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Effect of selected exercise program on vitamin D and parathyroid hormone level in high altitude residents

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Many external factors can affect hormonal level including physical activity and altitude level. Vitamin D (25(OH)D) and parathyroid hormone (PTH) hormone have critical role in regulation of many physiological and metabolic processes. 25(OH)D and PTH deficiencies usually treated by synesthetic substitutes which may have adverse effects. This study aimed to investigate the effect of selected exercise program on the regulation of these hormones. Forty females of high altitude residents and twenty females of low altitude residents participated in this study with age of 20-25 years. High altitude residents were randomly assigned into two groups (GI and GII) each contain 20 subjects. GI received the selected exercises for three months. GII didn't participate in the exercise program. GIII was the 20 low altitude residents and didn't participate in the exercise program. Serum level of the hormones was measured for all groups at the beginning and at the end of the study. Three-way mixed MANOVA revealed significant increase in 25(OH)D level after the exercise program in GI while PTH showed non-significant decrease after exercise. The difference between the hormonal levels at high and low altitude was non-significant. We concluded that skeletal muscles and muscular activities play an important role in vitamin D metabolism.

Keywords: exercises, vitamin D, parathyroid hormone, high altitude

INTRODUCTION

Vitamin D (Vit D) has a significant role in calcium metabolism, bone formation and bone health (Bikl, 2014) and (Nakamichi et al., 2018). There are many forms of Vit D including Vit D3 and Vit D2. Vit D3 (*cholecalciferol*) is produced in skin by the effect of ultraviolet light (UV) from 7-dehydrocholesterol (Bikl, 2014). Plant sterol ergosterol is the source of Vit D2 (*ergocalciferol*). Vit D is converted initially to 25 hydroxy Vit D (25OHD), then to the hormonal form 1,25-dihydroxy vitamin D (Webb, 2006), (Bikl, 2014) and (Xue et al., 2015). Vit D itself is not an active substance. Vit D needs to be converted into 1, 25-dihydroxycholecalciferol as active form of Vit D in liver and kidney, this occurs in the existence of Parathyroid hormone (PTH). PTH is an important agent in the metabolism of Vit D as PTH increases the absorption of calcium ions from gastrointestinal tract (GIT) through indirect action. PTH increases the synthesis of 1,25dihydroxycholecalciferol in kidneys, which increases the absorption of calcium from GIT (Sembulingam and Sembulingam, 2012) and (Christakos et al., 2015).

The activation of Vit D3 occurs in the existence of PTH in two steps. The first step, Vit D3 is transformed into 25-hydroxycholecalciferol in liver. This action is inhibited by 25-hydroxycholecalciferol itself through the feedback mechanism. The second step, 25-hydroxycholecalciferol is transformed into 1,25dihydroxycholecalciferol (*calcitriol*) in kidneys, which is the active form of Vit D3. This second step needs the PTH (Holick et al., 2011), (Sembulingam and Sembulingam, 2012), and (Carmina, 2017). This strong relation between Vit D and PTH made it necessary to study Vit D and PTH in conjunction with each other.

For evaluating Vit D status in patients who are liable to Vit D deficiency, it is preferable to use a reliable assay to determine serum circulating 25-hydroxyvitamin D (25(OH)D) level (Faridi et al., 2017), (Rabenberg et al. 2018), and (van Schoor, and Lips, 2018). Vit D deficiency is known as the decrease of serum 25(OH)D level below 20 ng/ml and insufficiency are reached when serum 25(OH)D level is between 21–29 ng/ml (Lefevre, 2015) and (Rabenberg et al., 2018). It was not recommended to use the serum 1,25-dihydroxyvitamin D [1,25(OH)2D] assay for the reliability of the assay and in favor of using it only in monitoring some specific conditions, such as acquired and inherited disorders of Vit D and phosphate metabolism (Rabenberg et al., 2018).
Muscular affection such as sarcopenia can be resulted from 25(OH) deficiency and increased PTH levels. Sarcopenia can improve using Vit D as a treatment (Visser, 2003), (Bauer et al., 2015) and (Skaaby et al., 2018). In addition, low Vit D and high PTH concentrations may increase the incidence of cardiovascular disease (Reis et al., 2009) and (Scragg et al., 2017). Lips et al, 2005 reported that, Vit D metabolites may have an important role in the prevention of autoimmune diseases and cancer (Lips et al. 2005), (Paolino et al., 2016) and (Lappe et al. 2017).

Higher secretion of PTH might be due to low serum 1,25(OH) D and low serum calcium, this causes high bone resorption causing bone loss. Bone loss occurs mainly in the cortical bone, which may be one of the pathogenesis of osteoporosis (Lips, 2006). Apparently, Vit D receptor present in most cells, and so, can convert the primary circulating form of Vit D into the active form (Bouillon, 2013) and (Staniszewski et al., 2018). This can indicate the extra skeletal functions of Vit D.

Vit D and PTH levels are related to physical performance. It was found that, physical activities and muscle strength are significantly correlated to Vit D level (Anderson et al., 2003) and (Wanner et al., 2015). In addition, several observational studies concluded that presence of positive association between serum 25(OH)D concentration and physical performance and muscle strength (Bischoff et al., 1999) and (Scott et al., 2015). On the other hand, lack of sports or lack of physical activity is associated with reduced serum calcium or 25(OH)D (Herrmann et al.,2015). Also, it was found that moderate intensity swimming exercise improved Vit D status in rats with induced type II diabetes (Aly et al., 2016).

High altitude can affect the level of Vit D formation. Vit D production improved at high altitude due to increased exposure to UV. Webb and Engelsen, 2006, studied the levels of UV exposure necessary for suitable Vit D status and they found that, every kilometer increase in altitude reduces the needed UV exposure time by about 7% (Webb and Engelsen, 2006) and (Kimlin, 2008). The combination of high altitude and physical activity may have an impact on the level of Vit D and PTH. Therefore, the aim of the current study was to investigate the effect of selected exercise program and high altitude on both Vit D and PTH. Our research question of this study was: "is there any effect of exercises and high altitude on 25(OH)D and PTH in high altitude residents". This may help to regulate the hormonal action using exercise, which is much beneficial than using synthetic forms.

MATERIALS AND METHODS

Study setting:

The study was conducted at College of Applied Medical Sciences, Taif University at the physical therapy and laboratory departments. The practical part conducted from February till April 2018.

Study design:

Randomized controlled trial was used to investigate the effect of selected exercise program and high altitude on the levels Vit D and PTH. Randomized controlled trial is considered as the gold standard for clinical trials. The participants who were residents of Taif City (a city of 1879 m above the sea level in Mecca province) were volunteers, and they were randomized into two groups. A third group was volunteers of Jeddah city (a city of 12 m above the sea level in Mecca Province).

Study participants:

Forty female subjects who are residents of Taif City and 20 female who are residents of Jeddah City participated in this study. They aged from 20-25 years. Taif residents were randomly assigned into two groups, GI and GII, with 20 subjects in each group. Subjects in GI practiced the selected exercises for three months. GII and GIII (Jeddah residents) subjects were the control groups where their hormonal levels were measured at the beginning and at the end of the study without any exercise intervention.

The inclusion criteria of the participated subjects in this study was, i- cognitively and mentally normal, ii- physically well intact, iii- they did not suffer from any chronic disease, and vi- they were within normal body mass index. Subjects were excluded if they had any of the following, i-medical condition that prevents independent weight bearing or severely affects balance, ii- continuous symptoms of vertigo or dizziness, iii- they suffered from any disease which can affect Vit D or PTH, or they are under any treatment that affects Vit D or PTH.

Instrumentation and procedures:

For evaluation:

ELISA reader:

ELISA (Enzyme Linked Immuno Sorbent Assay) test techniques have been developed and improved for detecting organic constituents of biological fluids including blood. The use of ELISA techniques has increased greatly having regard to the relative simplicity of the procedure, speed, sensitivity, reliability and as a means to avoid the use of radioactive assays. The ELISA technique is based upon three primary principles; firstly, the ability of proteins to bind to coated plastic; secondly, the high specificity and affinity of antigen/antibody reactions and finally, the ability of enzymes to modify a substrate (Moro, 1993).

For treatment:

Treadmill

TUNTURI treadmill was used. It has the following general characteristics: Motor power of 2.5 HP DC, speed of 1.0–16 km/h, inclination of 10%, running surface of 46x130(cm), running deck type (mm) 18, power supply of 230V AC / 50 / 60Hz_Direct current, foldable soft drop system, shock absorbing system T-Flex, and assembled dimensions (LWH cm) 168*79*137

Procedures

For evaluation:

Each data sheet of each participant was filled in data including her name, age, height, weight, body mass index (BMI), address, telephone number and identification number. Any participant was allowed to ask any question about any part of the study or the benefits of performing the testing procedures. A written informed consent form was completed and signed by all participants (appendix I). Blood samples were withdrawn from all participants in the three groups before starting the study and at the end of the study duration at the same time from the day (9 AM) to avoid the effect of diurnal rhythm of hormone. Three cubic centimeters of blood were withdrawn after fasting more than 10 hours. Subjects in the GI engaged in the following a pre designed treadmill-training program, illustrated in table (1). They participated in three sessions per week for three months.

Table (1): Treadmill training parameters for the study group.

Week	Speed-(mph)	Incline	Time (min)
1,2	3-3.5	9%	22
3,4	3.5- 4	11%	24
5,6	4 - 4.5	13%	26
7,8	4.5-5	15%	28
9,10	5-5.5	17%	30
11,12	5.5-6	19%	32

At the end of the three months, the blood samples were collected from all subjects in the three groups for measuring the serum level of both hormones with consideration of the effect of diurnal rhythm by collecting samples at the same time of the day following same procedure as the pre-exercise analysis.

Data analysis:

All statistical measurements were performed through the Statistical Package for Social Studies (SPSS) version 20**.** MANOVA was conducted to detect any significant differences in the mean values of age and body mass index of the subjects in the three groups. Also, three ways mixed design MANOVA (with using syntax for pairwise comparisons) were used to measure the statistical differences between and within the three groups regarding the hormonal levels at the beginning and at the end of the study duration.

RESULTS

The aim of this study was to investigate the effect of selected exercise program and high altitude on Vit D and PTH. Before starting statistical analysis, all data were tested for normal distribution using Shapiro–Wilk test which indicated normal distribution of the data (P>0.05). The mean age was 21.7 ± 3.2, 22.1 ± 2.9 and 21.3 ± 2.8 for GI, GII and GIII respectively. The body mass index (BMI) of GI, GII and GIII was 22.4 ± 4.7, 21.7 ± 3.9 and 22.2 ± 3.2 respectively. MANOVA test showed non-significant difference between groups regarding age and BMI (P>0.05), Table (2).The levels of 25(OH)D and PTH were measured before and after the exercise program for GI. The hormonal levels for GII and GIII were measured in conjunction with the measurement for GI. Levene's and Box's tests indicated equality of variance for the variables of the study (P>0.05) Therefore, three way mixed design MANOVA was used to compare the levels of both hormones within and between the three groups. Comparing the 25(OH)D and PTH levels between the three groups before starting the study, pairwise comparison revealed non-significant difference between the three groups (P>0.05). However, when we the levels of hormones were compared between GI, GII and GIII after the exercise intervention only 25(OH)D level of GI showed significant increase compared to that of the GII and GIII (P<0.05), Table (3). In addition, the results of the within subjects comparison for GI also indicated significant increase in the 25(OH)D level after the exercise program (P<0.05). While, PTH showed non-significant decrease (P>0.05). Regarding the comparison of GI and GII within subjects, there was no significant changes occurred during the study comparing the hormonal levels before starting and at the end of the study period Table (4).

Table (2): Demographic data of the study and control groups.

Parameters	GI M±SD	GII M±SD	GIII M±SD	P value of MANOVA test
Age	21.7±3.2	22.1±2.9	21.3±2.8	0.42
BMI	22.4±4.7	21.7± 3.9	22.2±3.2	0.44

Table (3): Between subjects comparison for 25(OH)D (ng/ml) and PTH (pg/ml) levels in three groups. Significance: *, p≤0.05

Parameters	Means ± stander deviation			P value		
	GI	GII	GIII	GI vs GII	GI vs GIII	GII vs GIII
25(OH)D before	18.6±1.6	18.48±1.5	19.03±3.6	0.3	0.29	0.24
25(OH)D after	21.8±2.1	18.19±1.45	19.1±4.3	0.02*	0.04*	0.31
PTH before	36.2±5.9	36.7±6.7	35.8±8.6	0.5	0.2	0.37
PTH after	35.2±6.8	35.4±9.4	35.4±9.7	0.41	0.19	0.42

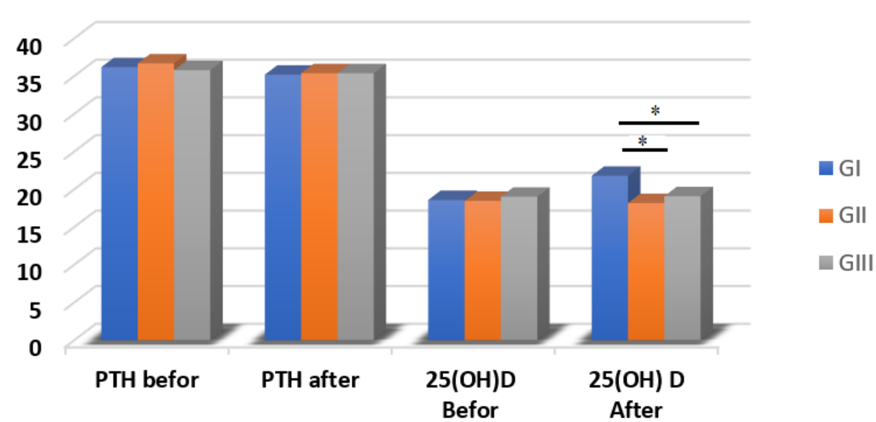

Figure (1): Between subjects' comparison for 25(OH)D (ng/ml) and PTH (pg/ml) levels in three groups. 25(OH)D after has shown significant P value when GI compared to GII (P value 0.02) and to GIII (P value 0.04).

Table (4): Within subjects comparison for 25(OH)D (ng/ml) and PTH (pg/ml) levels in three groups. Significance: *, p≤0.05

	Before	After	P value
25(OH)D GI	18.6±1.6	21.8±2.1	0.03*
25(OH)D GII	18.48±1.5	18.19±1.45	0.15
25(OH)D GIII	19.03±3.6	19.1±4.3	0.51
PTH GI	36.2±5.9	35.2±6.8	0.12
PTH GII	36.7±6.7	35.4±9.4	0.14
PTH GIII	35.8±8.6	35.4±9.7	0.2

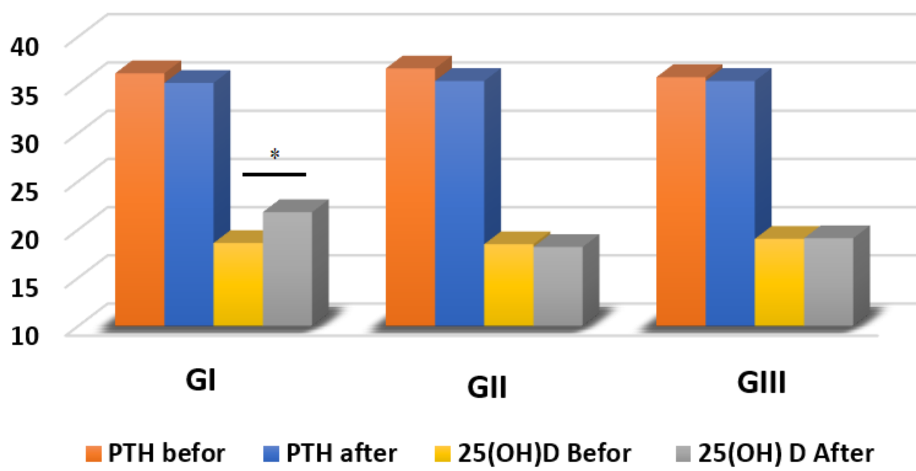

Figure (2): Within subjects' comparison for 25(OH)D (ng/ml) and PTH (pg/ml) levels in three groups. 25(OH)D has significantly (P value o.o3) increased in GI.

DISCUSSION

This study was conducted in order to investigate the effect of selected exercise program and high altitude on Vit D and PTH. Interestingly, the results denoted an increase in the plasma 25(OH)D after three months of selected treadmill training program while the PTH showed non-significant decrease. The elevated 25(OH)D levels is considered a positive effect of the selected exercise program. 25(OH)D serum level decreases with lowered activity level and increases in subjects with higher activity level as indicated by Al-Othman et al, 2012 (Al-Othman et al. 2012), after studying the effect of activity level and sun exposure on 25(OH)D.

Our results are concomitant with Sun et al, 2017, who studied the effect of an endurance exercise program on 25(OH)D level and their results indicated elevation of serum 25(OH)D levels. Also, they found that PTH level reduced one hour after exercise. Sun et al, 2018, as well concluded that, 5 weeks of endurance training could inhibit the seasonal reduction in serum 25(OH)D concentrations without changes in body fat.

Furthermore, Iwamoto et al, 2004, conducted a study to investigate the effect of treadmill exercise program on bone mass, bone metabolism, and calciotropic hormones in rats. Their results came in agreement of our results as they concluded an increase in the serum 1,25-dihydroxyvitamin D3 level and decreases the serum PTH level after application of treadmill exercise program for eleven weeks.

The increase in the circulating 25(OH)D after exercise may be attributed to adipose tissue loss (Gangloff et al., 2015). The mobilization of fat as a source of energy during exercise may have a role in increasing the circulating 25(OH)D. Adipose cells is considered as store for Vit D as it can be found in the analysis of fatty tissue, Vit D is not released readily from the triacyl glycerol matrix of the fatty cells due to the robust lipophilic properties of the fatty cells. The release of Vit D occurs only if there are net mobilizations of fatty acids to meet energy requirements (Wortsman et al., 2007).

Also, it was found that exercise indoors also provides higher concentrations of 25(OH)D compared with non-exercising individuals (Scragg et al., 1992). Skeletal muscles may have a role in storage and release of 25(OH)D in blood (Abboud et al., 2013). Skeletal muscle could be a site of an extravascular reservoir of 25(OH)D. This was confirmed by in vitro studies of the capability of differentiated C2 murine muscle cells to store and release 25(OH)D, in comparison with other types of the cells and the involvement of the membrane protein megalin in these mechanisms. It was established that myoblast contain Vit D receptors binding protein, which can bind nearly all 25(OH)D in the blood. Vit D receptors binding protein has a high affinity for actin in skeletal muscle. It can be concluded that 25(OH)D of muscles is the largest reservoir of this metabolite in the body (Abboud et al., 2018).

One of the most important benefits of storing a circulating 25(OH)D within muscle cells is the protection of 25(OH)D. These impounded molecules are protected from degradative hepatic catabolism, which is the main cause of destroying 25(OH)D (Wang et al., 2013). The stored 25(OH)D in the skeletal muscle may be released in the circumstances of low plasma levels of 25(OH)D such as low sun exposure which can explain elevated plasma level of 25(OH)D in individuals with high activity level compared with individuals with sedentary life (Abboud et al. 2018). Further studies are required to understand the mechanism of the release of 25(OH)D in skeletal muscle and the link to elevate serum level of this hormone which will contribute in regulating and improving various biological processes in the body. In addition, further investigation of the effect of different exercise intensities and durations on the plasma level of 25(OH)D is required. This will aim to determine the exercise level which can lead to the optimal level of plasma 25(OH)D.

In the current study our participants were suffering from Vit D deficiency (25OHD below 20ng/ml) at both high and low altitude. It is common that, Residents of high altitude suffer from Vit D deficiency despite the time of UVB exposure required for sufficient Vit D production is reduced at high altitude (Webb and Engelsen, 2006) and (Kimlin, 2008). This finding comes in agreement with Kapil et al, 2017, who found that, high prevalence of Vit D deficiency was found in children residing in high altitude regions. They stated that ninety-three percent of school-age children had Vit D deficiency (serum 25(OH) D levels of <20 ng/ml). The incidence was significantly higher amongst females. In addition, Kasprzak et al, 2015, found significant reduction in Vit D in Alpinists during 2-week high-altitude climb. This deficiency may be attributed to rare exposure of the female who participated in the current study to sun.

On the other hand, Rostand et al, 2016, found an increase in Vit D production associated with high altitude during studying the effect of Vit D level on decreasing the systolic blood pressure. They found decrease in the systolic blood pressure in high altitude residents and attribute this to sufficient production of Vit D at high altitude. We recommend further investigation of the effect of high altitude on the Vit D production by studying samples at different altitude and the correlation to systolic blood pressure. Also, it is important to consider the time of sun exposure in the study.

CONCLUSION

To conclude, Vit D level was not affected in the high altitude, but skeletal muscles and muscular activities play an important role in Vit D metabolism. Further study is highly recommended with consideration of consider the time of sun exposure in the study.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

L.E designed and performed the exercise program. H. H.A wrote the manuscript. A.S, M.a, and K.M.I collected recruited the subjects and collect the blood samples and performed the blood analysis. Y.O.A.A collect and the data and performed the data analysis. L.E and H.H.A designed experiments and reviewed the manuscript. All authors read and approved the final version.

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