International Journal of Science and Technology

Seventh edition

Volume 3, Issue 2

(1-22) 2026

The effect of increased contraction and relaxation (muscle stress) during skeletal muscle exercise on physiological parameters and it is impact on muscle tissue.

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Published on: Published online on 4, May, 2026

Abstract :

In this study, 90 men aged 20 to 40 were sampled and classified into three groups: the first group exercised for approximately two hours daily and injected testosterone and growth hormone; the second group exercised for approximately one hour daily and did not inject testosterone or growth hormone; and the third group neither exercised nor injected the hormones. The results of the calcium test were similar in the three categories. As for creatinine, which is a waste product excreted by the kidneys and results from the breakdown of creatine in skeletal muscles, there was a slight difference between the three categories. Also, when creatinine rises in athletes, it is not considered dangerous because it results from increased breakdown of creatine in the muscles due to increased muscle contraction and relaxation. As for body mass, the difference was clear, as it increased in athletes. One of the observed symptoms is that individuals who, in addition to intramuscular injections, also received local injections to increase muscle size and shape, suffer from hardening and fibrosis of that part of the muscle. Furthermore, this fibrosis increases in temperature and may potentially develop into serious complications. Therefore, we may see more noticeable differences if the volumes of injected hormones are increased. This is in addition to changes in voice tone and appearance due to a deficiency in memory and growth vitamins. From this, we conclude that hormone injections should be administered medically and under medical supervision, according to the athlete's needs. However, they should not be injected into anyone whose hormone levels are normal and whose muscle mass is appropriate for their size, in order to avoid complications and to obtain the health benefits from the exercise they practice.

Key words: (contraction, relaxation, muscle, tissue, skeletal)

Introduction

Mobility is a critical indicator of longevity in human health,[1–3] and exercise can sustain a level of skeletal muscle composition necessary for bodily resilience (such as impact from falls), strength, and cardiovascular health.[4] Beyond a biomechanical role, recent research has implicated muscle contraction in regulating biochemical communication with many organ systems. While glucose uptake and thermogenic pathways have been understood for decades, recent studies have highlighted that exercising muscles also release signaling molecules, "myokines," that influence vascularization, bone formation, immune system function, peripheral nerve growth, and even glucagon-like peptide-1 (GLP-1) secretion.[5–8] Tissue engineering can help advance understanding of muscle physiology by enabling high-throughput in vitro observation of changes in muscle fiber morphology, contractility, and gene expression. [9-10] Reproducible protocols for culturing and exercising skeletal muscle tissues in vitro can address current drawbacks of in vivo studies, in which exercise regimens can be difficult to uniformly apply across individuals, and muscle-specific responses can be hard to isolate.[11-12]Most in vitro exercise studies have leveraged electrical stimulation to trigger repeated muscle contraction. In this approach, applying an electric field to cell culture media opens voltage-gated ion channels in the cell membrane, including the voltage-gated calcium ion channels that trigger excitation-contraction coupling. Studies have reported that prolonged electrical stimulation of engineered muscle tissues leads to deterioration of contractile performance over time, potentially due to electrolysis of the culture media that can change pH.[13–16] Moreover, electrical stimulation typically generates bubbles that reduce optical accessibility and the ability to record precise functional readouts via optical microscopy. Finally, electrical stimulation requires placing tissues between pairs of precisely spaced electrodes, often requiring extensive manual handling steps and multi-step electrode sterilization protocols. Advances in optogenetics have enabled a less invasive alternative to electrical stimulation of skeletal muscle. In this approach, muscle cells can be engineered to express light-gated membrane calcium ion channels that can be specifically activated to initiate contraction. Optical stimulation also offers precise spatial control and could be used to pattern parts of a tissue, rather than the tissue as a whole, aligning with in vivo studies that highlight the benefits of spatially targeted muscle stimulation.[17-18] A current drawback of optogenetic exercise approaches is that the lack of commercially available optogenetic cell lines limits broader access. However, several papers have reported effective methods for researchers to engineer optogenetic muscle cells and also highlighted best practices for avoiding off-target effects of genetic engineering on downstream muscle contractility.[19-20] In previous studies, we have leveraged optical stimulation to exercise tissue-engineered 3D muscle constructs in vitro and in vivo, and demonstrated that daily optical stimulation can increase contractile force by _300%.[18-21] However, these studies did not systematically investigate or optimize how different optical exercise training regimens impact muscle gene expression. Moreover,

given the opacity of 3D muscle tissues, such culture platforms do not enable uniformly stimulating all cells within a tissue at the same light intensity. Precise investigation of muscle transcriptional responses to prolonged optical stimulation thus requires scalable methods for culturing and uniformly optically stimulating 2D monolayers of contractile muscle.[22–26] Historically, 2D skeletal muscle cultures have rarely been leveraged for exercise studies, as muscle monolayers typically delaminate from underlying substrates within a few days of culture due to generated passive tension and active contractile forces.[27] To address this challenge, we have recently demonstrated that differentiating 2D skeletal muscle monolayers on micro-topographically patterned hydrogels enables longitudinal studies of aligned contractile tissues over several weeks.[7,28] In this study, we leverage this tissue fabrication protocol and combine it with open-source hardware for optical stimulation of standard multi-well tissue culture plates.[29,30] We first establish minimum intensity thresholds, or rheobase, for inducing twitch in optogenetic skeletal muscle fibers derived from the widely used C2C12 line expressing the blue light sensitive calcium ion channel ChR2(H134R).[13,17,21,23,26,31–36] To our knowledge, optical rheobase is not currently documented in the optogenetic muscle literature. We then conduct high-throughput optical exercise experiments of varying stimulation frequency (1–10 Hz) and duration (0–60 min) and leverage bulk RNA sequencing (RNA-seq) to examine how modulating training regimen impacts muscle physiological and pathological gene expression. Our findings establish the diverse effects of optically exercising muscle tissues, with the goal of establishing a practical foundation for researchers to leverage optogenetics to promote maturation of tissue engineered skeletal muscle.

Methodology

Ninety men aged 20 to 40 were selected and divided into three groups:

Group 1: (30 male) who exercised and received hormone injections.
Group 2 :(30 male) who exercised but did not receive hormone injections.
Group 3 :(30 male) who neither exercised nor received hormone injections.

Study design

The study design in this research was based on cross-sectional analysis, and we used the one-away ANOVA function of the SPSS statistical program and calculated the p-value as an indicator of the significant factor.

Patient and sample

A venous blood sample was taken from individuals aged 20 to 40 years old (males only). The sample was centrifuged, and the serum was separated for use in the required measurements at a private laboratory in Baghdad, Iraq.

Measurements

Blood Creatinine levels were measured using a Cobas 411 analyzer, with a normal range of 0.6 to 1.2 mg/dL.

Blood calcium levels were also measured using a Cobbas 411 analyzer, with a normal range of 8.5 to 10.5 mg/dL.

Testosterone levels were measured after intramuscular and local injections. The normal range for testosterone in men was 1.91-8.41 ng/ml, and for growth hormone in men, it was 0.05- 3 ng/ml.

Calculation of BMI

Body Mass Index (BMI) is calculated by dividing weight in kilograms by the square of height in meters (KG/M^2) to assess a healthy weight for adults. The equation is:

$BMI= WIEGHT/LENGTH^2$

The result determines whether the weight is normal (18.5-24.9), overweight (25-29.9), or obese (>OR=30).

Result and discussion

The statistical results for the three groups showed that training hours had an effect on body mass for individuals who exercised, regardless of whether the hormone was injected or not. The other statistical results and their relationship will be shown below.

Table 1: Descriptive statistics show the variables included in the statistics.

Descriptive Statistics					
	Minimum	Maximum			
variables	value	value	Mean	Std. Error	Std. Deviation
BMI(m2/kg) sport &inj. H	30.5	54.3	36.876	1.1125	6.0935
Ca(mg/dl) sport &inj. H	8.4	10.6	9.641	.1251	.6851
creatinine .sport &inj. H	.4	1.2	.700	.0323	.1767
BMI(m2/kg) no sport& no inj. H	30.1	45.0	35.245	.7750	4.2447
Ca(mg/dl) no sport& no inj. H	8.4	10.6	9.872	.1161	.6359
Creatinine . no sport& no inj. H	.4	1.2	.705	.0426	.2334
BMI(m2/kg) sport &no inj. H	19.1	29.4	26.313	.4724	2.5872
Ca(mg/dl) sport &no inj. H	8.4	10.6	9.753	.1278	.6998
creatinine . sport &no inj. H	.4	1.9	1.010	.0720	.3942

Table2: Comparison of body mass index (BMI) in exercisers with injection hormones Creatinine and blood calcium levels using a one-away ANOVA function.

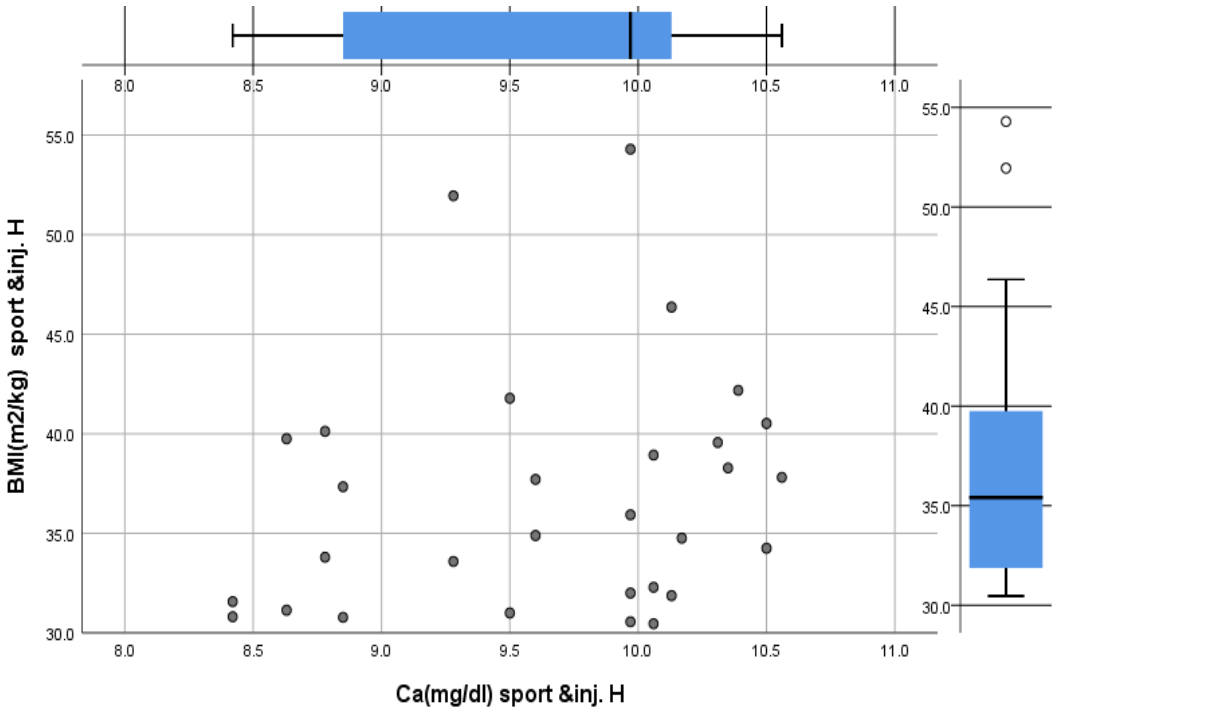
Variables		Sum of Squares	Mean Square	F	Sig.
Ca(mg/dl) sport &inj. H	Between Groups	13.613	0.469	0.0	0.0
creatinine .sport &inj. H	Between Groups	0.906	0.031	0.0	0.0

Table3: Comparison of body mass index (BMI) in no exercisers with no injection hormones Creatinine and blood calcium levels using a one-away ANOVA function.

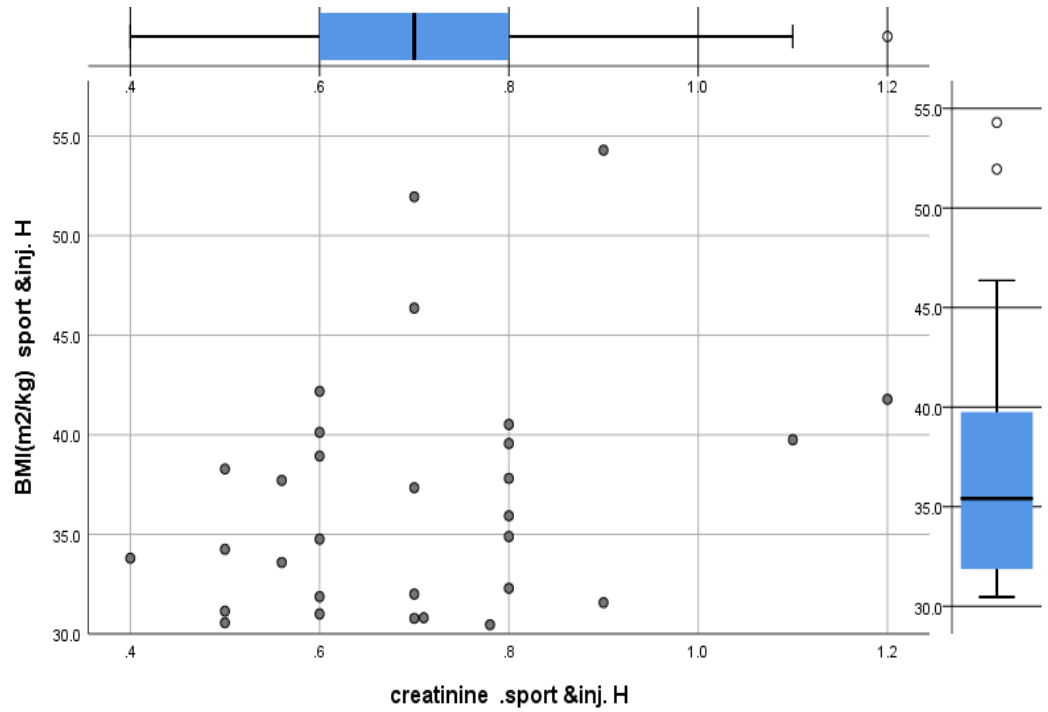
Variables		Sum of Squares	Mean Square	F	Sig.
Ca(mg/dl) no sport& no inj. H	Between Groups	10.181	0.377	0.488	0.851
Creatinine . no sport& no inj. H	Between Groups	1.505	0.056	1.496	0.479

Table4: Comparison of body mass index (BMI) in exercisers with no injection hormones Creatinine and blood calcium levels using a one-away ANOVA function.

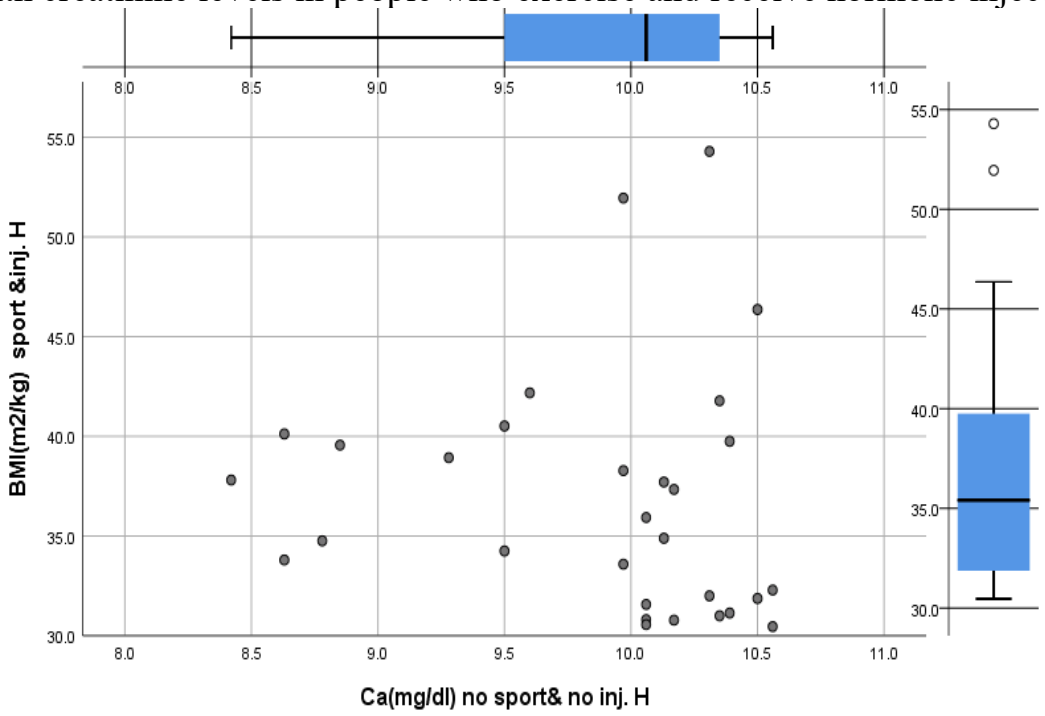
Variables		Sum of Squares	Mean Square	F	Sig.
Ca(mg/dl) sport &no inj. H	Between Groups	12.338	0.441	0.237	0.951
Creatinine . sport &no inj. H	Between Groups	4.487	0.160	8.013	0.273



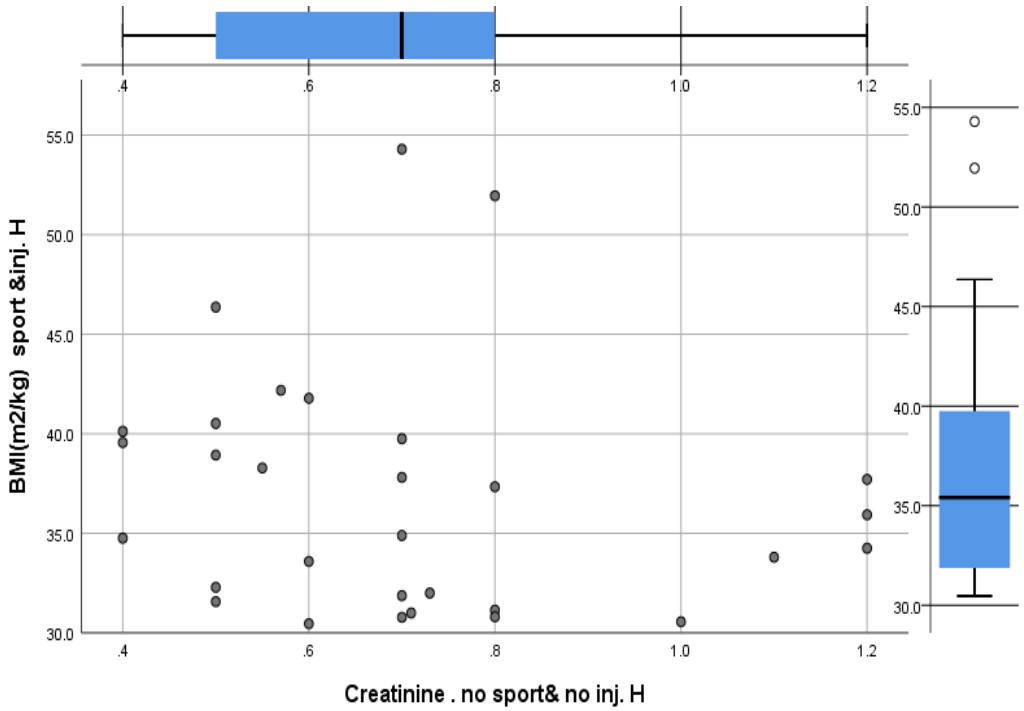
Graph 1: Show Comparison of BMI in people who exercise and receive hormone injections with calcium levels in people who exercise and receive hormone injections.



Graph 2: Show Comparison of BMI in people who exercise and receive hormone injections with creatinine levels in people who exercise and receive hormone injections.

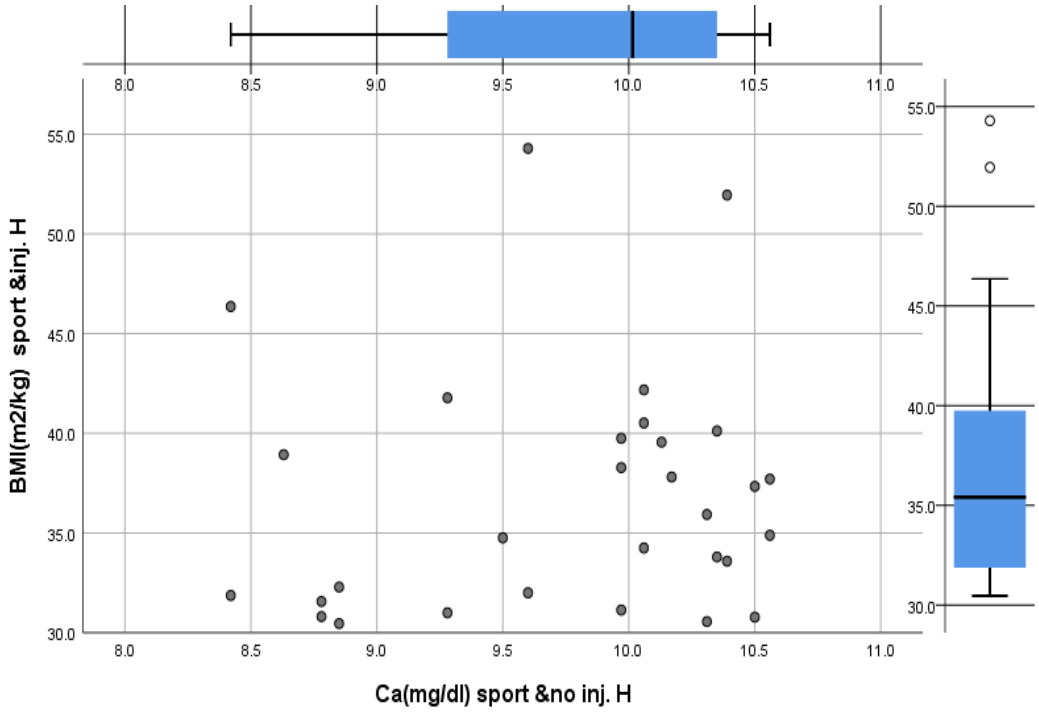


Graph 3: Show Comparison of BMI in people who exercise and receive hormone injections with calcium levels in people who no exercise and no receive hormone

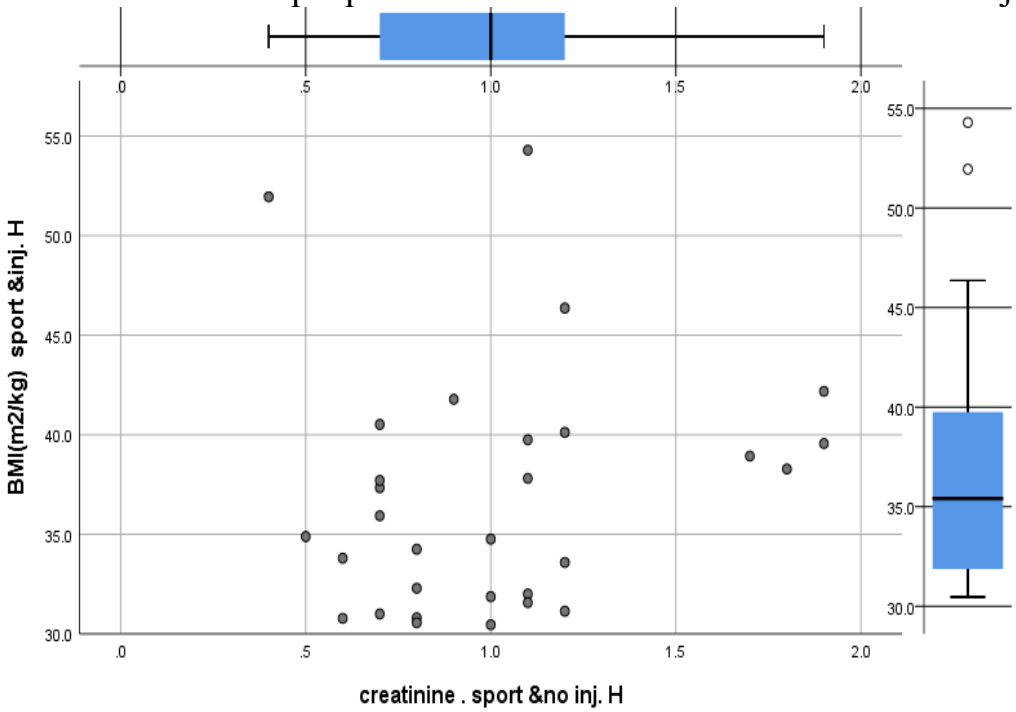


injections.

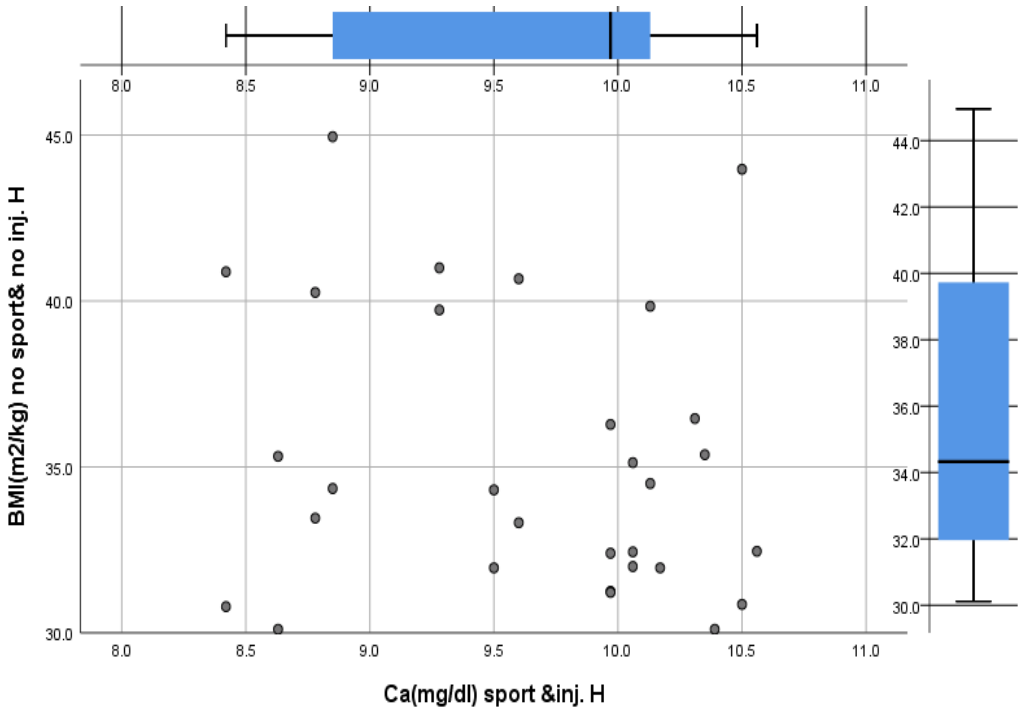
Graph 4: Show Comparison of BMI in people who exercise and receive hormone injections with creatinine levels in people who no exercise and no receive hormone injections.



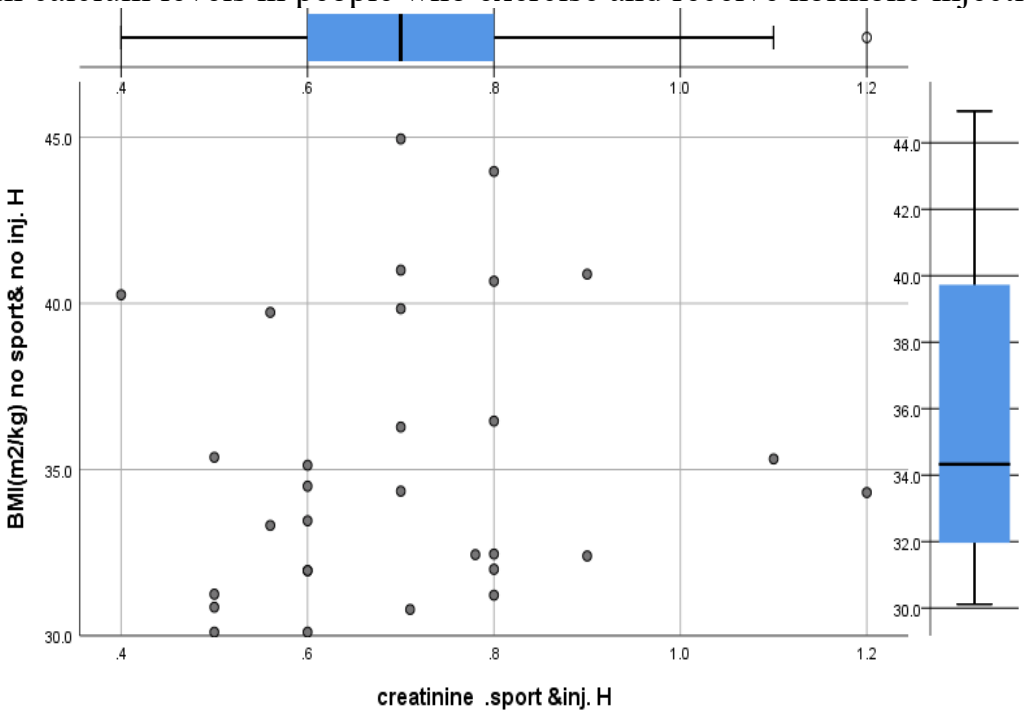
Graph 5: Show Comparison of BMI in people who exercise and receive hormone injections with calcium levels in people who exercise and no receive hormone injections.



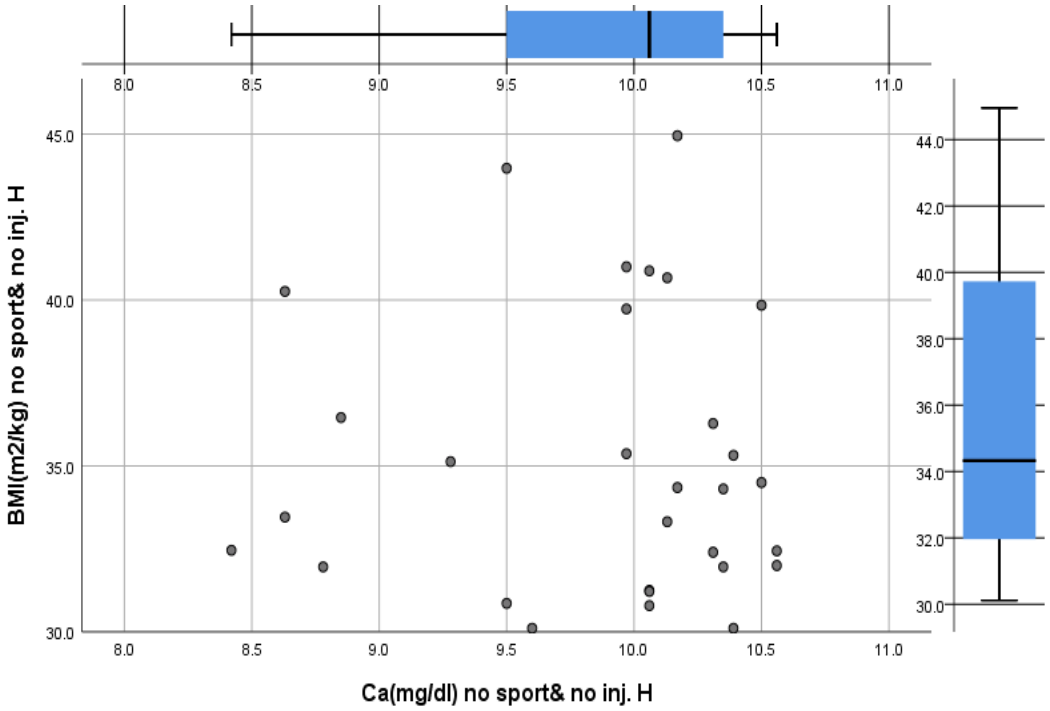
Graph 6: Show Comparison of BMI in people who exercise and receive hormone injections with creatinine levels in people who exercise and no receive hormone injections.



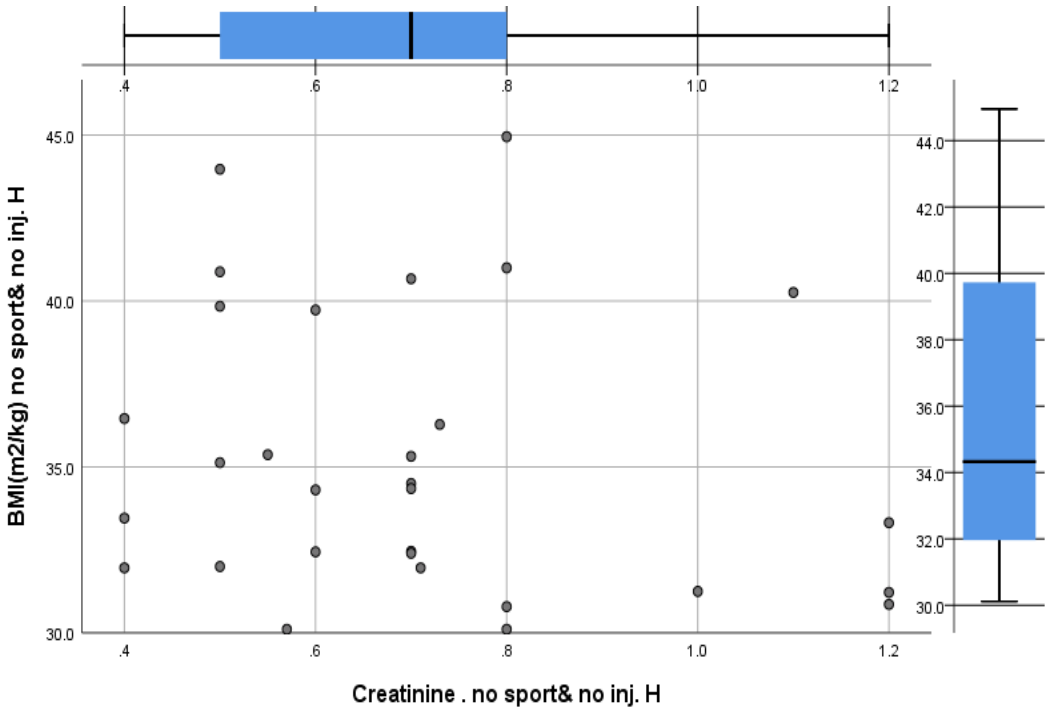
Graph 7: Show Comparison of BMI in people who no exercise and no receive hormone injections with calcium levels in people who exercise and receive hormone injections.



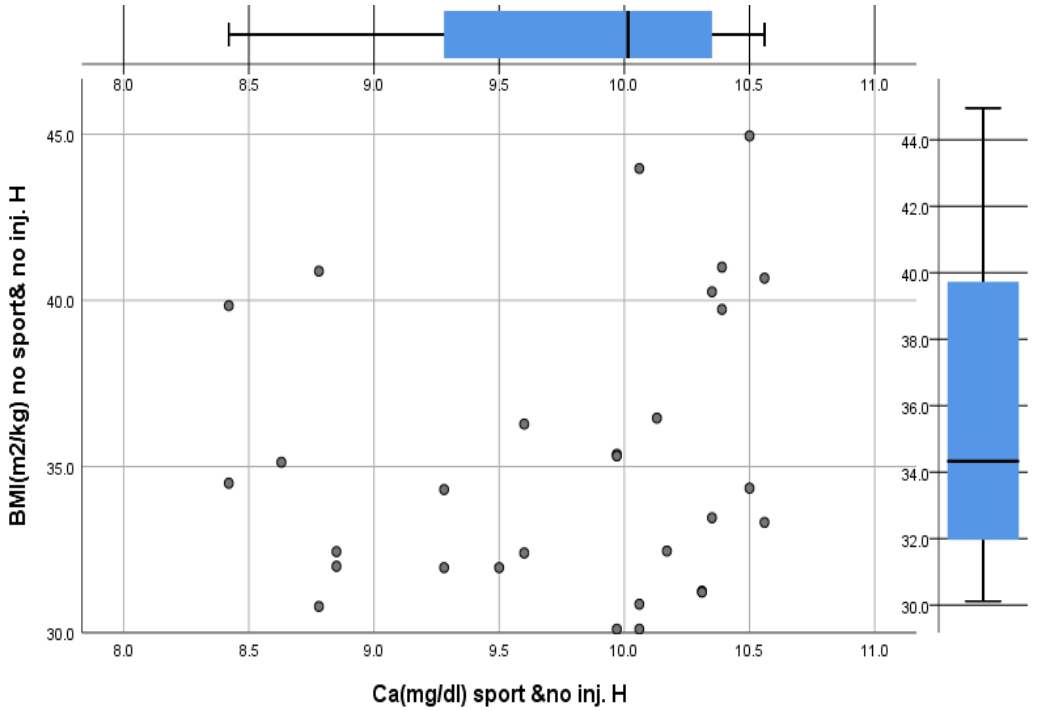
Graph 8: Show Comparison of BMI in people who no exercise and no receive hormone injections with creatinine levels in people who exercise and receive hormone injections.



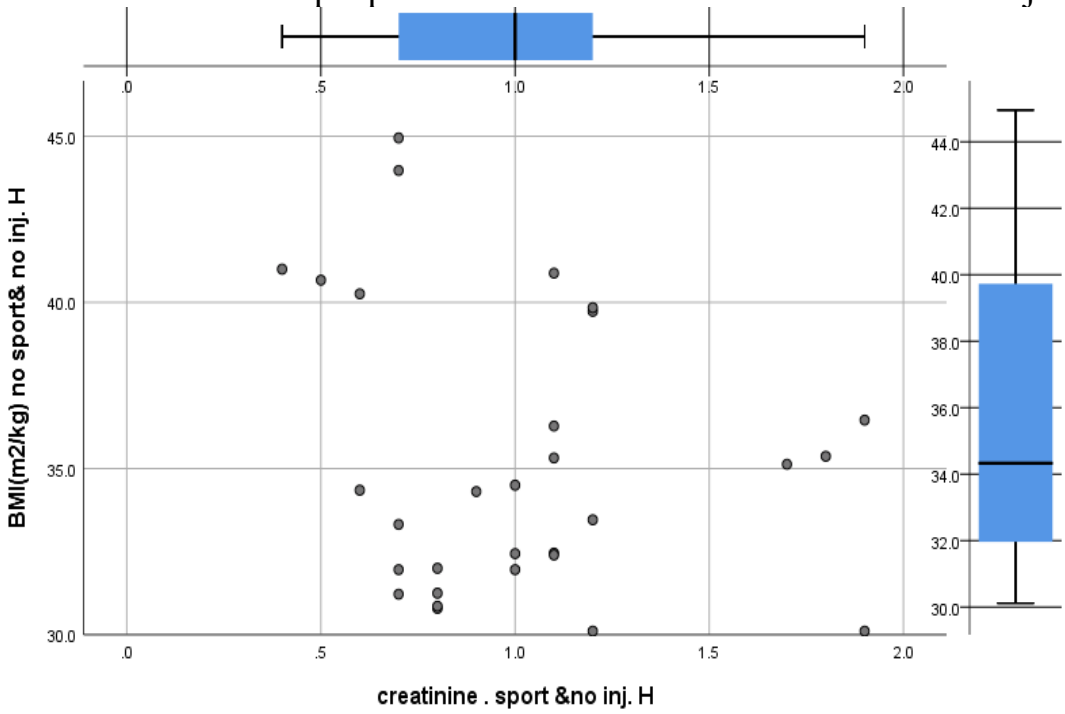
Graph 9: Show Comparison of BMI in people who no exercise and no receive hormone injections with calcium levels in people who no exercise and no receive hormone injections.



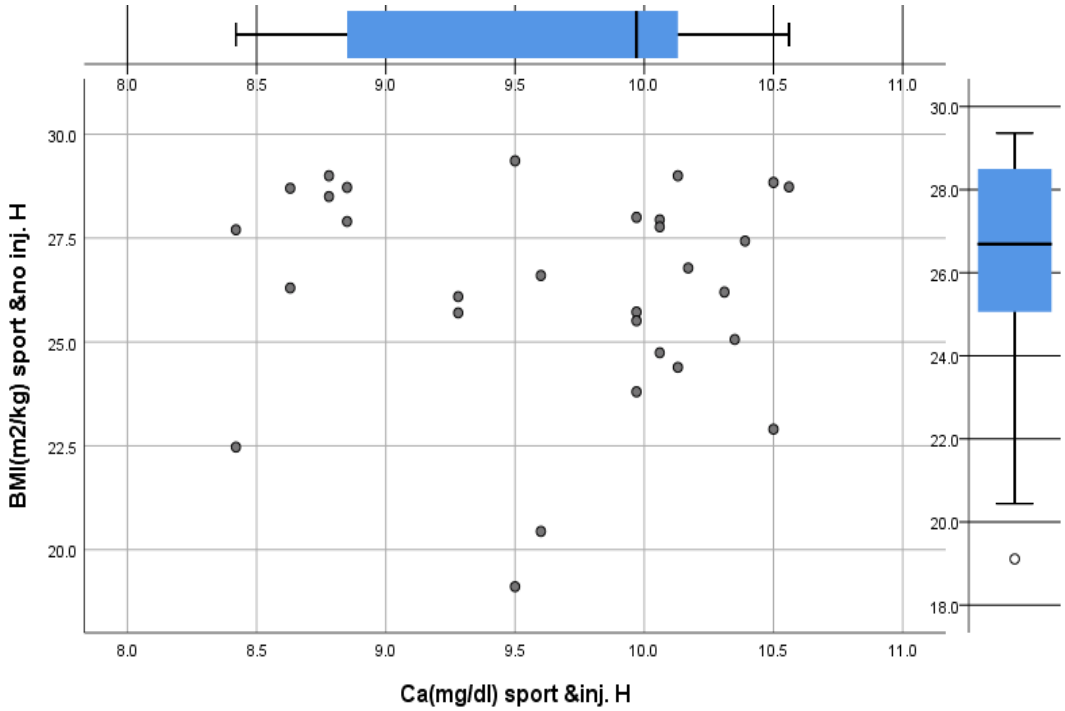
Graph 10: Show Comparison of BMI in people who no exercise and no receive hormone injections with creatinine levels in people who no exercise and no receive hormone injections.



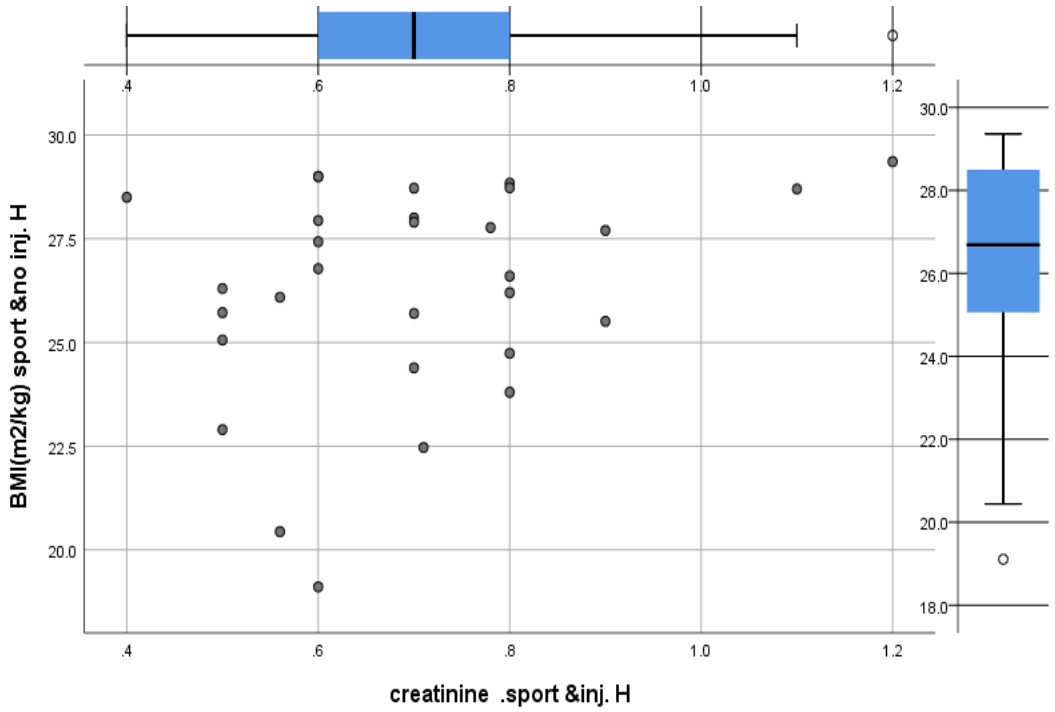
Graph 11: Show Comparison of BMI in people who no exercise and no receive hormone injections with calcium levels in people who exercise and no receive hormone injections.



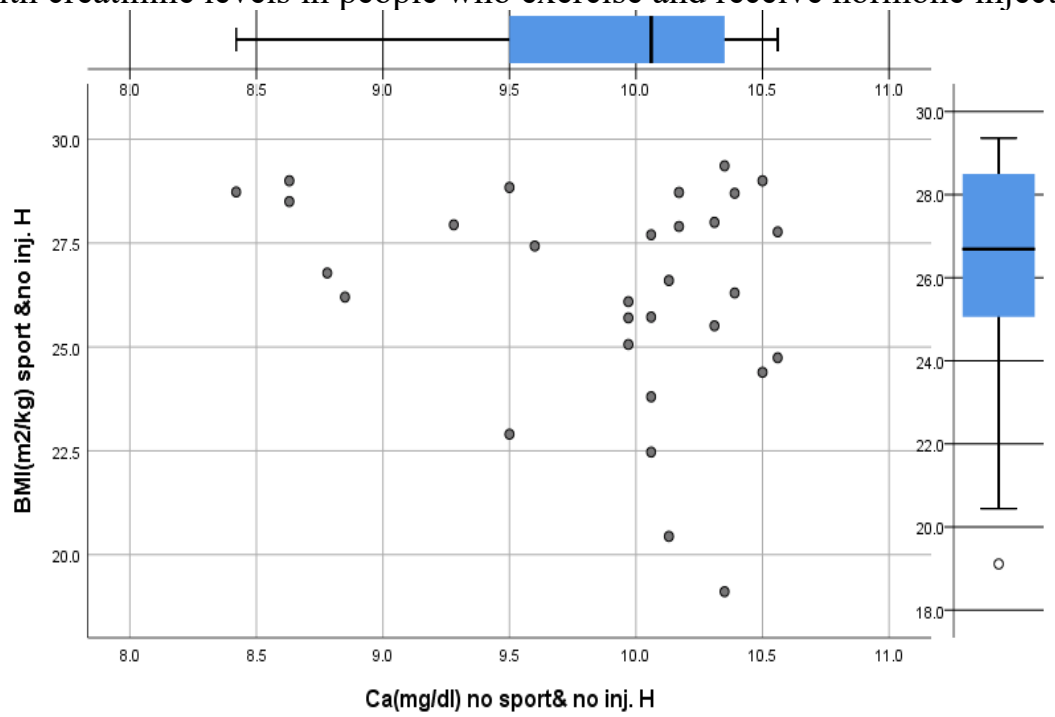
Graph 12: Show Comparison of BMI in people who no exercise and no receive hormone injections with creatinine levels in people who exercise and no receive hormone injections.



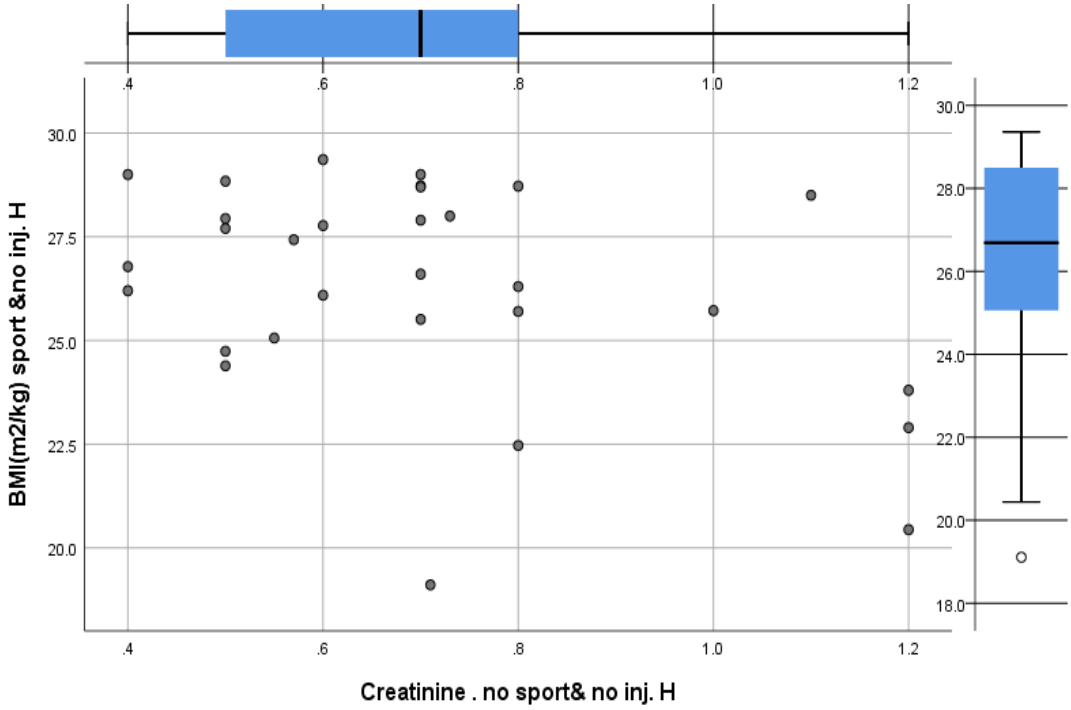
Graph 13: Show Comparison of BMI in people who exercise and no receive hormone injections with calcium levels in people who exercise and receive hormone injections.



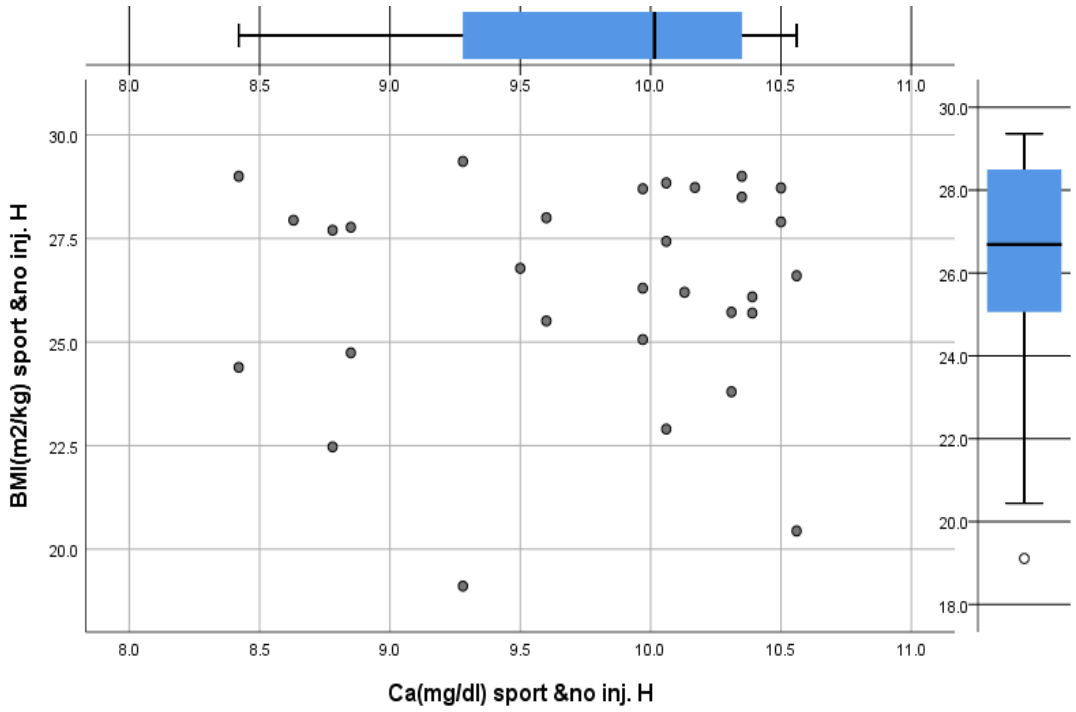
Graph 14: Show Comparison of BMI in people who exercise and no receive hormone injections with creatinine levels in people who exercise and receive hormone injections.



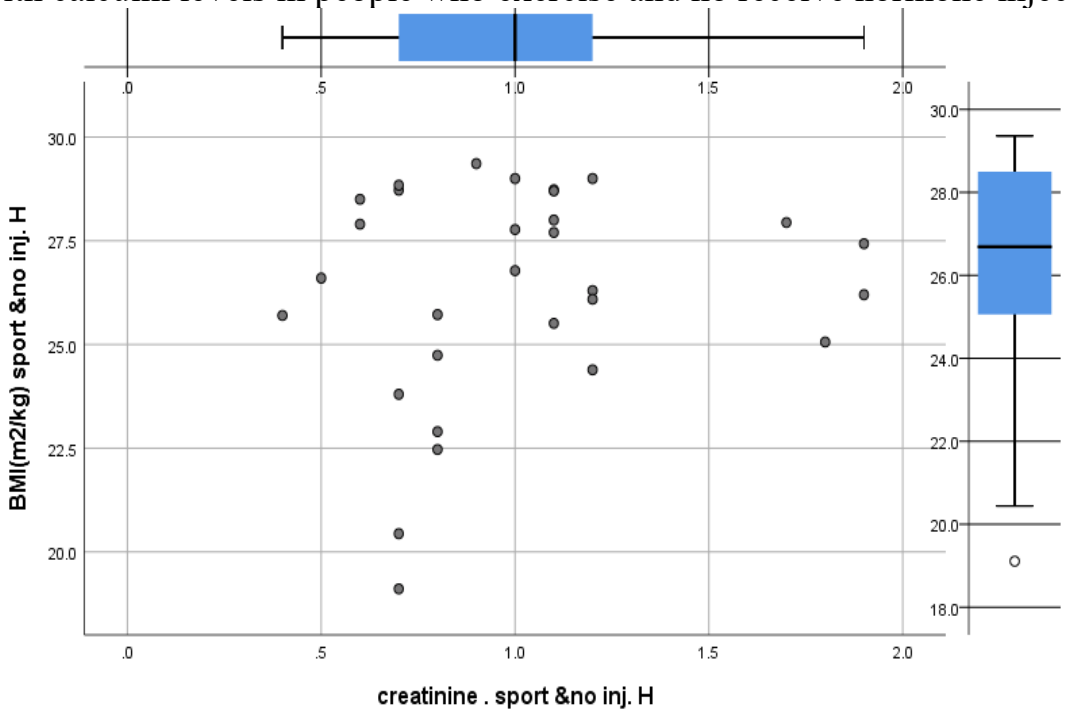
Graph 15: Show Comparison of BMI in people who exercise and no receive hormone injections with calcium levels in people who no exercise and no receive hormone injections.



Graph 16: Show Comparison of BMI in people who exercise and no receive hormone injections with creatinine levels in people who no exercise and no receive hormone injections.



Graph 17: Show Comparison of BMI in people who exercise and no receive hormone injections with calcuim levels in people who exercise and no receive hormone injections.



Graph 18: Show Comparison of BMI in people who exercise and no receive hormone injections with creatinine levels in people who exercise and no receive hormone injections.

Discussion

The descriptive statistics table shows us the maximum and minimum values, the mean, as well as the standard deviation and standard error for each of the BMI, calcium level, and creatinine level values for the three groups[2].

The statistical results in Table 2 show that the relationship between BMI, calcium, and creatinine in individuals who exercise and receive intramuscular hormone injections is 0.0, indicating a significant relationship between them. This relationship indicates an increase in calcium levels as a result of increased muscle fiber production during daily exercise, and an increase in creatine conversion to creatinine for the same reason, but the increase is considered slight[6-35].

The statistical results in Table 3 show the relationship between BMI in individuals who do not exercise or inject muscle hormones and their calcium and creatinine levels; there is no significant correlation, which confirms that the muscles were not stimulated. The p-values were 0.851 and 0.479 respectively, indicating that exercise has a significant effect on increasing muscle tissue and stimulating muscle growth[14-34].

Regarding Table 4, the statistical results show that there is no statistically significant relationship between the BMI of individuals who do not exercise and do not inject the hormone, and their calcium and creatinine levels, with p-values of 0.951 and 0.273 respectively. This confirms the absence of any stimulus for muscle fiber production and the lack of muscle mass increase[15-33].

Regarding the graphs, graphs one through six illustrate the effect of exercise on increasing muscle mass and calcium creatinine levels in individuals who exercise and inject the hormone[24].

Regarding the graphs, graphs seven through twelve illustrate the impact of inactivity on muscle mass reduction and the maintenance of calcium and creatinine levels in individuals who do not exercise and do not inject the hormone[25].

Regarding the graphs, graphs 13 through 18 illustrate the effect of exercise on increasing muscle mass, though not to a significant degree, and maintaining calcium and creatinine levels in individuals who exercise and do not inject hormones[32].

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