ISSN 2063-5346

CORRELATION BETWEEN SMOKING, SERUM SEROTONIN LEVEL, PERIPHERAL FATIGUE OF KNEE EXTENSORS AND KNEE PROPRIOCEPTION: CROSS-SECTIONAL STUDY

Mohsen Mohamed El Sayyad[1], Mohamed Abd El Hameed Mohamed Ali[2], Yasser Ramzy Lasheen[3]

Article History: Received: 02.07.2023 Revised: 15.07.2023 Accepted: 23.07.2023

ABSTRACT:

Background: Smoking is widely recognized as a contributor to numerous health problems; Furthermore, the knee extensors contribute greatly to the stability as well as proprioception of the knee joint. **Purpose:** This study was carried out to examine the correlation of serum cotinine level, serum serotonin level, peripheral fatigue of Knee extensors as well as proprioception of the Knee joint. **Materials and methods:** Sixty-six healthy males were divided into three groups of similar size based on their serum cotinine levels, 22 non-smokers Group (A) (control group) having a mean age (30.63 ± 8.43) years, mean body mass index (BMI) (21.64 ± 1.24) kg/m^2 and mean cotinine level (6.36 ± 1.43) ng/mL, 22 moderate smokers Group (B) having a mean age (29.32 ± 7.65) years, mean BMI (21.97 ± 1.01) kg/m^2 and mean cotinine level (32.86 ± 5.88) ng/mL, as well as 22 heavy smokers Group (C) having a mean age (32.77 ± 5.93) years, mean BMI (21.95 ± 1.29) kg/m^2 in addition their mean cotinine level (364.13 ± 18) ng/mL. Each participant had a blood sample obtained to test for cotinine as well as serotonin levels in the lab, accuracy in repositioning was evaluated using an isokinetic dynamometer (joint reposition error) and compute the fatigue index as a means of quantifying a person's vulnerability to fatigue. **Results:** The fatigue index demonstrated statistically significant differences among the three groups, with the greatest mean value being reported in the heavy smokers' group, and the amount of serotonin in the blood varied significantly. There was a negative correlation between serum cotinine as well as serotonin levels, with the greatest mean value being reported in the nonsmokers group. Moreover, the joint reposition error was significantly larger in the smokers' group compared to the non-smokers' group Error rates were lower among non-smokers compared to smokers. The correlation between serum cotinine and serum serotonin was a strong negative significant correlation, The correlation between serum cotinine with fatigue index of right and left knee extensors and JPE of right and left knee at 70º and 30º knee flexion were strong positive significant correlation and The correlation between serum serotonin with fatigue index of right and left knee extensors and JPE of right and left knee at 70º and 30º knee flexion were strong negative significant correlation. **Conclusion:** Cotinine has substantial impacts on the peripheral fatigue, repositioning accuracy of knee extensors as well as on serum serotonin level.

KEYWORDS: Cotinine level, Serotonin, Knee extensors, Peripheral fatigue, Repositioning accuracy.

1 Professor of Physical Therapy Department of Basic sciences Faculty of Physical Therapy, Cairo University.
2 M.Sc. of Physical Therapy Department of Basic sciences Faculty of Physical Therapy, Cairo University.
3 Assistant Professor of Physical Therapy Department of Basic sciences Faculty of Physical Therapy, Cairo University.

DOI:10.48047/ecb/2023.12.9.242

INTRODUCTION

The negative effects of smoking on human health and quality of life are well-documented. Smoking is a major public health issue with far-reaching economic and health consequences **[1].** Latest estimates indicate that by 2030, tobacco use will be responsible for over eight million annual deaths around the world. Additionally, smokers have a life expectancy that is ten years lower than that of nonsmokers **[2, 3].**

In epidemiological studies, researchers often use cotinine, a byproduct of nicotine, to differentiate between smokers and nonsmokers. Cotinine is a molecule produced by the body through nicotine, which can be found in tobacco smoke. Since nicotine is the only substance capable of producing cotinine, and since nicotine enters the blood when one smokes cigarettes, cotinine levels are a good indicator of how much nicotine one takes in **[4].**

There is evidence linking cigarette smoking to other unhealthy habits, such as inactivity, which in turn lowers aerobic power as well as overall muscle strength in smokers compared to nonsmokers **[5].** It is known that smoking significantly reduces skeletal muscle fatigue resistance in young male smokers compared to controls whose physical activity levels are similar **[6].**

The detrimental effect of smoking on fitness **[7]** suggests that smoking over an extended period of time can lead to a cumulative decline in skeletal muscle fatigue resistance. The ability to quickly extend the knee and maintain balance in one's legs are both hindered by fatigued knee muscles [**8].** The knee muscle reflex as well as proprioception are both affected by a lack of knee extensor strength **[9].**

Serotonin is a hormone that regulates many processes throughout the body, including mood, behavior, depression, sleep, appetite, learning, memory, and even the direction of body temperature, as well as some endocrine regulation and muscular contraction. The serum serotonin levels fluctuated between 101 and 283 ng/ml **[10, 11].**

It was unclear how cotinine and serotonin were connected; some research suggested that cigarette smoking increased serotonin release, while other research suggested the opposite [**12],** while others have found that smoking depletes serotonin in the brain. Cigarette smoking is associated with a 50% reduction in serotonin production. Nicotine also inhibits receptor activity and desensitizes receptors **[13].**

Muscle fatigue can result from peripheral variations in the degree of muscle activation or from a central nervous system (CNS) failure to activate the motor neurons effectively [**14].** It's a safeguard that keeps muscle glycogen stores from being depleted and inhibits the accumulation of potentially hazardous metabolic byproducts. It also prevents the constant production of strong forces that can injure the contractile parts [**15].** Fatigued muscle tissue transfers more of the work to the joint capsule as well as ligaments, raising the likelihood of an injury **[16].**

Exercise-induced decrease in maximal voluntary muscular force is what we call muscle fatigue. Potential causes include peripheral alterations in muscle action **(peripheral fatigue)**, or failure of CNS to activate the motoneurons sufficiently **(central fatigue) [14].** Basically, it is a safety mechanism that keeps us from running out of metabolic reserves in our muscles and keeps harmful metabolic products from building up.

Proprioceptors are located in muscles and joint tissues including capsular and ligamentous structures and free nerve endings, Pacinian like receptors, ruffini like receptors, muscle spindle and Golgi tendon organ (GTO). Pacinian like receptors could detect accelerating and small motion; muscle spindle respond to change in muscle length; GTO could detect position and direction of motion; finally, ruffini like receptors could detect speed and direction of motion (**17).** Serotonin, that is a master regulator in the cortex, is released when

serotonin receptors are stimulated by proprioceptive input.

As a neurotransmitter, serotonin not only delivers impulses within the brain, but it also regulates the rate at which all other neurotransmitters in the brain are activated [**18].** The neurotransmitter serotonin is reduced by nicotine, and research suggests that smoking cigarettes could reduce serotonin production by as much as half. Nicotine has a number of effects on receptors, including desensitization as well as inhibition **[13].**

The faculty of physical therapy at Cairo University has a number of modern computerised technologies accessible for use in the current investigation, including a Biodex isokinetic dynamometer. It has many attachment as well as isolated straps for the knee, shoulder, trunk, etc., making it one of the most complete computing devices. It offers isokinetic, eccentric, isometric and passive mood for all joints of the body. It records and prints out test data, graphs, and results with detailed information including torque, speed, duration, work power, peak torque, ROM, as well as repositioning errors **[19].** So, we used it in our study to measure is any repositioning accuracy and fatigue of knee extensor muscles.

PATIENTS AND METHODS:

This research was carried out between March 2022 and May 2023 at the Cairo University Faculty of Physical Therapy's isokinetic laboratory. Blood samples were collected and the fatigue index of the knee extensors was calculated in order to investigate the relationship among serum cotinine level, serum serotonin level, fatigue susceptibility, as well as knee proprioception.

- **Design:**

It was a cross-sectional design. Post test control group 1x3 design was used in the current study (independent variable was serum cotinine level and dependent variables were peripheral fatigue, repositioning accuracy, and serum serotonin levels).

- **Participants:**

Sixty-six untrained normal male subjects from Nile Hospital for Health insurance were divided into three groups with similar characteristics based on their cotinine levels A) non-smokers (control group), B) light, moderate smokers and C) heavy smokers. Each group consisted of 22 subjects.

- **Sample size calculations:**

It was done using F-test ANOVA one way with 80% power at $\alpha = 0.05$ level, for 3 groups and effect size = 0.4. A minimum of 66 participants is required for a reliable sample size, with 22 in each group. Using G*Power, an appropriate sample size was determined (version 3.0.10).

- **Inclusion Criteria:**

Only male smokers with age ranged between 20 and 45 years and their BMI ranged 18.5- 24.9 km/m^2 were included [**4]**. Regular smokers have averaged 5-6 cigarettes each day during the past five years. Cigarettes were all that were allowed to be smoked.

- **Allocation:**

Participants were divided into three equal groups based on their serum cotinine level, which was determined by drawing blood and doing tests in the lab: (a) nonsmokers (control group) having their serum cotinine levels lower than 10 ng/ml; (b) light, moderate smokers having their serum cotinine levels of 10–100 ng/ ml; as well as (c) heavy smokers having their serum cotinine levels higher than 300 ng/ml [**4]**. The average size of a group was 22 participants **(Fig.1).**

- **Exclusion criteria:**

Individuals who participated in athletics, Individuals who take antidepressants, individuals having a previous history of Knee injuries, Diabetes Mellitus [**20]**, disorders of the heart, lungs, or circulatory system, recent vestibular dysfunction, inner ear infection leading to coordination as well as balance issues **[21]**.

Each participant received a handout with additional details. Participants were recruited into the study if they met the

selection criteria but also provided informed written consent. The Physical Therapy Department at Cairo University approved this study (with the permission number **(P.T.REC/012/003084)**.

Data was also collected on the subjects' height, weight, as well as BMI.

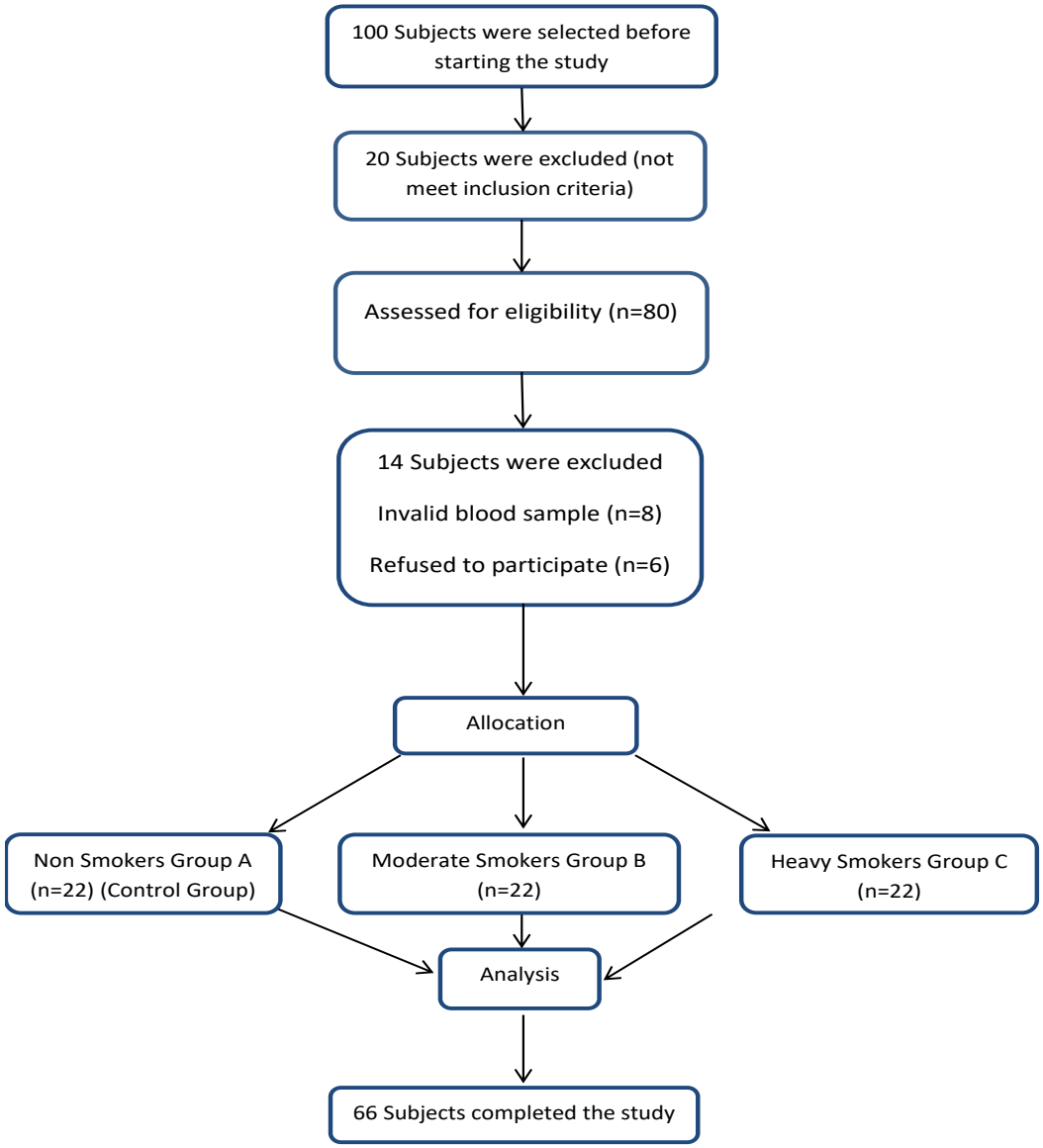

Fig. (1). Flow chart of study participants.

- **Instrumentation:**

Biodex System 3 Pro Isokinetic Dynamometer (Biodex Medical Inc., Shirley, New York, USA) utilized to quantitatively evaluate muscle function and performance (torque, peak torque, angle specific torque, work, power, as well as angle acceleration energy) which is difficult to acquire using conventional manual testing methods [**22].** Peak torques of the knee extensors as well as proprioception were measured before and after exhaustion using a Biodex isokinetic dynamometer fitted with a forward reclined knee attachment. It is one of the most advanced computer-controlled biomechanical systems for skeletal muscle conditioning as well as rehabilitation [**23].**

- **Procedure:**

All participants were given information about the study and had blood drawn for chemical analysis of cotinine as well as

serotonin levels. Serum cotinine levels were determined using a Human Cotinine ELISA Kit **(Cat.No E2043Hu)** in addition Human serotonin ELISA Kit was used to measure serum serotonin **(Cat. No E1128Hu)** analyzed by ELISA reader device **(Li StarFish S.r.l. Camillo Benso Conte di Cavour, Cernusco S/N (MI), Italia, 2015).**

-Measurement of knee repositioning accuracy:

Each participant sat on the Biodex system's testing chair, positioning their tested leg so that their knee was at a right angle to the dynamometer's axis (starting position), The participant was blindfolded and held in place during testing with straps around their trunk, pelvis, and thighs, The tibial pad was attached to the Shank three cm above the lateral malleolus [**24].** The selected test type required active repositioning of the test at his speed of 30 °/s, and each test was conducted three times. Each participant took two practice exams before the actual test **[25].**

The subject's leg was initially positioned at an angle between 30 and 70 degrees from the anatomical reference angle before being returned to the initial position. For standardization, the tested limb was allowed to move to target angles (30 °, 70 °) actively [**26]** The subject's limb was held in place for 10 seconds during the attachment process, during which time the subject was instructed to memorize the position before the device released the limb to return to its original position [**24].**

The subject was given a 5-second rest and then instructed to voluntarily move his limb to the target angles of 30 degrees and 70 degrees, The subject would press the Hold/Release button once he reached the desired angles voluntarily. Individuals were not allowed to make any adjustments to the angle **[24, 26].**

There were three attempts, each separated by a 30-second risk period [**27].** The main angular differences of the three trials among the target angle position as well as the subject received in range position (absolute error) was documented in degrees as the deficit in repositioning accuracy also, it was used in the statistical analysis **[28].**

-Measurement of knee extensor muscle Fatigue:

Pre fatigue test

First, the investigator moved the subject's knee so that it bent at an angle of 60°. This position was held for five seconds. The knee was then moved passively until it was completely flexed. After that, the patient was asked to move his knee to a flexion angle of 60°. Both knees were examined **[29].**

Fatigue challenge

During the pre-fatigue test, the participants were instructed to repeatedly extend their knees to their fullest range of motion while carrying a weight that was 50% of their peak torque. The repetitions were carried out slowly and deliberately. It was recommended that each participant do as many reps as they could until they could no longer continue due to tiredness **[8].**

Post fatigue test

Immediately following the onset of fatigue, subjects were told to perform the post fatigue test in the exact manner as well as sequence as the pre fatigue test. Torques produced both before and after fatigue testing were collected and used to determine the fatigue index.

Fatigue index

It is the percentage change in maximum torque after the fatigue challenge

$$\text{Fatigue index} = \frac{\boldsymbol{\Sigma} Pre fatigue\ torques - \boldsymbol{\Sigma} post fatigue\ torques}{\boldsymbol{\Sigma} Pre fatigue\ torques} \times 100$$ **[30].**

DATA ANALYSIS

Subject characteristics were compared between groups by one way ANOVA. The Shapiro-Wilk test was used to ensure that the data followed a normal distribution. The homogeneity of the groups was tested using Levene's test for homogeneity of variances. One-way MANOVA was performed for

comparison of serum serotonin level, fatigue index of knee extensors and knee JPE between groups. Pearson's correlation coefficient was conducted to investigate the correlation between serum cotinine as well as serum serotonin, fatigue index & JPE. All statistical tests were conducted with a p value of less than 0.05 considered significant. The Windows version of the SPSS statistical software (version 25) was used for all analyses (IBM SPSS, Chicago, IL, USA).

RESULTS:

- ***Subject characteristics:***

Sixty-six male subjects took part in this study. A total of 66 subjects were categorized into three groups of 22. Group A who were nonsmokers, group B who were moderate smokers and group C who were heavy smokers. **Table (1)** presented the subject characteristics of group A, B &C. There was no substantial difference among groups in age as well as BMI ($p > 0.05$).

Table 1. Basic characteristics of participants.

	Group A	Group B	Group C	p-value
Age, mean ± (SD), years	**30.63 ± 8.43**	**29.32 ± 7.65**	**32.77 ± 5.93**	**0.30**
BMI, mean ± (SD), kg/m²	**21.64 ± 1.24**	**21.97 ± 1.01**	**21.95 ± 1.29**	**0.57**

SD, standard deviation; **p-value,** level of significance

-Effect of smoking on serum serotonin level, fatigue index of knee extensors and knee JPE

There was a substantial improvement in the level of serum serotonin of group A in contrast to Groups B and C ($p < 0.001$) also a substantial improvement in the level of serum serotonin of group B in contrast to Group C ($p < 0.001$).

There was a substantial decline in fatigue index of right as well as left knee extensors in Group A as opposed to Groups B and C ($p < 0.001$) in addition a substantial decline in fatigue index of right as well as left knee extensors in Group B as opposed to Group C ($p < 0.001$).

There was a substantial decline in JPE of right as well as left knee at 70º & 30º knee flexion in Group A as opposed to Groups B and C ($p < 0.001$) in addition a substantial decline in JPE of right as well as left knee at 70º & 30º knee flexion of group B compared in contrast to Group C ($p < 0.001$). **(Table 2).**

Table 2. Mean serotonin, fatigue index and JPE of group A, B and C*:*

	Group A	Group B	Group C	p-value		
	mean ± SD	**mean ± SD**	**mean ± SD**	**A vs B**	**A vs C**	**B vs C**
-Serum serotonin (ng/ml)	175.45 ± 10.41	87.59 ± 7.61	64.82 ± 6.79	0.001	0.001	0.001
-Fatigue index of right knee extensors (%)	35.87 ± 2.54	50.23 ± 2.34	62.61 ± 2.41	0.001	0.001	0.001
-Fatigue index of left knee extensors (%)	33.37 ± 2.85	49.34 ± 3.44	62.31 ± 3.55	0.001	0.001	0.001
-JPE of right knee at 70º knee flexion (degrees)	0.71 ± 0.27	1.51 ± 0.34	3.58 ± 0.97	0.001	0.001	0.001
-JPE of right knee at 30º knee flexion (degrees)	0.52 ± 0.30	1.59 ± 0.31	3.67 ± 0.63	0.001	0.001	0.001
-JPE of left knee at 70º knee flexion (degrees)	0.67 ± 0.34	1.63 ± 0.33	3.85± 0.94	0.001	0.001	0.001
-JPE of left knee at 30º knee flexion (degrees)	0.54 ± 0.24	1.45 ± 0.32	3.74 ± 0.58	0.001	0.001	0.001

SD, Standard deviation**; p-value**, Level of significance

- Relationship between serum cotinine as well as serum serotonin, fatigue index and JPE:

The correlation between serum cotinine and serum serotonin was a strong negative substantial correlation (r = -0.701, p = 0.001).

The correlation among serum cotinine with fatigue index of right and left knee extensors and JPE of right and left knee at 70º and 30º knee flexion were strong positive substantial correlation (r = 0.843: 0.935, p = 0.001).

The correlation among serum serotonin with fatigue index of right and left knee extensors and JPE of right and left knee at 70º and 30º knee flexion were strong negative significant correlation (r = -0.756: -0.22, p = 0.001). **(Table 3).**

Table 3. Correlation between serum cotinine and serum serotonin, fatigue index and JPE:

Variable	Serum cotinine (ng/ml)		Serum serotonin (ng/ml)	
	r value	p value	r value	p value
Serum serotonin (ng/ml)	-0.701	0.001		
Fatigue index of right knee extensors (%)	0.854	0.001	-0.922	0.001
Fatigue index of left knee extensors (%)	0.843	0.001	-0.920	0.001
JPE of right knee at 70º knee flexion (degrees)	0.885	0.001	-0.756	0.001
JPE of right knee at 30º knee flexion (degrees)	0.907	0.001	-0.825	0.001
JPE of left knee at 70º knee flexion (degrees)	0.898	0.001	-0.782	0.001
JPE of left knee at 30º knee flexion (degrees)	0.935	0.001	-0.805	0.001

r value: Pearson correlation coefficient**, p value:** Probability value

DISCUSSION

The purpose of this research was to detect the correlation of serum cotinine level (the metabolite of nicotine), serum serotonin level, peripheral fatigue of Knee extensors as well as proprioception of the Knee.

In the recent research, the findings revealed that there was a substantial association among the smokers' groups for the fatigue index of Knee extensors. When comparing groups, heavy smokers had a greater fatigue index because their cotinine levels were higher. Furthermore, the results demonstrated a statistically significant correlation between the three groups with regards to serum serotonin level. Serum serotonin levels were shown to be higher in the nonsmokers group, and to be decreasing with increasing cotinine levels in the smokers.Moreover, the findings revealed that there was a substantial association among the smokers' groups for serum serotonin level as well as the fatigue index. In smokers, peripheral knee extensor fatigue was proportional to a decrease in serum serotonin levels.

Smoking use is a documented contributor to numerous health problems as well as this socioeconomic impact, our study tried to shed a light about more hazard of smoking habit. This study had several limitations; **First,** it was feasible that smokers may exhibit symptoms of vascular disease during the diagnostic process. Furthermore, the impact of smoking on the cardiovascular system may have an adverse effect on physical performance, masking the underlying impact of smoking on muscular exhaustion. **Secondly,** the findings might be influenced by other factors that were connected with smoking status as well as were not controlled in this study including such depression, anxiety, as well as lower socioeconomic status **[8].**

In the current study the results indicated rejection of the study hypothesis that there was no statistical substantial difference in fatigue index of both knees extensors among three groups **(**non- smokers group having mean value of serum cotinine level is **6.36**, moderate smokers group having mean value of serum cotinine level is **32.86**

and heavy smokers group having the mean value of serum cotinine level is **364.13)** as there was statistical substantial difference among non, moderate as well as heavy smokers for the fatigue index of knees extensors. Average levels of fatigue were found to be highest among heavy smokers.

Also, the findings revealed rejection of the study hypothesis that there was no statistical substantial difference for precise repositioning (Joint Reposition Error) among three groups as there was statistically substantial difference for precise repositioning (Joint Reposition Error) between non- smokers' group as well as (moderate, heavy smokers groups). But the results indicated acceptance of this hypothesis between moderate and heavy smokers' groups as there was no statistical substantial difference for precise repositioning (**Joint Reposition Error**) among moderate as well as heavy smokers groups.

In addition, the findings indicated rejection of the research hypothesis that there was no substantial difference in terms of the concentration of serotonin in the blood across three groups as there was a statistically substantial difference across the three groups in terms of the concentration of serotonin in the blood. In terms of serum serotonin levels, non-smokers had the highest mean value.

Our results match with the work of **Chan et al., (2020) [31]** discovered that young male smokers had a decrease in their muscle's resistance to fatigue during triggered contractions of the quadriceps. The fatigue index was 17% greater among smokers than among non-smokers. The decrease in fatigue resistance explained by 1) Muscle mitochondrial enzyme activity has been shown to be reduced in smokers, this data points to a link between cigarette smoking and changes in oxidative enzyme activity as well as fatigue resistance. Smoke contains a number of harmful chemicals, including cyanide and carbon monoxide, both of which have the ability to diminish a person's resistance to fatigue. These chemicals work by inhibiting cytochrome oxidase, which leads to a decrease in mitochondrial activity and oxidative capacity. 2) The blood's ability to transport oxygen is diminished due to an increase in carboxyhemoglobin (COHb) caused by cigarette smoke.

Our findings corroborated those of **Seyedsadeghi et al., (2023) [32]**, who found an inverse association between serum serotonin as well as cotinine level, with the non-smokers group having the greatest mean value of serum serotonin. They discovered that nicotine elevates neurotransmitter levels by binding to nicotinic acetylcholine receptors; elevated dopamine with in brain's reward circuits is hypothesized to be responsible for smoking's sedative effects. However, nicotine decreases serotonin production in the brain; tobacco use can reduce serotonin production by as much as 50%.

Also, the outcomes of the current investigation have an agreement with the work of **Kuan et al., (2021) [33]** who revealed that smoking has been linked to an elevated risk of age-related muscle deterioration more than any other factor. They concluded that cigarette smoking was the single most important factor in the development of age-related muscle degeneration, with the oxidative damage caused by cigarettes being the probable explanation.

Furthermore, study by **Decker et al., (2023) [34]** who studied the effects of smoking upon skeletal muscle properties as well as fatigue resistance. According to the findings of this study, smokers experience more peripheral muscular fatigue, report a greater overall feeling of fatigability, and also the effect would rise with increasing smoking volume, Smokers may fatigued more quickly because 1) their muscles have less oxidative capacity because their mitochondrial enzyme activity is lower, or 2) oxygen delivery to their muscles is impaired because of reduced blood flow or lower-than-normal

blood oxygen content brought on by the nicotine in cigarettes.

Moreover, the work of **Zhang et al., (2022) [35]** who looked into how cigarette smoke affected fibres in the deep extensor digitorum longus as well as the superfecialis. The extensor digitorum longus regions of the extensor digitorum longus muscle in wister-kyoto as well as spontaneously hypertensive rates at three different dose levels utilizing a smoking machine. The results showed that only chronic exposure to high levels of cigarette smoke altered the morphological and metabolic features of fibers in the extensor digitorum longus at both rates. Two explanations are offered for this result: 1) Hypoxia brought on by cigarette smoke causes blood carbon monoxide (HbCo) levels to rise. Second, the nicotine in cigarettes causes a reduction in blood and oxygen supply due to vasoconstriction of the blood vessels.

This study supported by the work of **Håglin, (2015) [36]** who found that long-term exposure to nicotine from cigarettes causes insulin resistance, which in turn hinders the body's ability to absorb nutrients and hence reduces energy generation, fatigue resistance, as well as sports performance.

Also, our results are in line with **Su et al., (2020) [37]** who investigated the effects of smoking on the contractile as well as fatigue properties of skeletal muscle among COPD patients, and found that smokers were more likely to experience muscle fatigue compared with non-smokers. They speculated that this was due to a combination of factors, including impaired neuromuscular transmission and the presence of COHb, which limits the oxygen available to muscles. The research of **Darabseh et al., (2021), [29]** supported this finding, finding that inhaling carbon monoxide (CO) from cigarette smoke results COHb, which has an immediate effect on the muscle's resistance to fatigue as well as causes hypoxemia. COHb may reach level of 9% in smokers inhibiting the release of oxygen from Hb to muscle decreasing oxygen supply to the muscles.

In addition, the present study's findings are consistent with the research of **Al-Bashaireh et al., (2018) [38]** who compared the muscular fatigability of athletes who smoke with those who don't and discovered that athletes who smoke have greater rates of muscular fatigability as well as decreased knee strength after knee extensors tasks. These findings suggest that changes in the fiber type composition as well as the oxidative capacity of the knee extensor muscles may contribute to the increased muscle fatigability and reduction of knee strength observed in cigarette smokers.

Another possible explanation by the work of **Harrison et al., (2020) [39]** studied the association of cigarette smoking on higher risk for suicide and attempted suicide. Smoking cigarettes may be linked to decreased serotonin activity in the brain, which may explain why people with significant psychiatric problems are more likely to engage in suicidal thoughts and behaviors. It has been established that acute administration of nicotine increases serotonin release, while chronic administration of nicotine leads to serotonin depletion within brain areas like the hippocampus formation and decreases firing of serotonergic neurons emerging in the midbrain raphe.

This study's results lined up with those of **Sharma et al., (2022) [40]** who discovered that nicotine stimulates neurotransmitters (norepinephrine, dopamine as well as serotonin). in the brain within 8 seconds after inhalation. Despite the fact that smoking temporarily increases serotonin levels, these elevated levels only remain as long as the cigarette is being smoked, suggesting that smoking causes a physical alteration in the brain that ultimately suppresses serotonin production.

The proprioceptive accuracy of knee extensors was evaluated using an active repositioning test and just a Biodex system 3pro isokinetic dynamometer in this study.

It is generally agreed that this is the best way to evaluate joint position sense **[41, 42]** because proprioceptive information is mostly contributed by muscle receptors, this test is valid. Therefore, the input from this receptor is best utilised when active testing is employed rather than passive testing. Additionally, active testing has greater practical value than passive testing [**43]**. Reproducing a joint's position actively was also found to be more accurate than passively **[44].**

This significant decrease in knee proprioceptive sense accuracy in smokers than non- smokers may be attributed to direct and indirect mechanisms. The direct mechanism could be explained by the work of **Rehman et al., (2021) [45]** who concluded that nicotine in cigarettes decreases peak expiratory flow rate in humans, which may help to explain why long-term smokers are more likely to develop emphysema, based their findings on the hypothesis that nicotine produces an initial stimulant phase at autonomic ganglia as well as the neuromuscular junction, which is then rapidly followed by neuromuscular blockade but also receptor desensitisation. That could have an impact on fatigability as well as proprioception of the muscles.

The fact that nicotine alters proprioception may also be explained by the fact that nicotine alters the myelin sheath, a protecting fatty acid covering on the nerve cell, and that nicotine stops the axons, or nerve impulse transmitters, within the nerve cells from firing appropriately [**46].** Also, **Decker et al. (2023) [34]** found that direct muscle stimulation overlaid on a nerve resulted in a failure in neuromuscular transmission among smokers.

Since the release of serotonin, the brain's master regulator neurotransmitter, is triggered by proprioceptive information, decreased proprioception accuracy of the knee joint is an indirect result of cigarette smoking and cotinine's impact on serotonin levels in the blood. Serotonin acts as a neurotransmitter, which mean that it directly sends signals to the brain, the level of activity of all other neurotransmitters is determined by serotonin [**47].** So when level of serotonin decreased this function neurotransmitter affect proprioception input.

Other indirect factors contribute to reduction of knee proprioception accuracy in smokers' groups than non- smokers group related to negative effect of cotinine on fatigue resistance of knee extensors causing higher fatigue index in smokers' groups. And the fatigue may reduce proprioception sense of knee extensors. This can be explained by several mechanisms. Firstly, it is logically assumed that the fatigue protocol directly affects elements of the knee muscles as well as the receptors within it. Therefore, muscles as well as their receptors (muscle spindle and golgi tendon organ) may be dysfunctional and their ability to actively reproduce an established position was inhibited **[25].**

Another explanation which may account for the alteration in proprioceptive ability after muscle fatigue may be due to the change in local metabolism of the muscle. **Proske, (2019) [48]** who revealed that elevated intramuscular concentrations of lactic acid, potassium chloride, bradykinin, as well as arachidonic acid have been found to influence the muscle spindle system and, in turn, proprioceptive acuity in fatigued muscles. In addition, **Lima et al., (2021) [49]** observed that muscle fatigue alters muscle spindle output as a measure of reflex arc due to increased intramuscular concentrations of numerous contractile chemicals.

Muscle spindles were proposed as the primary receptors for sensory information transmitted to the brain. Absolute changes in muscle length are best sensed by group 1 and 2 afferents from the muscle spindle. That's why sensory afferent information used to initiate movement primarily comes from muscle spindles **[50].**

The finding of this study had an agreement with **Ehlert, (2021) [51]** who found that functional fatigue had a substantial

outcome on joint position sense acuity of the upper limb in overhead throwing athletes as measured by the active multi joint position reproduction.

Also, the results of the current study are consistence with **Rossi et al., (2021) [25]** who found that muscular fatigue impairs proprioceptive accuracy in the shoulder because fatigued muscles are less sensitive to mechanical stimulation of the tendons that make up the rotator cuff. Shoulder proprioception may be impaired because to rotator cuff desensitisation. **Ghamkhar and Kahlaee (2019) [52]** examined the impact of paraspinal muscle fatigue on sensitivity to lumbar position changes, and their findings corroborate ours. Also found that the ability to notice a change in lumber position due to a change in the afferent input was diminished following lumbar muscle fatigue.

In addition, **Tong et al., (2017) [53]** assessed association among lumbar muscle strength as well as proprioception afterwards fatigue in men suffering from low back pain, position sense was observed to be moderately linked with muscle fatigue among healthy group. Muscle fatigue as well as dysfunction in the trunk could alter afferent input to the affected muscles, leading to the observed effect.

Our results confirmed by **Jalayee et al., (2020) [54]** who evaluated smokers' lumbar extensor muscle fatigue index found a correlation among lumber extensor fatigue index as well as cigarette smoking; more cigarette consumption was associated with a higher fatigue index, indicating an elevated risk of lumbar injury. Based on their findings, **Darabseh et al., (2021) [29]** concluded that sedentary male smokers have a greater percentage of low oxidative type II fibers in fast vastus lateralis muscle and a smaller percentage of high oxidative type I fibers. They postulated that a switch in fiber type from high oxidative towards low oxidative occurs in skeletal muscle as a result of reduced blood as well as (0_2) supply brought on by cigarette smoking.

Also, this study results were in an agreement with the work of **Mohamed and Lasheen, (2017) [55]** whom investigated the association among serum cotinine, serum serotonin as well as peripheral fatigue of back extensors. They found that the heavy smokers subjects have a higher fatigue index than the moderate and non-smokers groups. Also **Mohamed, (2012) [56]** found that repositioning accuracy of back extensors in heavy smokers' subjects were more affected than both moderate and non-smokers subjects.

In addition, our results contradicted by the work of **Farmer et al., (2020) [57]** who looked into how paraspinal muscle fatigue affected reflex delay as well as amplitude. It was also shown that the reflex delay was unaffected by fatigue, suggesting that the increased reflex amplitude may be an attempt to compensate for the decrease in muscular force capability that occurs with fatigue.

Toro et al. (2019) [58] also, tried to draw the conclusion that a lack of neuromuscular transmission is caused by smoking by observing a negative correlation between muscle fatigue and serotonin levels.

On the other hand, the outcomes of the current investigation were in a disagreement with **Fields and Image, (2005) [59]** who looked at how muscle fatigue affected shoulder proprioception and found no significant variation in proprioceptive ability with fatigue among moderate as well as heavy smokers and also no difference among dominant as well as non-dominant limbs.

Also, our findings disagreed with a study of **Olmedo and Rodríguez, (2009) [60]** who investigated the instantaneous impacts of an upper body fatigue protocol on knee joint position sense using a position matching technique, and found that the fatigue group showed a statistically significant reduction in absolute angle error (AAE) for the angles measured between pre- and post-tests, while the control group (non-smokers) as well as light smokers found no significant differences.

Furthermore, results of our study were against these of **Miura et al., (2004) [61]** who measured AAE at matched defined index angles before as well as after general in addition local fatigue protocols and showed a significant increase of AAE after general fatigue, implying that reduced reproduction ability after general load is not due to the smoking effect but rather to other factors, particularly deficiency of central processing of proprioceptive signals.

Our research aimed to shed light on the topic and expand on the risks associated with smoking due to the fact that it is a major risk factor in the spread of several illnesses and also has a significant economic impact.

LIMITATIONS

There were some limitations to this study. For one, participants could have been asymptomatic concerning vascular problems since they were smokers. Furthermore, the effect of smoking upon that vascular system may influence levels of physical performance, masking the true impact of smoking upon muscular fatigue. Furthermore, the outcomes may have been altered by other characteristics related with smoking status that were not controlled for in this study, such as depression, nervousness, as well as lower socioeconomic status.

CONCLUSION

Cotinine (the metabolite of nicotine) had a significant effect on both of peripheral fatigue and proprioception (repositioning accuracy) of knee extensors and joints. In addition, Cotinine also was proved to have a significant effect on serum serotonin level.

Acknowledgements

The authors express their sincere gratitude to all patients who kindly participated in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Vega Palma MI, Klivinyi C, Lampl T, Lang-Illievich K, Bornemann-Cimenti H, Szilagyi IS. The Effect of Smoking Cessation on Acute Pain: A Systematic Review. Pain and Therapy. 2022 Dec 7:1-3.
2. World Health Organization. WHO report on the global tobacco epidemic. Geneva, Switzerland: World Health Organization; 2011.3 Jha P, Ramasundarahettige C, Landsman V. 21st Century hazards of smoking and benefits of cessation in the United States. N Engl J Med 2013; 368:341–350.
3. Benjamin EJ, Muntner P, Alonso A, Bittencourt MS, Callaway CW, Carson AP, Chamberlain AM, Chang AR, Cheng S, Das SR, Delling FN. Heart disease and stroke statistics—2019 update: a report from the American Heart Association. Circulation. 2019 Mar 5; 139(10):e56-28.
4. Hoch D, Majali-Martinez A, Bankoglu EE, Stopper H, Glasner A, Desoye G, Gauster M, Hiden U. Maternal smoking in the first trimester and its consequence on the early placenta. Laboratory Investigation. 2023 Jan 10:100059.
5. Gomez M, Jeudy J, Petit A, Brunet J, Saint-Cast Y, Rabarin F, Cesari B, Raimbeau G, Bigorre N. Acute pain after digital crushing and nicotine intoxication. Cigarettes are always a false friend. A prospective study. Orthopaedics & Traumatology: Surgery & Research. 2022 Sep 29:103425.
6. Weng W, Li H, Zhu S. An Overlooked Bone Metabolic Disorder: Cigarette Smoking-Induced Osteoporosis. Genes. 2022 Apr 30; 13(5):806.

7. Ho CC, Lee PF, Xu S, Hung CT, Su YJ, Lin CF, Wu MC, Chen YT. Associations between cigarette smoking status and health-related physical fitness performance in male Taiwanese adults. Frontiers in Public Health. 2022 Aug 18; 10:880572.
8. Xiaogang L, Di G, Lianlei Z. Impact of the Cigarette Smoking on Athlete's Health: A Systematic Review. Tobacco Regulatory Science. 2021 Nov 3; 7(6):6222-7.
9. Griffioen M, van Drunen P, Maaswinkel E, Perez RS, Happee R, van Dieën JH. Identification of intrinsic and reflexive contributions to trunk stabilization in patients with low back pain: a case–control study. European Spine Journal. 2020 Aug; 29(8):1900-8.
10. McConville J, Chambers R, Hassed C, Morris E, Hahne A. Predictors of stress, anxiety, depression, study engagement and academic performance in physiotherapy students, including a subgroup receiving a mindfulness training program. Focus on Health Professional Education: A Multi-Professional Journal. 2022 Dec 16; 23(4):52-72.
11. Sinenko SA, Kuzmin AA, Skvortsova EV, Ponomartsev SV, Efimova EV, Bader M, Alenina N, Tomilin AN. Tryptophan Hydroxylase-2-Mediated Serotonin Biosynthesis Suppresses Cell Reprogramming into Pluripotent State. International Journal of Molecular Sciences. 2023 Mar 2 ; 24(5):4862.
12. Shen L, Qiu HB, Xu HH, Wei K, Zhao L, Zhu CC, Li CJ, Lu ZJ. Nicotine withdrawal induces hyperalgesia via downregulation of descending serotonergic pathway in the nucleus raphe magnus. Neuropharmacology. 2021 May 15; 189:108515.
13. Suchanecka A, Chmielowiec J, Chmielowiec K, Trybek G, Jaroń A, Czarny W, Król P, Masiak J, Grzywacz A. Serotonin Receptor HTR3A Gene Polymorphisms rs1985242 and rs1062613, E-Cigarette Use and Personality. International Journal of Environmental Research and Public Health. 2022 Apr 14; 19(8):4746.
14. Amann M, Sidhu SK, McNeil CJ, Gandevia SC. Critical considerations of the contribution of the corticomotoneuronal pathway to central fatigue. The Journal of Physiology. 2022 Dec; 600(24):5203-14.
15. Park D, Sahai E, Rullan A. SnapShot: cancer-associated fibroblasts. Cell. 2020 Apr 16; 181(2):486-.
16. Lemme NJ, Kuczmarski AS, Goodman AD, Ready LV, Dickens JF, Owens BD. Management and outcomes of in-season anterior shoulder instability in athletes. JBJS reviews. 2019 Nov 1; 7(11):e2.
17. McPherson AL, Nagai T, Webster KE, Hewett TE. Musculoskeletal injury risk after sport-related concussion: a systematic review and meta-analysis. The American journal of sports medicine. 2019 Jun; 47(7):1754-62.
18. Boyce TG, Ballone NT, Certa KM, Becker MA. The use of β-adrenergic receptor antagonists in psychiatry: a review. Journal of the Academy of Consultation-Liaison Psychiatry. 2021 Jul 1; 62(4):404-12.
19. Thomas RJ, Timmins RG, Tofari PJ, Williams MD, Opar DA. Assessing isometric kicking force and post-match responses using the Kicker test. Journal of Sports Sciences. 2022 Jun 3; 40(11):1275-81.
20. Reddy RS, Alahmari KA, Samuel PS, Tedla JS, Kakaraparthi VN, Rengaramanujam K. Intra-rater and inter-rater reliability of neutral and target lumbar positioning tests in subjects with and without non-specific lower back pain. Journal of Back and Musculoskeletal Rehabilitation. 2021 Jan 1; 34(2):289-99.
21. Shahvarpour A, Preuss R, Larivière C. The effect of extensible and non-extensible lumbar belts on trunk

postural balance in subjects with low back pain and healthy controls. Gait & Posture. 2019 Jul 1; 72:211-6.
22. Muff G, Dufour S, Meyer A, Severac F, Favret F, Geny B, Lecocq J, Isner-Horobeti ME. Comparative assessment of knee extensor and flexor muscle strength measured using a hand-held vs. isokinetic dynamometer. Journal of physical therapy science. 2016; 28(9):2445-51.
23. Torpey D, Murray E, Hughes T, Sergeant J, Callaghan M. The reliability of and agreement between devices used to measure eccentric hamstring strength: a systematic review protocol. Systematic Reviews. 2022 Sep 23; 11(1):204.
24. Oleksy Ł, Królikowska A, Mika A, Reichert P, Kentel M, Kentel M, Poświata A, Roksela A, Kozak D, Bienias K, Smoliński M. A Reliability of Active and Passive Knee Joint Position Sense Assessment Using the Luna EMG Rehabilitation Robot. International Journal of Environmental Research and Public Health. 2022 Jan; 19(23):15885.
25. Rossi C, Bastian AJ, Therrien AS. Mechanisms of proprioceptive realignment in human motor learning. Current Opinion in Physiology. 2021 Apr 1;20:186-97.
26. Akbar UU, Bhatti ZM, Asim HM, Khan I. Effects of mild, moderate and intense physical activity on knee joint proprioception in healthy young adults. Pakistan Journal of Rehabilitation. 2023 Jan 4; 12(1).
27. Goh SL, Persson MS, Stocks J, Hou Y, Welton NJ, Lin J, Hall MC, Doherty M, Zhang W. Relative efficacy of different exercises for pain, function, performance and quality of life in knee and hip osteoarthritis: systematic review and network meta-analysis. Sports Medicine. 2019 May 1; 49:743-61.
28. Henry M, Baudry S. Age-related changes in leg proprioception: implications for postural control. Journal of neurophysiology. 2019 Aug 1; 122(2):525-38.
29. Darabseh MZ, Maden-Wilkinson TM, Welbourne G, Wüst RC, Ahmed N, Aushah H, Selfe J, Morse CI, Degens H. Fourteen days of smoking cessation improves muscle fatigue resistance and reverses markers of systemic inflammation. Scientific reports. 2021 Jun 10;11(1):1-1.
30. Hedström AK, Olsson T, Alfredsson L. Cotinine as a measure of smoking in observational studies of multiple sclerosis. Multiple Sclerosis Journal. 2021 Jul; 27(8):1293-6.
31. Chan SM, Cerni C, Passey S, Seow HJ, Bernardo I, van der Poel C, Dobric A, Brassington K, Selemidis S, Bozinovski S, Vlahos R. Cigarette smoking exacerbates skeletal muscle injury without compromising its regenerative capacity. American Journal of Respiratory Cell and Molecular Biology. 2020 Feb;62(2):217-30.
32. Seyedsadeghi M, Arabzadeh A, Entezariasl M, Shahbazzadegan B, Dindar S, Isazadehfar K. The Effect of Nicotine Patch on Reducing Nausea, Vomiting, and Pain Following Laparoscopic Cholecystectomy: A Randomized Clinical Trial. Addiction and Health. 2023 Jan 1;15(1):39-44.
33. Kuan V, Warwick A, Hingorani A, Tufail A, Cipriani V, Burgess S, Sofat R, Fritsche LG, Igl W, Bailey JN, Grassmann F. Association of smoking, alcohol consumption, blood pressure, body mass index, and glycemic risk factors with age-related macular degeneration: a Mendelian randomization study. JAMA ophthalmology. 2021 Dec 1;139(12):1299-306.
34. Decker ST, Alexandrou-Majaj N, Layec G. Effects of acute cigarette smoke concentrate exposure on mitochondrial energy transfer in fast-and slow-twitch skeletal muscle. Biochimica et

Biophysica Acta (BBA)-Bioenergetics. 2023 Aug 1; 1864(3):148973.
35. Zhang W, Lin H, Zou M, Yuan Q, Huang Z, Pan X, Zhang W. Nicotine in inflammatory diseases: anti-inflammatory and pro-inflammatory effects. Frontiers in immunology. 2022:376.
36. Håglin L. High serum phosphate concentration as the result of smoking might underlie the lower risk of Parkinson's disease. Medical Hypotheses. 2015 Sep 1;85(3):287-90.
37. Su J, Li J, Lu Y, Li N, Li P, Wang Z, Wu W, Liu X. The rat model of COPD skeletal muscle dysfunction induced by progressive cigarette smoke exposure: a pilot study. BMC Pulmonary Medicine. 2020 Dec;20:1-3.
38. Al-Bashaireh AM, Haddad LG, Weaver M, Kelly DL, Chengguo X, Yoon S. The effect of tobacco smoking on musculoskeletal health: a systematic review. Journal of environmental and public health. 2018 Jul 11;2018.
39. Harrison R, Munafò MR, Smith GD, Wootton RE. Examining the effect of smoking on suicidal ideation and attempts: triangulation of epidemiological approaches. The British Journal of Psychiatry. 2020 Dec;217(6):701-7.
40. Sharma S, Rahman Archie S, Kanchanwala V, Mimun K, Rahman MA, Zhang Y, Abbruscato T. Effects of nicotine exposure from tobacco products and electronic cigarettes on the pathogenesis of neurological diseases: impact on CNS drug delivery. Frontiers in Drug Delivery. 2022 Apr 26;2:8.
41. Ager AL, Roy JS, Roos M, Belley AF, Cools A, Hébert LJ. Shoulder proprioception: how is it measured and is it reliable? A systematic review. Journal of Hand Therapy. 2017 Apr 1;30(2):221-31.
42. Horváth Á, Ferentzi E, Schwartz K, Jacobs N, Meyns P, Köteles F. The measurement of proprioceptive accuracy: A systematic literature review. Journal of Sport and Health Science. 2023 Mar 1;12(2):219-25.
43. Azevedo J, Rodrigues S, Seixas A. The influence of sports practice, dominance and gender on the knee joint position sense. The Knee. 2021 Jan 1;28:117-23.
44. Reddy RS, Tedla JS, Alshahrani MS, Asiri F, Kakaraparthi VN, Samuel PS, Kandakurti PK. Reliability of hip joint position sense tests using a clinically applicable measurement tool in elderly participants with unilateral hip osteoarthritis. Scientific reports. 2022 Jan 10;12(1):1-9.
45. Rehman OU, Amjad F, Waqas M. Effects of smoking on lung function in students of university of Lahore, Pakistan. Rawal Medical Journal. 2021 Oct 14;46(4):803.
46. Nishanth K, Tariq E, Nzvere FP, Miqdad M, Cancarevic I, Katukuri N, Miqdad MA. Role of smoking in the pathogenesis of multiple sclerosis: a review article. Cureus. 2020 Aug 5;12(8).
47. Moncrieff J, Cooper RE, Stockmann T, Amendola S, Hengartner MP, Horowitz MA. The serotonin theory of depression: a systematic umbrella review of the evidence. Molecular psychiatry. 2022 Jul 20:1-4.
48. Proske U. Exercise, fatigue and proprioception: A retrospective. Experimental brain research. 2019 Oct;237(10):2447-59.
49. Lima CR, Sahu PK, Martins DF, Reed WR. The neurophysiological impact of experimentally-induced pain on direct muscle spindle afferent response: a scoping review. Frontiers in Cellular Neuroscience. 2021 Feb 19;15:649529.
50. Feng J, Hung TM, Huang R, Hou S, Ren J. Role of proprioception in slow and rapid movements. Perceptual and motor skills. 2020 Apr;127(2):281-98.

51. Ehlert A. The correlations between physical attributes and golf clubhead speed: A systematic review with quantitative analyses. European Journal of Sport Science. 2021 Oct 3;21(10):1351-63.
52. Ghamkhar L, Kahlaee AH. The effect of trunk muscle fatigue on postural control of upright stance: A systematic review. Gait & posture. 2019 Jul 1;72:167-74.
53. Tong MH, Mousavi SJ, Kiers H, Ferreira P, Refshauge K, van Dieën J. Is there a relationship between lumbar proprioception and low back pain? A systematic review with meta-analysis. Archives of physical medicine and rehabilitation. 2017 Jan 1;98(1):120-36.
54. Jalayee A, Gholami T, Dehghan A, Zakeri H. Prevalence of Low Back Pain and Lifestyle Risk Factors in Opioid Users and Non-Opioid Drug Users in Fasa Persian Cohort Population. Journal of Advanced Biomedical Sciences. 2020 Nov 10;10(4):2826-36.
55. Mohamed GI, Lasheen YR. Correlation between smoking, serum serotonin level, and peripheral fatigue of back extensors: cross-sectional study. Bulletin of Faculty of Physical Therapy. 2017 Dec;22(2):83-8.
56. Mohamed G. Effect of serum cotinine level on peripheral fatigue and repositioning accuracy of back extensors. Bulletin of Faculty of Physical Therapy. 2012 .
57. Farmer AD, Strzelczyk A, Finisguerra A, Gourine AV, Gharabaghi A, Hasan A, Burger AM, Jaramillo AM, Mertens A, Majid A, Verkuil B. International consensus based review and recommendations for minimum reporting standards in research on transcutaneous vagus nerve stimulation (version 2020). Frontiers in human neuroscience. 2021 Mar 23;14:568051.
58. Toro SF, Santos-Cuadros S, Olmeda E, Álvarez-Caldas C, Díaz V, San Román JL. Is the use of a low-cost sEMG sensor valid to measure muscle fatigue?. Sensors. 2019 Jul 20;19(14):3204.
59. Fields G and Image V. MaryL Search Results. American Journal of Physical Medicine & Rehabilitation, 2005; 84: 649-654.
60. Olmedo A.C, Rodrígue z J.R: Effects of upper body exercise on knee joint position sense. Miguel Angel García de la Concepción. Revista Digital - Buenos Aires, Septiembre de 2009.
61. Miura K, Ishibashi Y, Tsuda E, OkamuraY, Otsuka H and Toh S: The effect of local and general fatigue on knee proprioception. Arthroscopy. 2004; 20, 414-418.