

## Examination of the ACE gene and some cardiovascular variables in Divers

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**Abstract: The aim of the study:** This study examined whether there are any significant frequencies of the ACE variants in divers versus a group of sedentary subjects. Also examination of some cardiovascular variables in both groups. **Hypothesis:** It may be hypothesized with respect of ACE gene, that the D allele will be observed with greater percentage in divers when compared to the sedentary group. In addition the cardiovascular variables are expected to be in the favour of the divers when compared to the sedentary group. **Materials and Methods:** The descriptive method was used in this study. The participants were divided into divers (n = 10) and sedentary group (n = 10), in Hurghada area. Their age ranged from 20 to 25 years old. Blood sample was drawn from each subject containing EDTA, DNA was extracted, PCR was applied using primers for ACE genotype analysis and agarose gel. Echocardiography two dimensional Echo is recorded for PWT, SWT and LV mass were performed, also pulse rate, BMI. **Results:** revealed that divers genotype was ACEDD 80%, ID 20%, control genotype was ACEDD 20%, ID 50%, II 30%, also there was a significant change in case of PWT, SWT, LV mass of the divers compare to control. **Conclusion:** It may be concluded that training affect cardiovascular system of divers and it is also affected by genetic factors such as ACE genotype.

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### 1. Introduction

Diving is a competitive sports of aquatic activities, and include the events of swimming with fins, underwater swimming with breathing apparatus, compass guidance, saving, and the diving as a sport differ from other sports activities with its characteristic style of performance and site of practice, it requires high level of swimming with physical and physiological abilities enabling the player to perform efficiently in addition to functional ability on modification to face stress of continuous performance of swimming under water.

Diving sport is a marked activity in many of Arab Republic of Egypt clubs, with increased importance after establishing of sports federation for it in (1983) after return of Sinai and Egypt become one of the most important world areas for diving.

Diving is one of the aquatic spots practiced under water using special apparatuses with certain system, which requires from the practitioners special physical abilities to enable them achieving high level mainly in competitive field (**El Tarabily, 2006**).

**Butler (2001)** stated that a column of sea water 33 feet deep exerts the same pressure at its bottom as the pressure of the atmosphere above the sea. Therefore, a person 33 feet beneath the ocean surface is exposed to 2 atmospheres pressure, 1 atmosphere of pressure caused by the weight of the air above the water and the second atmosphere by the weight of the water itself. At 66 feet the pressure is 3 atmospheres, and so forth.

Growing evidence suggests that genetic variants of certain genes are linked to athletic performance. This study presents and analysis of genetic variation of the ACE gene in diving.

The ACE gene codes for the Angiotensin Converting Enzyme (ACE), which is an integral part of the Renin-Angiotensin system. It is responsible for regulating blood volume, arterial pressure, electrolyte balance and cardiac and vascular function. Polymorphisms of the ACE gene effect serum and tissue levels of the enzyme and are distinguished genetically by either an insertion (I-allele) or deletion (D-allele). The insertion allele is thought to be beneficial to endurance athletes and the deletion allele is thought to be beneficial to sprint athletes (**Yang et al., 2003, Ian Mayne, 2006**).

The ACEI/D polymorphism corresponds to the presence (I) or absence (D) of a 287 bp alu repetitive sequence. The D allele is associated with a 190 bp PCR fragment targeting the polymorphism site in intron 16 of the ACE gene. Individuals homozygous for the D allele have elevated ACE serum levels (**Sonna et al., 2001**). Higher levels of ACE increase the conversion of Angiotensin I to II and accordingly vasoconstriction is the result. The I allele is associated with a 490 bp PCR fragment and is related to lower ACE serum levels (**Cam et al., 2005, Chiu et al., 2005**).

The ACE I/D polymorphism have been examined extensively in the field of medicine for a large number of condition such as Diabetes,

Alzheimer's and many cardiovascular diseases. It has been studied in populations all over the world because of its central role in the overall functioning of the body. The importance of ACE has led researchers to examine its effects on elite athletes to determine if the variants of the ACE gene are related to human performance (Nazarov *et al.*, 2001, Scanavini *et al.*, 2002).

#### The aim of the study:

This paper examined whether there are any significant frequencies of the ACE variants in divers versus a group of sedentary subjects. Also examination of some cardiovascular variables in both groups.

#### Hypothesis :

It may be hypothesized with respect of ACE gene, that the D allele will be observed with greater percentage in divers when compared to the sedentary group. In addition the cardiovascular variables are expected to be in the favour of the divers when compared to the sedentary group.

## 2. Materials and Methods

The descriptive method was used in this study. The participants were divided into athletes (divers) (n = 10) and sedentary group (n = 10). Both groups were selected purposely from divers in Hurghada area and a control group from the same area, their age ranged from 20 to 25 years old. All participants voluntarily signed an informed consent. Blood sample was drawn from each subject containing EDTA in Polyethylene tube, for genomic DNA to be measured and extracted, PCR was applied together with a sense and antisense primers for ACE genotype analysis by size determination and agarose gel electrophoresis. The protocol of acquiring the DNA samples was identical to that of diver and each control sample; they were given an assignment code to protect their identity.

#### Genotype determination:

Genomic DNA was isolated from lymphoblastoid cells lines following a standard protocol. The ACEID polymorphism was typed with a PCR-based method using primers specific. The final reaction mixture of 15 ul contained 100 mg of genomic DNA. PCR protocol, consisted of one cycle at 94°C for 3 min. 55°C for 1 min. and 72°C for 1 min. followed by 35 cycles at 94°C for 30 s, 55°C for 30 s. 72°C for 45 s and one cycle at 72°C for 10 min. The PCR products were separated on 3.5% agarose gel and visualized under light after ethidium bromide staining (Figure 1).

Echocardiography two dimensional Echo is recorded for PWT, SWT and LV mass were performed (posterior wall thickness, septal wall thickness, left ventricular mass). Pulse rate was

determined using pulse meter, BMI using body analyzer, restameter to measure height and a balance for weight.

Three sets of PCR primers were employed to target ACE I/D as described by Iane Mayne (2006). the study was conducted from 2/8/2012 to 10/8/2012.

#### Statistical analysis:

The data were presented as the mean  $\pm$  SD. Comparisons between groups were analyzed by Kruskal wallis test, *p* value < 0.05 were defined statistically significant.

## 3. Results:

#### Subjects characteristics:

Ten divers and 10 low active control males participated in the study. The two groups were matched for age, weight and height (Table 1). Non significant changes in basic characteristics and for BMI

**Table (1):** Basic characteristics

Variables	Divers n=10	Control n=10	Sig.
Age (yr)	24.3 $\pm$ 1.74	23.4 $\pm$ 1.32	NS
Height (cm)	178 $\pm$ 2.84	176 $\pm$ 1.96	NS
Weight (Kg)	76 $\pm$ 3.64	75 $\pm$ 2.4	NS
BMI	22 $\pm$ 1.6	23 $\pm$ 2.4	NS
Pulse Rate (count/m.)	68 $\pm$ 2.1	73 $\pm$ 2.5	S

Values are mean  $\pm$ SD *P* < 0.05

Table (2) revealed that divers genotype ACE DD 80%, ID 20%, control ACEDD 20%, ID 50%, II 30%, also there was a significant change in case of PWT, SWT, LV mass for the sake of the divers compared to control.

**Table (2):** Means and standard deviation in divers and control groups in cardiac and gene changes

Variables	Divers M $\pm$ SD	Control M $\pm$ SD	Sig.
PWT (mm)	8.9 $\pm$ 0.16	8.4 $\pm$ 0.34	S
SWT (mm)	8.68 $\pm$ 0.18	8.3 $\pm$ 0.21	S
LV mass/g	254 $\pm$ 5.9	242 $\pm$ 3.8	S
ACE variant			
DD	80%	20%	
ID	20%	50%	
II	-	30%	



**Figure 1.** Genomic DNA

#### 4. Discussion

**Robergs and Roberts (2000)** defined hyperbaria as an increased barometric pressure, they added that when the body is submerged to the sea water, the pressure increases one atmosphere (ATM) (760 mmHg) every 10 meters. They also added that when the body is submerged in water to the level of the neck, acute cardiovascular adaptations occur in response to the increased compressive forces exerted on the skin resulting in decreased cutaneous blood flow, increased central blood volume, increased venous return and a lowered heart rate. During immersion of the body in water an additional neurologic reflex (diving reflex) is excited that also lowers heart rate and lead to improve cardiovascular function. This theory is in accordance with the result in Table (1) and Table (2) as pulse rate decreased in case of divers compared to control and the heart dimensions increased in divers compared to control.

Prolonged and repeated exposures to exercise cause structural and functional changes in the cardiovascular system. The extent of these changes is dependent on the type and quality of the exercise training. Comment must also be made on the influence of genetics to cardiovascular capacities and adaptability. Data from a cross sectional evaluation of athletes and sedentary subjects, indicating that the cardiac mass index was larger in athletes compare to sedentary subjects (**Robergs and Roberts, 2000**).

**Guyton and Hall (2006)** stated that when human descend beneath the sea. The pressure around them increases tremendously. To keep the lungs from collapsing, air must be supplied at very high pressure to keep them inflated. Also, there are tremendous alternation in body physiology. They added that the individual gases to which a diver is exposed when breathing air are nitrogen, oxygen and carbon dioxide, each of these at times can cause significant physiologic effects at high pressures. They also added that hyperbaric oxygen can have valuable therapeutic effects in several important clinical conditions such as decompression sickness, arterial gas embolism and myocardial infarction, indicating the beneficial effect on cardiac muscles.

**Johanthan et al. (2000)** reported that the increased thickness of the athletes left ventricular wall and other heart dimensions may be due to the exercise training which induced cardiac hypertrophy. **Kanalis and Hickson (1980)** suggested that exercise is a potent stimulus for left ventricular hypertrophy, while **Myerson et al. (2001)** indicated that cardiac growth responses are influenced by different exercise intensities and types due to different prospective training schemes. **De Maria et al. (1998)** and **Dubach et al. (1997)** showed that even modest training changes may alter cardiac growth or responses.

The data presented in Table (2) indicated that the divers with ACE DD in majority of cases 80%, possessed higher calculated cardiac dimensions post wall thickness (PWT), septal wall thickness (SWT) and left ventricular mass compared with the control group, that have different forms of ACE (DD, ID, II). This was in accordance with the report of **Payne and Montgomery (2003)**, they demonstrated that cardiac activity is correlated with cardiac ACE mRNA levels and this activity may result in increased cardiac angiotensin II levels (**Andreas et al., 1997**) and **Heshmat et al. (2010)**. **Indra and Annapoorani (2008)** reported a(+) relationship between ACE gene polymorphism D and coronary artery disease.

**El Kib (2012)** reported that aerobic exercise program induced a positive effect on diabetic woman of the type II, she also reach the conclusion that the majority of the studied cases possessed ACEDD genotype, that has a relation to diabetes disease and cardiac infarction, as ACE increased which in turn decreased the blood supply of the vital organs.

Myocardial hypertrophy due to thickening of the left ventricle and dilation of its cavity is greater in athletes compared to non athletes, however, these changes are influenced by the athlete's training regimen and genetic factors (**Venchunas et al., 2006**).

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#### Conclusion:

It may be concluded that training affect cardiovascular system of divers and it is also affected by genetic factors such as ACE genotype.

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