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Effect of Immersion of Lower Limb in Marine Water on Testosterone Secretion in Adult Male Subjects

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Abstract
People swim either for leisure or as professionals/occupation. Some stay in water (mostly marine water) for several minutes to hours each day. This may have an effect that may be ignored based on the understanding that the skin barrier prevents entry of foreign materials into the body. This research tends to find the effects of immersion of lower limb in marine water on testosterone secretion in adult male subjects. 33 male students from Nnamdi Azikiwe University College of Health Sciences were recruited for the study. Ethical clearance was obtained from the faculty ethical committee. The volunteers were divided into three groups (A[marine water], B[fresh water], C[distilled water]) and were asked to immerse their lower limb in different water type according to their group for one hour every day for fifteen days. Blood samples were collected (before and after immersion) from the volunteers intravenously on 1st, 5th, 10th and 15th day. The levels of testosterone were measured by ELISA methods. The mean difference of data collected before and after immersion was established. Data obtained were compared with control group using student T-test method, P<0.05. Those exposed to marine water showed a negative response in plasma testosterone concentration. There are significant difference in plasma testosterone level between group A and group C on Day0 and group A and group C on Day10. According to the result, it could be suggested that immersion of lower limb in marine water could attenuate testosterone secretion in adult males; the mechanism of action for this effect is poorly understood.

Introduction

Swimming is one of the fast growing sporting activities in the world. Many people swim for leisure while some swim as professionals and others as occupation (fishermen and other off-shore workers). Some of these people stay in water (mostly marine water) for several minutes to hours each day. In marine water, one of the major characteristics is its high salinity. This may have an effect which may have been ignored based on its insignificant level or based on the understanding that the skin barrier (corneal layer) prevents entry of foreign materials into the body, especially substance with large molecular weight and polarised molecule.[1][2][3] Some researchers have found out that materials like allergens and substance with small molecular weight(less than 1,000 daltons) can pass through the skin.[1][4] Materials can also pass through the skin into the body via intracellular space.[1] Although there are no research works that shows that sodium or sodium chloride enters the body through the skin.

Researchers have shown that barrier function of the skin can be compromised if there is a compromise in the structure of the skin.[4] Other conditions that can cause materials to pass through the corneal layer includes; high concentration of penetrating substance, if the substance is non polar or semi polarised but small molecular size {<500 daltons}.[3] There are no literature on the effects of salt concentration and water (fluid) volume in the body in relation to male reproductive hormone secretion.

About one-third of those living in riverine area in southern Nigeria are offshore workers (including fishermen). This set of people is involved in swimming either directly or indirectly. Some engage themselves in swimming as a hobby while others like the professional swimmers do it in order to earn a living. They normally deep their lower limb in the water (marine) in the process of doing their job on daily bases. They come in contact with this high salinity water body. There are so many literatures on effects of swimming on other systems of the body of mammals but little in humans. There is little or no research done on the effect of swimming on endocrine and reproductive system especially on testosterone hormone. Testosterone is very important in the life and development of all male mammals following the central role it plays in fertility and development of secondary sexual characters in male mammals. There is need to have a research proof or an evidence based research on the effects of immersion of lower limb in marine water on testosterone secretion in adult male subjects.

Material and Methods

Thirty-three healthy young adult males that participated in this study were randomly selected based on acceptance, and they were recruited from Nnamdi Azikiwe University, Nnewi campus over a period of one month. Ethical clearance was obtained from the ethics and research committee of Nnamdi Azikiwe University, Faculty of Basic Medical Sciences, Nnewi Campus. Those who were aged 18 and above, that has not been diagnosed of diabetes, chronic obstructive pulmonary disease, pituitary tumor, sleep apnea or takes opoid, glucocorticiod drugs and does not take dopamine receptor stimulatory or inhibitory drugs were recruited in this research. Oral consent of the participant was obtained. The selected volunteer formed the study groups and a questionnaire was given to them to fill in other to collect their bio-data. A scale balance and a stadiometer were used to obtain their weight and height. The BMI of each participant was calculated using this formula according to Kolimechkov, 2016 and Centers for Disease Control and Prevention (CDC), 2009.

BMI = (weight in kilograms) divided by (height in meters X height in meters)

BMI= Weight(kg)/Height(m^2)[5][6]

Marine water was fetched from the Atlantic Ocean in Abonema, Rivers State and Fresh water was also fetched from "Nwosu" stream in Akpo Aguata Local Government Area Anambra State. Distilled water was bought from bridge-head market Onitsha a special market for medications and medical equipment in Anambra State. Fifteen litres buckets were rented, divided into three groups and labelled differently from other groups based on their water content. Buckets labelled "A" contained marine water obtained from Atlantic Ocean, buckets labelled "B" contained Fresh water obtained from Nwosu stream while those labelled "C" contained distilled water. The participants were divided into three groups based on the source of the water. First group which their buckets were labelled 'A' had marine water, and second group which there bucket were labelled 'B' had fresh water and the third group which there bucket were labelled 'C' had distilled water.

The participants were educated about the process of the experiment and the participants were asked to wash their legs with tap water before they immerse their legs into the water up to the kneel level according to their groups for one hour everyday for fifteen days, blood sample for serum testosterone analysis were taken from each participant twice (before immersion and after

immersion) on intervals as follows: 1st day, 5th day, 10th day and 15th day. The samples were taken in the morning between 8.00am and 10am intravenously from their arm using syringe. Blood sample collected was introduced into a plain tube. The blood samples were centrifuged at 2,500 rpm, serum separated, stored at -80°C in freezer, analysed in a single session to reduce interassay variation and by a lab technician.

The parameter used to determine testosterone hormone secretion in the subjects was the concentration of testosterone hormone in the blood serum. The serum levels of testosterone were assayed using ELISA technique.

Data obtained was statistically analysed using Students sample t-test method [SPSS version 21.0]. Data was expressed as mean ± SEM *p<0.05

Results

The mean Age and BMI of the subjects are shown in Figure 1 and Figure 2 respectively. There were no significant difference between ages in group A (marine water) vs group C/control (distilled water) (20.70±0.50yrs vs 20.55±0.59yrs; p-value 0.769) and group B (fresh water) and group C/control (distilled water)(21.00±0.55yrs vs 20.55±0.59yrs; p-value 0.817). BMI of the subjects in group B (fresh water) (22.47±0.40 kg/m) shows a significant difference when compared with group C/control (distilled water)(21.43±0.72 kg/m) p-value = **0.024*** while there were no significant difference between group A (marine water)(23.11±0.97kg/m) and group C/control (distilled water)(21.43±0.72 kg/m) p-value = 0.120.

The plasma testosterone response to lower limb immersion in marine water at variable time is shown in Figure 3 a negative response was observed from the result throughout the period of the exposure. There was a significant difference between the mean difference of Day-0 vs Day-5(-1.17 ± 0.43 vs 0.02 ± 0.51; p-value **0.043***) but Day-10 (-1.00 ± 0.32) and Day-15 (-0.46 ± 0.35) are higher than Day-0 (-1.17 ± 0.43) but not statistically significant p-value = 0.613 and 0.070 respectively.

The plasma testosterone response to lower limb immersion in fresh water at variable time is shown in Figure 4. The result shows a negative response on Day-0, Day-10 and Day-15 while Day-5 shows a positive response during the time of exposure. When the mean difference of Day-0 (-0.32 ± 0.39) is compared with others, it shows that day-5 (0.60 ± 0.41) is statistically higher than Day-0 (-0.32 ± 0.39) p-value = **0.046*** but Day-10 (-0.05 ± 0.18) and Day-15 (-0.23 ± 0.18) are higher but not statistically significant p-value = 0.172 and 0.634 respectively.

The plasma testosterone response to lower limb immersion in distilled water at variable time is shown in Figure 5. A positive response was observed on Day-0 (0.24 ± 0.36) and Day-10 (0.17 ± 0.28) while Day-5 (-0.16 ± 0.36) and Day-15 (-0.19 ± 0.16) shows a negative response during the time of exposure. When the mean difference of Day-0 (0.24 ± 0.36) is compared with others, it shows that day-15 (-0.19 ± 0.16) is statistically lower than Day-0 (0.24 ± 0.36) p-value = **0.020*** but Day-5 (-0.16 ± 0.36) and Day-10 (0.17 ± 0.28) are lower but not statistically significant, p-value = 0.293 and 0.813 respectively.

The Plasma testosterone response to lower limb immersion in different water sample on Day-0 is shown in Figure 6. Result shows that those exposed to marine water (-1.17 ± 0.43) had a statistically lower/negative response than those exposed to distilled water (control group) (0.24 ± 0.36) p-value = **0.009*** while those exposed to fresh water (-0.32 ± 0.39) were lower than those exposed to distilled water (0.24 ± 0.36) but not statistically significant, p-value = 0.183.

The Plasma testosterone response to lower limb immersion in different water sample on Day-5 is shown in Figure 7. Observation shows that those exposed to marine water (0.02 ± 0.51) and fresh water (0.60 ± 0.41) were higher than those exposed to distilled water (control group) (-0.16 ±

0.36) but not statistically significant, p-value = 0.731 and 0.091 respectively.

The Plasma testosterone response to lower limb immersion in different water sample on Day-10 is shown in Figure 8. Result shows that those exposed to marine water (-1.00 ± 0.32) had a statistically lower/negative response than those exposed to distilled water (control group) p-value= **0.006*** while those exposed to fresh water (-0.05 ± 0.18) were lower than those exposed to distilled water (0.17 ± 0.28) but not statistically significant, p-value = 0.259.

The Plasma testosterone response to lower limb immersion in different water sample on Day-15 is shown in Figure 9. Observation shows that those exposed to marine water (-0.46 ± 0.35) and fresh water (-0.23 ± 0.18) were lower than those exposed to distilled water (control group) (-0.19 ± 0.16) but not statistically significant, p-value = 0.455 and 0.811 respectively.

Fig. 1: Mean Age of the Subjects

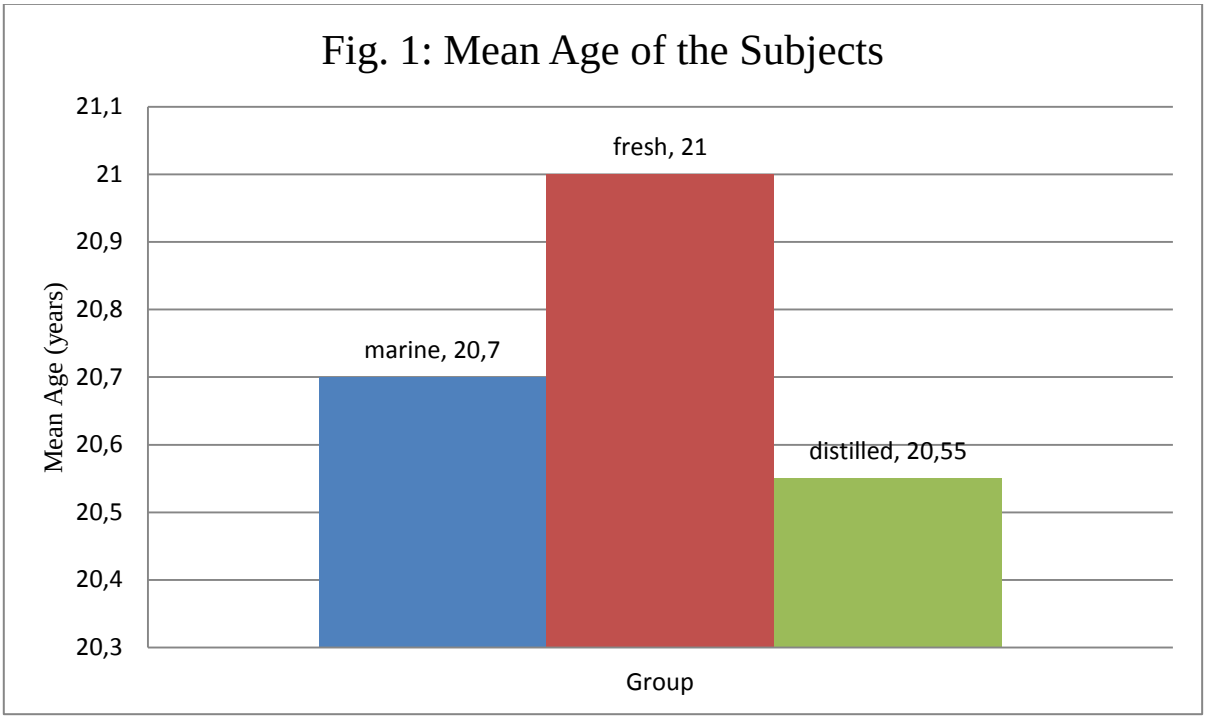


Fig. 2: Mean BMI of the Subjects

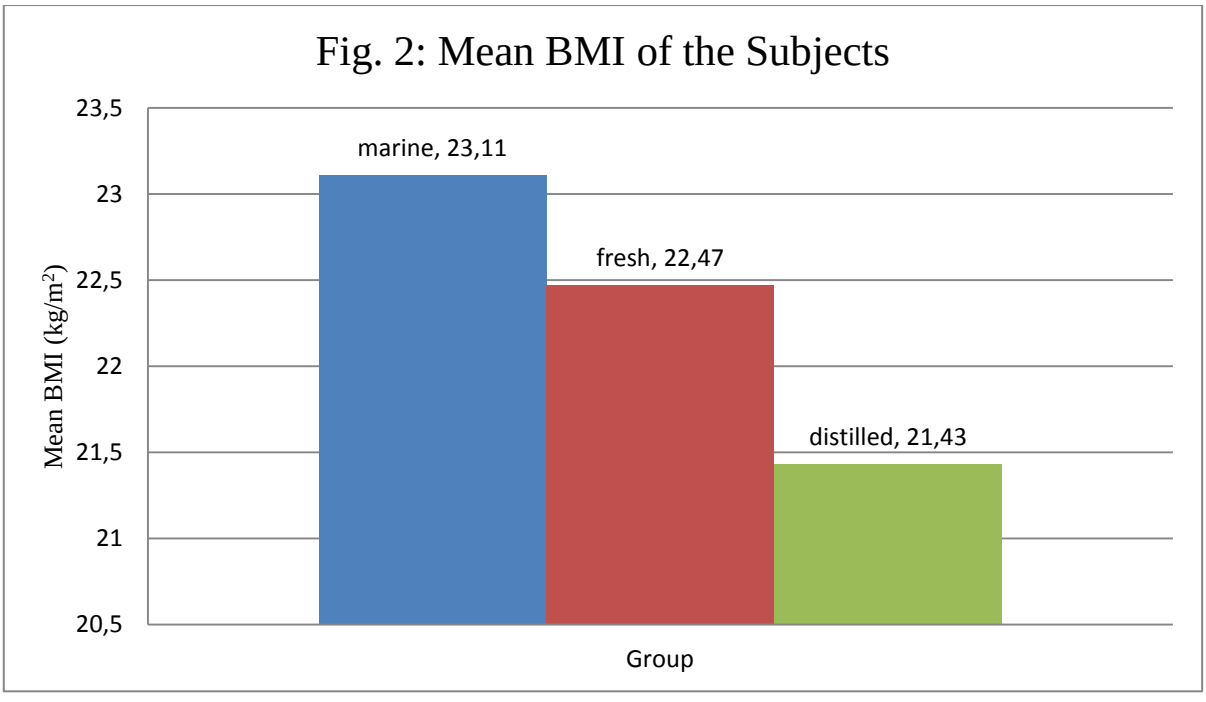


Fig. 3: Plasma testosterone response to lower limb immersion in marine water at variable time interval.

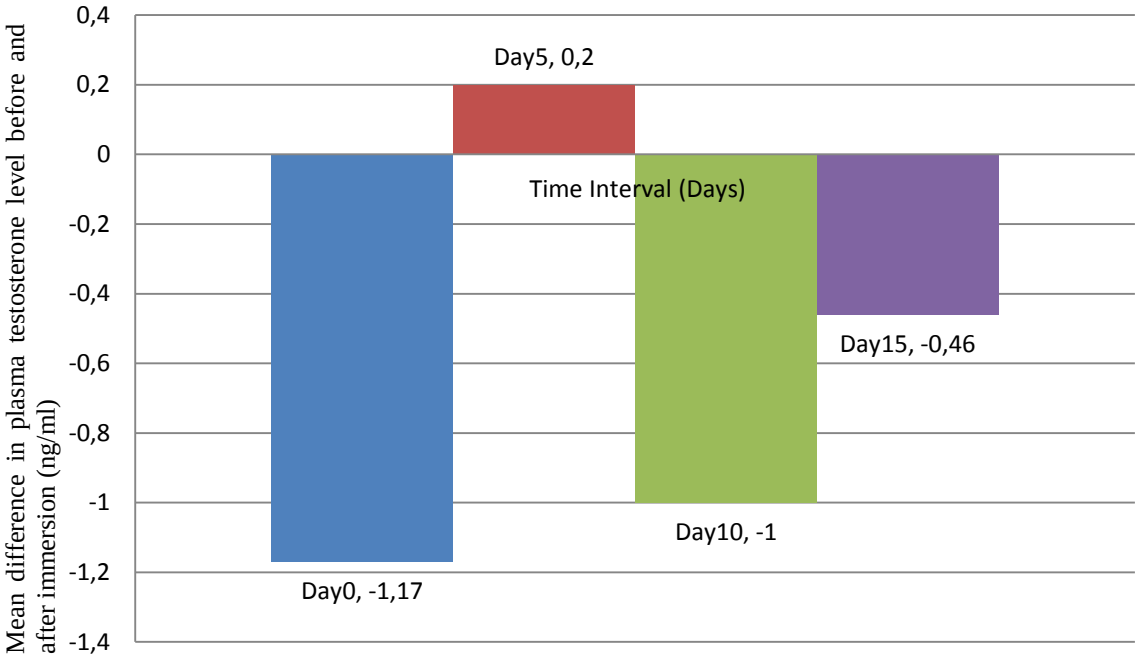


Fig. 4: Plasma testosterone response to lower limb immersion in fresh water at variable time interval.

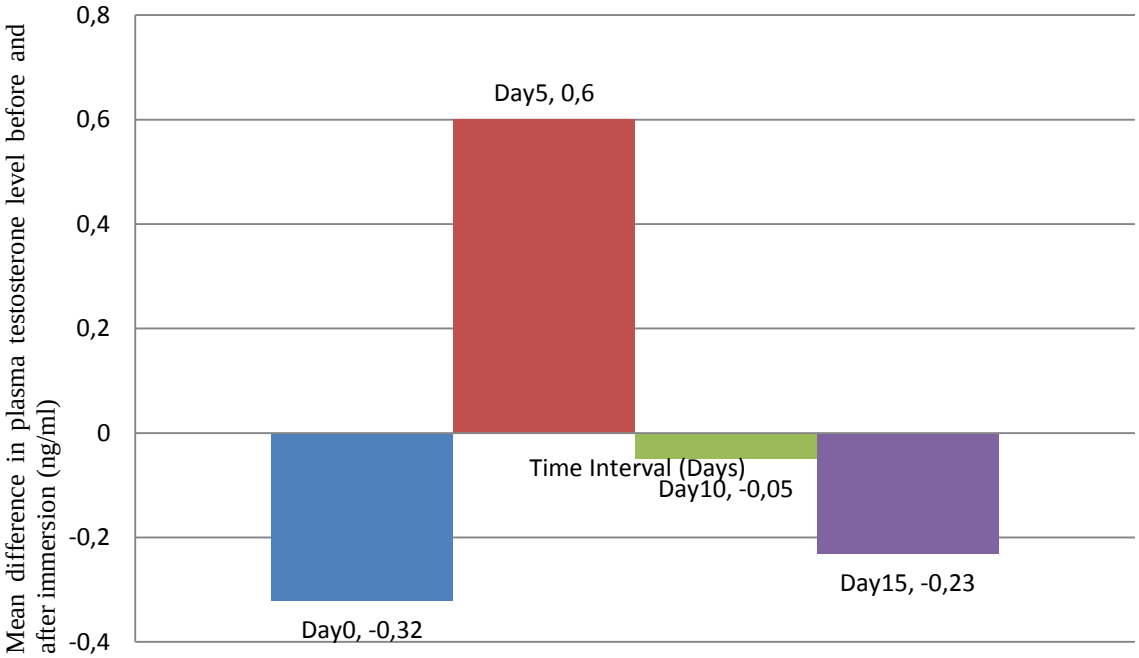


Fig. 5: Plasma testosterone response to lower limb immersion in distilled water at variable time interval.

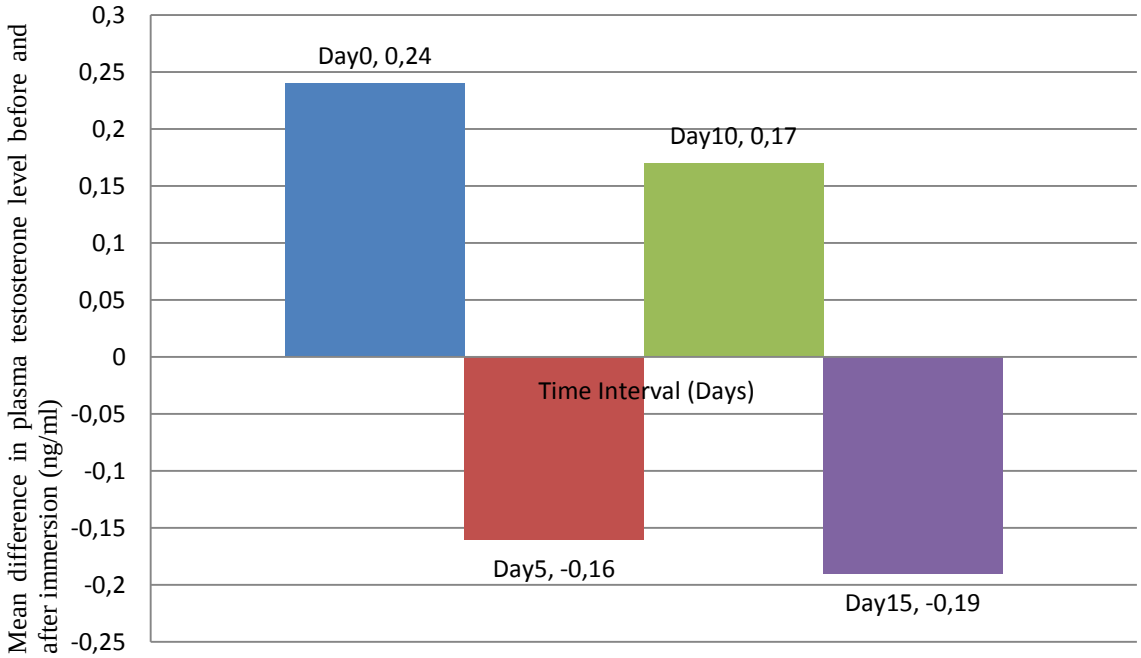


Fig. 6: Plasma testosterone response to lower limb immersion in different water sample on day-0.

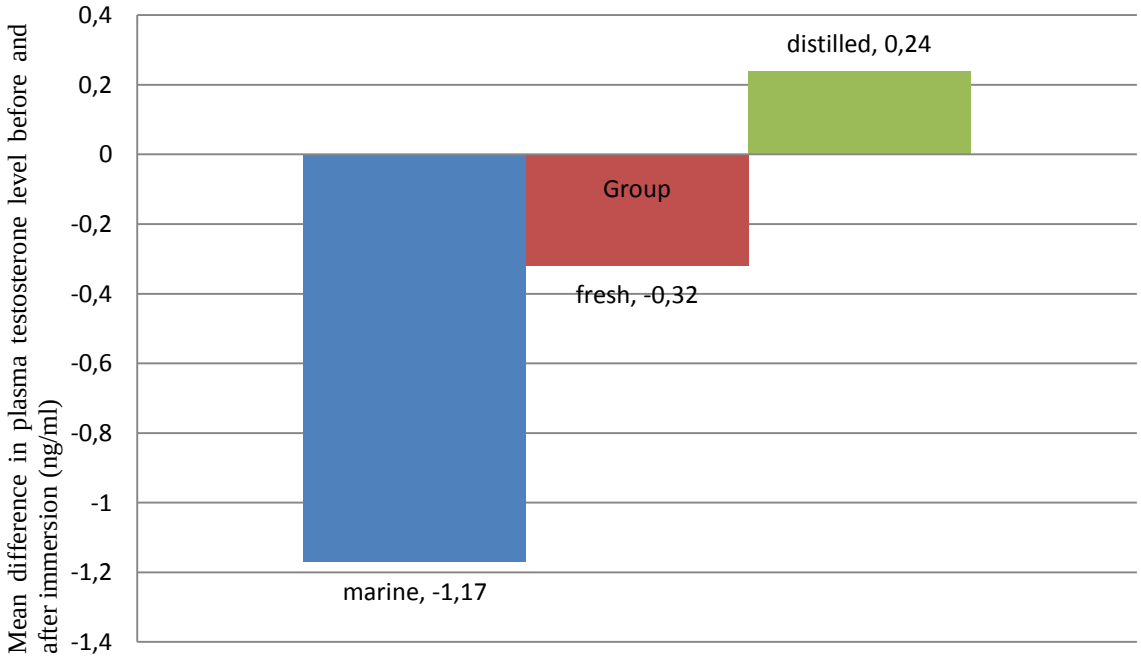


Fig. 7: Plasma testosterone response to lower limb immersion in different water sample on day-5.

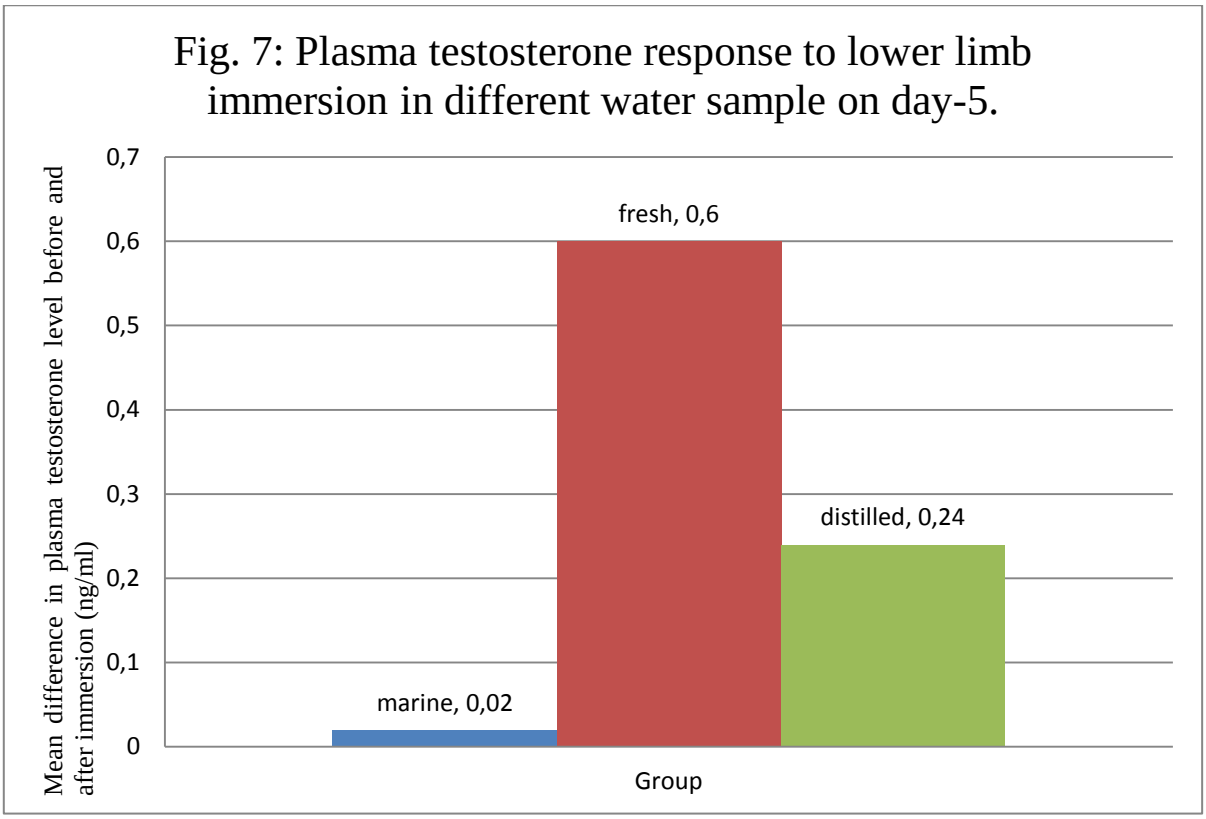


Fig. 8: Plasma testosterone response to lower limb immersion in different water sample on day-10.

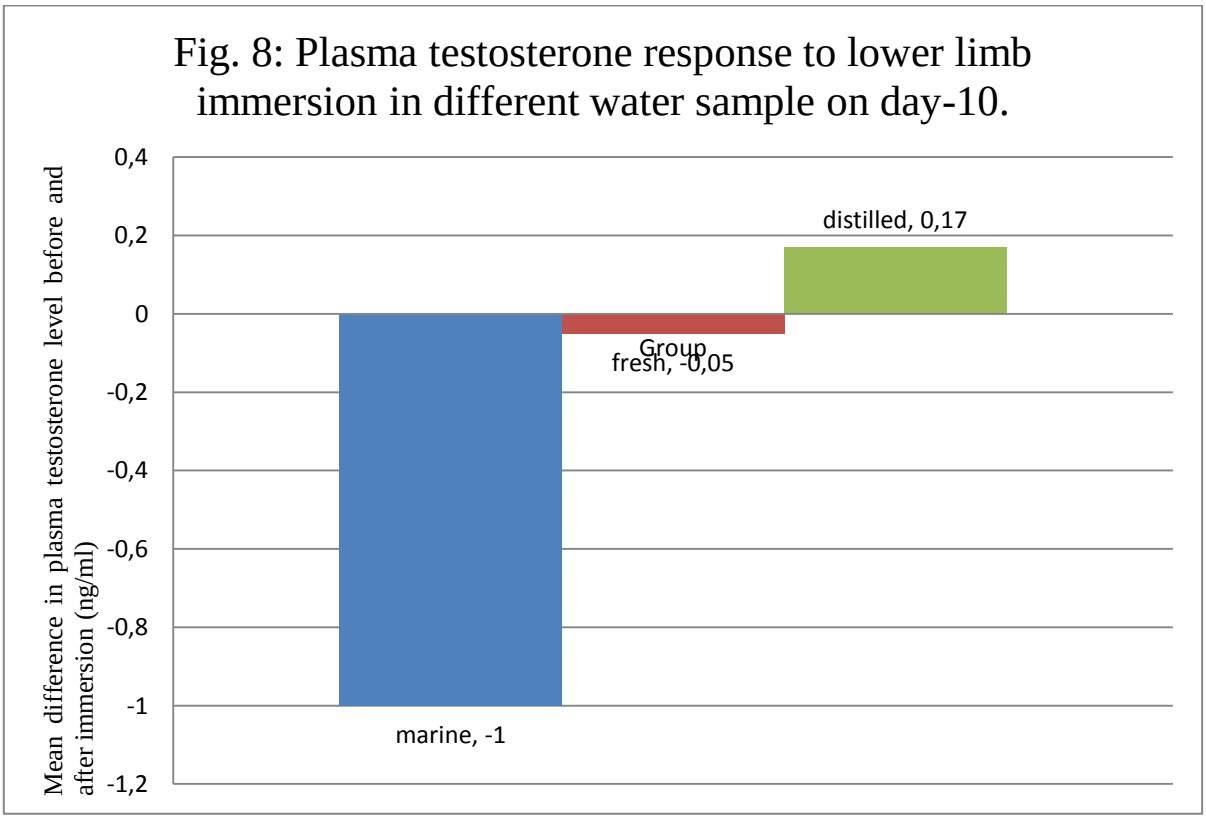
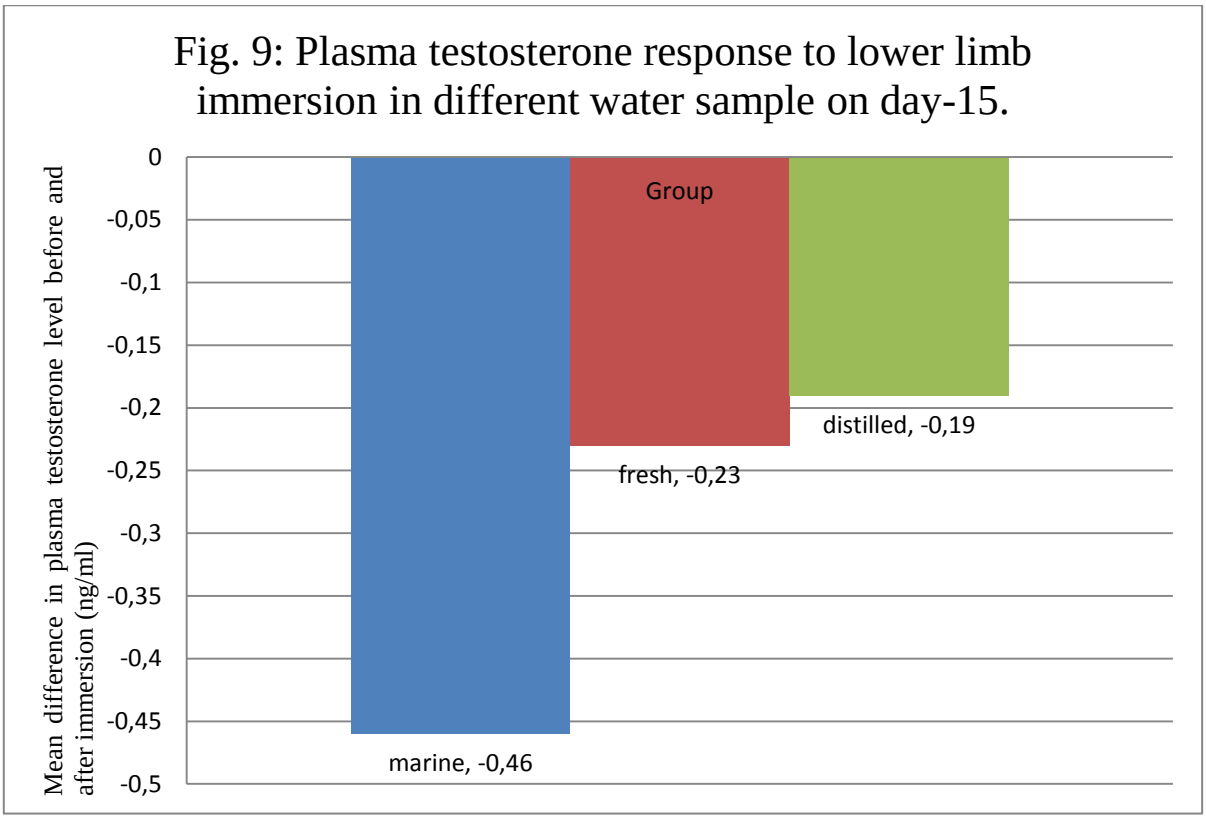


Fig. 9: Plasma testosterone response to lower limb immersion in different water sample on day-15.



Discussion

Normal BMI (BMI < 25kg/m^2) subjects were recruited to avoid incidence of low testosterone due to obesity.[7] Corona *et al*, has both reported in their different research that higher BMI leads to low serum testosterone.[8] Shamim *et al*,; Osuna *et al*, has both reported a negative correlation between testosterone and BMI in their various studies.[9][10]

The median and modal ages for all groups however lie between 19 and 21 years. The younger age used in this research help to eradicate the possibilities of low testosterone due to old age. Age above 50years has been reported to correlate with decrease serum testosterone.[11]

The negative response to testosterone secretion by the subjects as shown in (Figure 3) may be as a result of the nature of water (marine water). It is likely that substance (especially Na) passed through the skin into blood as a result of high concentration of sodium chloride which may alter the barrier function of the corneal layer. According to Frederic *et al*, barrier function of the skin can be compromised if there is a compromise in the structure of the skin.[4] In the research conducted by Bei and Daniel, and Reckelhoff *et al*, they reported that testosterone was implicated in reduced renal sodium excretion using male spontaneously hypertensive rats.[12][13] In a different research it was reported that testosterone seems to activate the rennin-angiotensin system, resulting in sodium retention, higher BP and renal injury.[14] Another research (Jonathan *et al*,) reported that, the presence of androgen receptor transcript in the kidney of each strain is consistent with the idea that testosterone binds to the androgen receptor in the kidney to play a role in controlling Na regulation.[15] With regards to localization of the AR studies have shown AR in proximal and distal tubules[16] and in other locations within the kidney (pars recta and cortical collecting duct (by real-time PCR) and low but detectable levels in medullary and cortical parts of the thick ascending limb of Henle's loop.[17] Quinkler *et al*, showed that testosterone increased the expression of the alpha-subunit of the epithelial sodium channel in a human renal proximal tubule cell line 2–3 fold which was blocked by flutamide.[18] This work is consistent with previous reports where the serum

testosterone level showed a negative response to exposure to high salt(Na) concentration, this may be as a result of the free testosterone in the blood has been bound to the androgen receptor in the kidney to trigger the mechanism for the reduction of Na excretion from the kidney in other to balance osmolarity and Na concentration in the body and outside the body. This may cause reduction in the number of free testosterone. Day-5 shows a positive response to testosterone secretion, this may be as a result of the body trying to augment for the deficit recorded previously as a result of the exposure by freeing more protein bound testosterone in the blood. When result obtained from Day-5, Day-10 and Day-15 is compared with Day-0 as shown in (Figure 3), it shows a significantly higher plasma testosterone concentration in Day-5 than in Day-0. This may likely be as a result of the body trying to re-adjust to the new environment.

The results for those exposed to fresh water were similar to those exposed to marine water but shows less negative response to testosterone secretion by the subjects as shown in (Figure 4). This may be as a result of concentration difference of individual substance present in the two water samples. Naturally, fresh water contains substance with less salinity like magnesium and potassium. Two separate research has reported that magnesium supplement caused an improvement on testosterone in male subjects [19][20] and the relationship between magnesium and testosterone was independent of body mass index.[21] This suggests that magnesium may also be responsible for more positive response in fresh water when compared with response in marine. This research supports the findings of previous reports effects of magnesium on serum testosterone level.

The fluctuating pattern observed in serum testosterone among those exposed to distilled water as shown in (Figure 5) is opposite of what was obtained in test group which may be attributed to the nature of water (distilled water). Normal pulsative pattern of testosterone secretion may be responsible for the fluctuations noticed in subjects in control group.

The result of this research shows that the plasma testosterone level in those exposed to marine water were significantly lower on Day-0 and Day-10 when compared with result of those exposed to distilled water (control group) while the plasma testosterone level of those exposed to marine water on Day-5 and Day-15 were lower than those exposed to distilled water (control group) as shown in (Fig. 6, Fig. 7, Fig. 8 and Fig. 9) but not statistically significant. This maybe as a result of concentration difference in salt (NaCl) present in the different water samples. Several research has reported that testosterone plays a role in sodium retention in the kidney.[14][15][18] The binding of free testosterone to androgen receptors in the kidney maybe responsible for the negative response noticed in subjects exposed to marine water.

The results for those exposed to fresh water when compared with those exposed to distilled water (control group), shows no significant difference in plasma testosterone level.

There were scarce literatures on this area, from the results of this research it could be suggested that immersion of lower limb in marine water has a negative effect on testosterone secretion in adult male but the mechanism of action for the depressive activities is not well understood.

Conclusion

After considering the results of the different groups, there was indication that plasma testosterone level was lower in those exposed to marine water than in fresh water and distilled water (control group) this suggest that the nature (marine) of the water may be responsible for the depressive action on testosterone secretion but physiological adaptation may tend to restore the effects with time.

Recommendation

Further research should be conducted in this area on long term duration of about six months and

above to understand the effects better. Also further research should be conducted to ascertain the composition of the different water and to ascertain the possible mechanism of action.

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