analysis of PTX3 and GDF-15 with the main descriptive and biochemical parameters.**

Groups		Age	BMI	HDL-C	Testosterone	hs-CRP	IGF-1
Sedentary	PTX3	r	0.209	-0.213	-0.106	0.115	0.198
		P	0.277	0.268	0.584	0.552	0.469
	GDF-15	r	0.400	0.351	-0.250	0.301	0.321
		P	0.032*	0.062	0.191	0.112	0.090
Swimmer	PTX3	r	-0.146	-0.415	-0.017	-0.173	0.060
		P	0.477	0.035*	0.935	0.397	0.770
	GDF-15	r	0.226	0.042	-0.166	0.123	0.042
		P	0.268	0.838	0.418	0.550	0.838

BMI – body mass index; HDL-C – high-density lipoprotein cholesterol; hsCRP – high sensitivity C-reactive protein; IGF-1 – insulin-like growth factor 1



sedentary controls. This might be depending on the sample size. In the previous studies (3, 16, 17) it has been demonstrated that both intense physical activity such as marathon running (8) or soccer match (16) and exercise training (3) increased GDF-15 levels in elite athletes. On the other hand, Munk et al (25) showed that moderate exercise training (i.e., 3 times a week, for 1 hour, over 6 months) did not affect plasma GDF-15 levels in patients with stable coronary artery disease. An elevated level of GDF-15 seems to reflect several underlying conditions, acute and/or chronic, associated with adverse cardiovascular outcomes or inflammatory response. Kempf et al (26) reported that GDF-15 concentrations were significantly higher in heart failure patients than in healthy control individuals, and they proposed to use 1200 pg ml<sup>-1</sup> as the upper limit of the reference interval in elderly individuals. In the present study, GDF-15 levels of both the sedentary and the athletes were much lower than this reference value.

We demonstrated that there was a significant positive correlation between PTX3 and GDF-15 concentrations in the swimmers but not in the sedentary controls. These findings suggest that, since both PTX3 and GDF-15 are produced in response to inflammation or injury, there might be a reciprocal interaction between these parameters in athletes due to the continuous exposure to the effects of exercise. However, no correlation was observed between PTX3 and GDF-15 levels in patients with stable angina over 6 months of exercise training (25). The differences between the results might be depending on the age, health status and training status of the participants.

The present study has the following limitations that should be underlined. First, this study was cross-sectional and therefore the results are preliminary. These results should be confirmed in a longitudinal study. Second limitation is gender. Due to the limited number of adolescent female swimmers we couldn't examine these variables. Another limitation is the lack of performance parameters such as maximal oxygen consumption and cardiac monitoring like echocardiography. These results should be confirmed with both performance parameters and cardiovascular monitoring.

## Conclusions

The current study for the first time indicated that PTX3 levels are higher and GDF-15 levels tended to be lower in the adolescent swimmers than in the sedentary adolescent controls. It is also showed that there is a positive correlation between the PTX3 and GDF-15 in adolescent male swimmers. However, more detailed research is needed, especially in adolescents, to further delineate reciprocal interaction of the PTX3 and GDF-15 at the molecular level and its relationship to other cardiovascular markers.

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