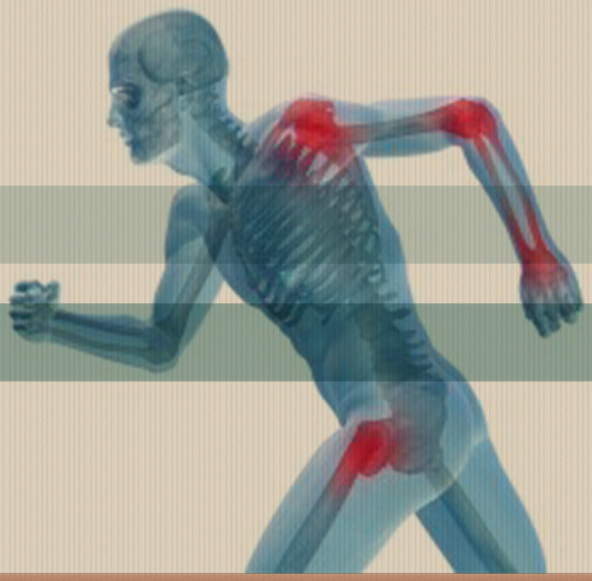
August, 2015 ● Volume 1, Issue 3

Openventio PUBLISHERS

ISSN 2379-6375



# SPORTS AND EXERCISE MEDICINE

Open Journal



www.openventio.org

SPORTS AND EXERCISE MEDICINE

Openventio PUBLISHERS

ISSN 2379-6375

Open Journal

# TABLE OF CONTENTS

SPORTS AND EXERCISE MEDICINE

Openventio PUBLISHERS

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-111

Research

*Corresponding author:
**Foong Kiew Ooi, PhD**
Associate Professor
Sport Science Unit
School of Medical Sciences
Universiti Sains Malaysia
Kubang Kerian 16150
Kelantan, Malaysia
**Tel.** +609-767 6931
**Fax:** +609-764 1945
**E-mail:** fkooi@usm.my

*Volume 1 : Issue 3*
*Article Ref. #: 1000SEMOJ1111*

***Article History:***
**Received:** May 25th, 2015
**Accepted:** July 20th, 2015
**Published:** July 27th, 2015

***Citation:***
Atiqah WAN, Ooi FK, Chen CK, Nudri WDW. Effects of chocolate malt drink consumption combined with aerobic dance exercise on blood bone metabolism markers, antioxidant enzymes and aerobic capacity in young females. *Sport Exerc Med Open J*. 2015; 1(3): 71-80.



# Effects of Chocolate Malt Drink Consumption Combined with Aerobic Dance Exercise on Blood Bone Metabolism Markers, Antioxidant Enzymes and Aerobic Capacity in Young Females

**Wadiah Azmi Nur Atiqah[1], Foong Kiew Ooi[1*], Chee Keong Chen[1] and Wan Daud Wan Nudri[2]**

*[1]Sports Science Unit, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan Malaysia*

*[2]Islamic Center, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan Malaysia*

## ABSTRACT

**Purpose:** The aim of this study was to investigate the effects of combined aerobic dance exercise and chocolate malt drink consumption on bone metabolism markers, antioxidant enzymes and aerobic capacity in young females.
**Methods:** Forty four physically healthy females (19-25 years old) were age and body mass matched and subsequently being assigned into four groups with n=11 per group: Control group (C), Chocolate malt drink group (Cmd), aerobic dance exercise group (Ex) and combined aerobic dance exercise and chocolate malt drink group (CmdEx). Participants' anthropometry and aerobic capacity were measured. Meanwhile, blood samples were taken in order to determine the concentrations of serum total calcium, serum osteocalcin and serum alkaline phosphatase (ALP) (bone formation marker), serum C-terminal telopeptide of type 1 collagen (1CTP) (bone resorption marker) and antioxidant enzyme activities, i.e. Glutathione peroxidase (GPx) and Superoxide dismutase (SOD).
**Results:** At the end of 8 weeks of the intervention period, the percentage of increment in serum osteocalcin was the highest in CmdEx group compared to the other experimental groups. Meanwhile, significant increased in glutathione peroxidase were observed in Ex ($p<0.05$) and CmdEx ($p<0.01$) groups after 8 weeks of intervention period. Additionally, the percentage changes in glutathione peroxidase and superoxide dismutase activity were the highest in CmdEx group compared to other groups. There were also significant increases in aerobic capacity in Ex and CmdEx groups.
**Conclusions:** The present study found that generally aerobic dance exercise alone and aerobic dance exercise combined with the consumption of chocolate malt drink elicited more beneficial effects on bone turnover, antioxidant enzyme activities and aerobic capacity compared to chocolate malt drink consumption alone or sedentary without chocolate malt drink consumption in young females.

**KEYWORDS:** Aerobic fitness; Antioxidant; Bone; Chocolate malt; Exercise.

## INTRODUCTION

Physical activity or exercise is believed to be important for prevention and treatment of bone loss and osteoporosis.[1] Besides exercise, bone health can also be affected by nutritional status of an individual. Chocolate malt powder (MILO®) is mainly made up of cocoa and malt. It also contains protomalt, actigen-E, protein, carbohydrate and fat. Protomalt is a special malt extract from barley, which provides carbohydrate and supplies energy to body needs. ActigenE

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-111

is a combination of 8 vitamins and 4 minerals, which helps to release energy from carbohydrate in the diet. Vitamins and minerals that contained in chocolate malt powder (MILO®) are vitamin B complex and vitamin C, calcium, phosphorus, iron and magnesium.[2] Some of these vitamins and minerals are believed to play important roles in maintaining bone health.

Chocolate malt powder (MILO®) contains cocoa, which is a good source of minerals including flavanoids, phosphorus and magnesium.[3] Flavonoids are reported to influence bone mass density and protect against osteoporosis in older women.[4] Phosphorus is important for bone formation,[5] and impairment of serum phosphate can lead to impair bone mineralization and osteoblast function. Magnesium plays a role in bone growth and stabilization and involved in bone and mineral homeostasis.[5] Several previous studies reported that magnesium intake has positive correlation with bone mineral density and bone resorption markers in middle-aged women.[6] Chocolate malt powder (MILO®) also contains milk. Milk consists of carbohydrates, proteins, fats, vitamins and minerals, which are important to prevent osteoporosis.[3] It was reported that protein can influence bone growth and bone mass in children and adolescents.[7]

Regarding chocolate malt powder (MILO®) and antioxidant properties, the cocoa contained in chocolate malt powder has flavonoids, which are the polyphenolic compound that have antioxidant effects.[8] Cocoa has been reported can increase antioxidant defense system.[9] Cocoa supplementation was reported can increase superoxide dismutase activity.[10] Cocoa was also reported can enhance antioxidant enzymes activities in the liver and heart tissues of the rat.[11]

The present research group has carried out a few previous studies to investigate the effects of exercise alone and also combined effects of exercise with nutritional supplementation on bone health. For instance, Ooi, et al.,[12] found that bone mass and strength were significantly higher in jumping rats compared to controls. These findings imply that exercise can enhance bone mass and strength. Additionally, it was found by Ooi, et al.,[13] that serum alkaline phosphatase (a bone formation marker) increased in jumping exercise group and serum 1CTP (a bone resorption marker) decreased in jumping exercise group and authors concluded that jumping exercise could increase bone formation and decrease bone resorption.

Regarding animal study on combination effect of exercise and nutritional supplementation, Somayeh, et al.,[14] found that tibial mass and tibial and femoral strength in combined honey supplementation and jumping rats were significantly increased, implying that combination of jumping exercise and honey supplementation give more beneficial effects on tibia and femur bones compared to either jumping alone or honey supplementation alone.

In humans, Ooi, et al.[15] found that combination of honey supplementation aerobic dance give beneficial effect on bone formation marker, i.e. serum alkaline phosphatase in young females.[15] Similarly, Ooi, et al. [16] found that there was decreased in 1CTP in combined honey with circuit training in young males, even though no significant changes in alkaline phosphatase and osteocalcin were observed. In year 2013, Marhasiyah, et al.[17] had conducted a study on "Effects of Combined Aerobic Dance Exercise and Honey Supplementation on Bone Metabolism in Women" (Malaysia). It was found that there was lowest percentage of increment in 1CTP in combined aerobic dance exercise with honey supplementation group compared to other groups, even though there were no discernable changes in alkaline phosphatase and osteocalcin were observed. The authors concluded that, combination of aerobic dance exercise and honey supplementation may elicit beneficial effect on bone health in adult women. Lau and Ooi.[18] found that serum 1CTP decreased in combined circuit training with chocolate malt supplementation group. Nevertheless, serum alkaline phosphatase and osteocalcin were not significantly affected by this combination. The authors concluded that circuit training combined with chocolate malt supplementation elicited greater effect on bone resorption in young males.

It is generally known that the type of exercise or physical activity which is necessary to improve and maintain bone density is weight bearing exercise. Weight bearing exercises include walking, running, dancing and jumping.[19] Dancing serves an ideal osteogenic stimulus because it has various jumps and landings which provide unusual impact and high-impact loads on the skeleton.[20] To date, no studies have been carried out to investigate the effects of chocolate malt drink consumption with aerobic dance exercise on blood bone metabolism markers, antioxidant enzymes and aerobic capacity in young females, thus the present was proposed. If the present study can show that chocolate malt drink combined with aerobic dance exercise can give positive effects on bone metabolism markers, antioxidant enzymes and aerobic capacity, it can be used for formulating guidelines in young females to plan their exercise and nutritional promotion program for maintaining bone health and reducing the risk of osteoporosis, enhancing antioxidant enzymes activities and increasing cardiorespiratory endurance.

## METHODS AND MATERIALS

### Participants

In this study, forty four physically healthy females, with age ranged from 19-25 years old were recruited. The participants should be leading sedentary lifestyles (not involved in regular physical activity for more than once per week) and do not have the habit of consuming chocolate malt drink (MILO®) as daily consumption prior to the experiment. Participants were matched in age and body mass before they were assigned randomly into the group.

All participants were fully informed by the researcher about the nature of the experiments, purpose of the study, pro-

cedures, benefits and risks of feeling discomforts experienced in this present study. All participants were required to fill up participants' information sheets and sign on the consent forms. The present study was approved by the Research Ethics Committee (Human) of Universiti Sains Malaysia.

**Experimental Design**

**Participant's grouping:** The participants were age and body mass matched and then being randomly divided into four groups, with 11 participants per group (n=11): 8 weeks of sedentary without chocolate malt consumption control (C), 8 weeks with chocolate malt drink consumption (Cmd), 8 weeks of aerobic dance exercise without chocolate malt drink consumption (Ex) and 8 weeks of chocolate malt drink consumption with aerobic dance exercise (CmdEx) groups. Participants in the control group did not perform neither exercises nor taking chocolate malt drink consumption. Participants of chocolate malt drink consumption group were required to consume 45 g of chocolate malt powder mixed with 300 ml of plain water, 7 days per week for 8 weeks duration. Meanwhile, aerobic dance exercise group were required to perform aerobic dance exercise for 1 hour per session, 3 times per week for 8 weeks. Participants in combined chocolate malt drink consumption with aerobic dance exercise were required to consume 45 g of chocolate malt powder mixed with 300 ml of plain water for 7 days per week for 8 weeks and performed aerobic dance exercise for 1 hour per session, 3 times per week for 8 weeks. The participants of CmdEx group were required to consume chocolate malt drink 30 minutes before performing aerobic dance exercise on the exercised days.

**Blood sample taking:** Before and after 8 weeks of intervention, blood samples were withdrawn from the participants, from an antecubital vein after a 12 hours overnight fast (drinking plain water was allowed) at 8.00-10.00 am. The blood was withdrawn by the laboratory technologist in the Sport Science Laboratory, School of Medical Science, Health Campus, Universiti Sains Malaysia to determine the concentrations of bone metabolism markers and antioxidant enzymes activities. Blood taking for participants in Ex and CmdEx were carried out 13 to 16 hours after performing aerobic dance exercise.

During each blood taking, 6 ml of resting venous blood sample was collected into a plain, without EDTA tube from each participant. Serum was obtained by centrifuging blood sample for 10 minutes of 3000 RPM at 4 ºC (Hettich Zentrifuger-Rotina 46RS, Germany). Then, the serum obtained was divided into equal portions in bullet tubes and stored at -80 ºC in a freezer (ThermoForma, Model 705, USA) until subsequent analysis of serum bone metabolism markers and antioxidant enzymes.

**Anthropometric measurements:** In this study, anthropometric measurements were recorded before the commencement of the study. Body mass and percentage of body fat were measured by using Body Composition Analyser (TANITA, Model TBF-410, Japan) to the nearest 0.1 kg and 0.1 % respectively. Meanwhile, body height was measured by using a scale (Seca 220, Gemany) to the nearest 0.1 cm.

**Chocolate malt drink consumption:** Chocolate malt drink was consumed by the participants in chocolate malt drink (Cmd) and combined chocolate malt drink and aerobic dance exercise (CmdEx) groups in the dosage of 45 g of chocolate malt powder (containing 4.5 g of fat, 5.3 g of protein, 30 g of carbohydrate, 234 mg of calcium, 248 mg of phosphorus, 76.5 mg of magnesium, 4.7 mg of iron) mixed with 300 ml of plain water. Participants consumed chocolate malt drink once per day, 7 days per week for 8 weeks. Participants in CmdEx group consumed 300 ml of chocolate malt drink 30 minutes before performing aerobic dance exercise.

**Aerobic dance exercise program:** The participants of aerobic dance exercise group (Ex) and combined chocolate malt drink consumption with aerobic dance exercise group (CmdEx) were required to attend aerobic dance classes for 3 sessions per week, one hour per session (from 5.30 pm to 6.30 pm) for 8 weeks.

The aerobic dance exercise program of this study consisted of one sessions of 'floor aerobic dance exercise' and two sessions of a 'step board' aerobic dance exercises in a week. The one hour session started with 10 to 15 minutes of warming up period, 30 to 35 minutes of dance period and ended with 5 to 7 minutes of cooling down, conditioning and toning.

The aerobic dance exercise program prescribed in the present study generally involved continuous, controlled movement of the legs and trunk and intermittent movement of the arms. These include movements that extend, flex, abduct, adduct and rotate the leg and foot like side stepping, fast walking, forward and backward stepping, leg lifts, placing foot to the front, side and behind, forward and side-lunging, heel rises and also some high impact exercises like jumping. In the floor aerobic dance exercise sessions, participants were required to do upper and lower limbs movements according to the beat of the music played. In the 'step board' exercise sessions, participants were required to step up and step down the step board while dancing. Heart rate monitor (polar watch, $710, US) were worn by the participants throughout the dancing sessions to estimate the intensity of aerobic dance exercise.

**Measurement of aerobic capacity:** Twenty meter shuttle run test was conducted to determine participants' predicted maximal oxygen uptake ($VO_{2max}$). This test was conducted before and after 8 weeks of intervention period. The equipments that had been used in this test were measuring tape, pre-recorded CD, CD player and marker cones. The test procedure began with 5 min of warm-up. The participants ran on a flat, non-slippery area which was marked with cones separated by 20 meters distance. Participants' predicted $VO_{2max}$ were calculated based on the number of completed shuttles by using an online formula (http://www.

topendsports.com/testing/beepcalc.htm).

**Blood biochemical analysis:** Serum total calcium was analysed calorimetrically by using an automatic analyzer (Hitachi Automatic Analyzer 912, Bohringer Mannheim, Germany) with commercially available reagent kits (Randox, UK). Serum osteocalcin is a bone formation marker, which was analysed by using a commercially available enzyme immunological test kit (N-MID® Osteocalcin ELISA). Serum ALP is a bone formation marker, which was analysed colorimetrically by using a chemistry analyser (Architec C 8000, USA) with commercially available reagent kits (Randox, UK). Serum 1CTP is a bone resorption marker, which was analysed by an available enzyme immunoassay kit (Human C-telopeptide of type 1 collagen (1CTP), ELISA Kit). The concentration was determined by using a photometric reader (Molecular Device, Versamax tunable micro reader, USA). Reagent kits from BioAssay Systems, USA were used to determine Superoxide dismutase enzyme activity (EnzyChromTM Superoxide Dismutase Assay Kit, ESOD-100) and glutathione peroxidase enzyme activity EnzyChromTM Glutathione Peroxidase Assay Kit ,EGPX-100).

**Statistical analysis:** Statistical analysis was done by using Statistical Package for Social Science (SPSS) version 20.0. All values are presented as mean±standard deviations (SD). Repeated measure Analysis of variance (ANOVA) and Bonferroni post hoc test were performed to determine the significance of the differences between and within groups. The difference was considered statistically significant at $p<0.05$.

## RESULTS

### Anthropometric Characteristics of the Participants

A total of 44 healthy young female's participants which are recruited in the present study had completed the study. Anthropometric characteristics of the participants are illustrated in Table 1. No participant had discontinued the program during the experimental period.

### Bone Metabolism Markers

**Bone formation markers: Serum osteocalcin (OC) and serum alkaline phosphatase (ALP):** The bone formation markers of serum osteocalcin (OC) and serum alkaline phosphatase (ALP) concentrations in all the groups at pre-and post tests are presented in Table 2 respectively. After 8 weeks of intervention period, there was no significant simple effect of intervention on serum osteocalcin (OC) concentrations ($F=0.34$, $p>0.05$) between all the experimental groups. Furthermore, no significant main effect of time on serum osteocalcin (OC) concentrations ($F=0.599$, $p>0.05$) between pre- and post tests for all experimental groups. The present results showed that percentage increment of serum osteocalcin (OC) concentrations was the highest in CmdEx group (+7.90%) compared to Ex (+2.25%), C (+1.27%) and Cmd (-1.21%) groups. After 8 weeks of intervention period, no significant simple effect of intervention on serum ALP concentrations ($F=0.117$, $p>0.05$) was observed between all the groups. Statistically significant main effect of time on this serum bone formation marker was observed ($F=37.78$, $p<0.01$) between pre- and post tests in Cmd, Ex and CmdEx groups. In post test, there were significant greater values of serum ALP as compared to the pre test values in Cmd, Ex and CmdEx groups.

**Bone resorption marker: Serum C-terminal of type 1 collagen (1CTP):** Mean serum C-terminal of type 1 collagen (1CTP) concentrations of all groups is presented in Table 2. After 8 weeks of intervention period, no significant simple effect of intervention on serum 1CTP concentrations ($F=0.087$, $p>0.05$) was observed between all the groups. Statistically significant main effect of time on serum 1CTP concentrations was observed ($F=3.136$, $p<0.05$) between pre- and post tests in Ex group, in which there was significant lower post test serum 1CTP value as compared to the pre test value in Ex group. The percentage reduction of mean serum 1CTP concentrations was the highest in Ex group (-25.02%).

**Serum total calcium:** Mean serum total calcium concentrations for all groups are presented in Table 3. There were no significant simple effect of intervention on serum total calcium concentrations ($F=0.547$, $p>0.05$) between all the experimental groups after 8 weeks of intervention period. Furthermore, no significant main effect of time on serum total calcium concentrations ($F=4.029$, $p>0.05$) between pre- and post tests was observed among all the experimental groups.

### Antioxidant Enzyme Activities: Glutathione Peroxidase (Gpx) and Superoxide Dismutase (SOD) Activity

Mean of glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity of all groups are presented in Table 4. There was no significant simple effect of intervention on GPx

| Groups (N=44) | Height (cm) | | Weight (kg) | | Body fat (%) | | Body mass index (kg/m²) | |
|---|---|---|---|---|---|---|---|---|
| | Pre-test | Post-test | Pre-test | Post-test | Pre-test | Post-test | Pre-test | Post-test |
| Control (C) | 155.21±5.20 | 154.96±5.19 | 56.66±7.98 | 56.19±7.57 | 29.96±7.57 | 30.92±7.45 | 23.16±3.69 | 23.47±3.63 |
| Chocolate malt drink (Cmd) | 152.79±5.63 | 152.87±5.70 | 51.47±9.88 | 52.36±9.56 | 28.14±6.46 | 29.36±6.11 | 22.02±3.44 | 22.26±3.21 |
| Exercise (Ex) | 152.60±3.63 | 152.73±3.70 | 55.21±14.79 | 55.21±13.84 | 32.33±13.39 | 32.16±12.38 | 23.70±6.11 | 23.57±5.80 |
| Combined (CmdEx) | 154.86±6.09 | 155.03±5.99 | 56.13±10.79 | 57.03±10.37 | 31.18±8.14 | 31.27±7.85 | 23.56±4.71 | 23.69±4.14 |

**Table 1:** Means age, body height, body weight and percentage of body fat of the participants.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-111

| Groups (N=44) | Serum osteocalcin concentration (µg/ml) (Mean±SD) | | | Mean percent difference (%) | Serum ALP concentration (µg/ml) (Mean±SD) | | | Mean percent difference (%) |
|---|---|---|---|---|---|---|---|---|
| | Pre-test | Post-test | Mean difference between pre- and post | | Pre-test | Post-test | Mean difference between pre- and post | |
| Control (C) | 25.12±9.19 | 25.44±7.34 | 0.32± 3.53 | 1.27 | 52.55±5.73 | 58.36±9.09 | 5.82±7.73 | 11.06 |
| Chocolate malt drink (Cmd) | 23.19±5.04 | 22.91±5.59 | - 0.28±5.21 | -1.21 | 56.82±13.86 | 63.91±17.82** | 7.09±5.17 | 12.48 |
| Exercise (Ex) | 21.79±4.80 | 22.28±6.73 | 0.49±4.70 | 2.25 | 59.55±17.1 | 67.18±21.83** | 7.64±7.27 | 12.81 |
| Combined (CmdEx) | 23.30±6.00 | 25.14±3.47 | 1.84±1.53 | 7.90 | 57.36±9.72 | 64.36±14.21** | 7.00±9.02 | 12.20 |
| Groups (N=44) | Serum 1CTP concentrations (µg/ml) (Mean±SD) | | | Mean percent difference (%) | Serum total calcium concentration (µg/ml) (Mean±SD) | | | Mean percent difference (%) |
| | Pre-test | Post-test | Mean difference between pre- and post | | Pre-test | Post-test | Mean difference between pre- and post | |
| Control (C) | 150.66±172.64 | 136.26±159.99 | -14.4±84.78 | -9.56 | 2.22±0.18 | 2.31±0.15 | 0.09±0.20 | 4.05 |
| Chocolate malt drink (Cmd) | 166.31±120.38 | 161.56±113.63 | -4.75±68.37 | -2.86 | 2.30±0.13 | 2.33±0.13 | 0.03±0.15 | 1.30 |
| Exercise (Ex) | 203.02±161.74 | 152.22±109.10* | -50.8±96.54 | -25.02 | 2.37±0.09 | 2.40±0.15 | 0.03±0.11 | 1.27 |
| Combined (CmdEx) | 76.97±51.41 | 65.39±34.79 | -11.58±46.27 | -15.04 | 2.30±0.13 | 2.33±0.12 | 0.03±0.09 | 1.30 |

*significantly different from pre-test (p<0.05)
**significantly different from pre-test (p<0.01)

**Table 2**: Mean serum osteocalcin, alkaline phosphatase (ALP), C-terminal telopeptide of type 1 collagen (1CTP), total calcium concentrations at pre- and post tests (Mean±SD).

| Groups (N=44) | Glutathione peroxidase activity (U/g) (Mean±SD) | | | Mean percent difference (%) | Superoxide dismutase activity (U/g) (Mean±SD) | | | Mean percent difference (%) |
|---|---|---|---|---|---|---|---|---|
| | Pre-test | Post-test | Mean difference between pre- and post | | Pre-test | Post-test | Mean difference between pre- and post | |
| Control (C) | 265.57±46.18 | 279.64±28.21 | 14.07±52.28 | 5.30 | 1.76±0.59 | 1.64±0.62 | -0.12±0.32 | -6.82 |
| Chocolate malt drink (Cmd) | 271.82±42.26 | 306.02±25.61 | 34.21±52.31 | 12.58 | 1.65±0.48 | 1.55±0.51 | -0.1±0.23 | -6.06 |
| Exercise (Ex) | 242.16±33.20 | 293.98±33.86* | 51.82±55.23 | 21.40 | 1.55±0.35 | 1.49±0.34 | -0.06±0.34 | -3.87 |
| Combined (CmdEx) | 231.02±78.50 | 291.14±47.03** | 60.11±111.37 | 26.02 | 1.43±0.22 | 1.64±0.64 | 0.20±0.62 | 14.69 |

*significantly different from pre-test (p<0.05)
**significantly different from pre-test (p<0.01)

**Table 3**: Mean of glutathione peroxidase (GPx) activity and superoxide dismutase (SOD) activity at pre- and post tests.

activity (F=0.876, p>0.05) for all the experimental groups after 8 weeks of intervention period. There were statistically significant main effect of time on GPx activity between pre- and post tests for Ex (F=13.493, p<0.05) and CmdEx (F=13.493, p<0.01) groups. In post test, there were significant greater values of GPx activity as compared to the pre test values in Ex and CmdEx groups. The present results showed that the percentage increment of mean of GPx activity was the highest in CmdEx group (+26.02%) compared to Ex (+21.40%), Cmd (+12.58%) and C (+5.30%) groups. There was no significant simple effect of intervention on SOD activity (F=1.509, p>0.05) for all the experimental groups after 8 weeks of intervention period. Furthermore,

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-111

| Groups (N=44) | Aerobic capacity (mL/kg/min) (Mean±SD) | | | Mean percent difference (%) |
|---|---|---|---|---|
| | Pre-test | Post-test | Mean difference between pre- and post | |
| Control (C) | 23.41±2.23 | 24.16±3.27 | 0.75±1.79 | 3.20 |
| Chocolate malt drink (Cmd) | 25.47±3.81 | 26.12±4.13 | 0.65±2.57 | 2.55 |
| Exercise (Ex) | 23.49±0.002 | 25.9±2.10*** | 2.41±1.38 | 10.26 |
| Combined (CmdEx) | 25.46±3.60 | 27.86±4.35***, + | 2.4±1.63 | 9.43 |

***; significantly different from pre-test ($p<0.001$),
+; significantly different from respective control (C) group ($p<0.05$)

**Table 4:** Mean of aerobic capacity at pre- and post tests.

no significant main effect of time on SOD activity ($F=0.082$, $p>0.05$) between pre- and post tests for all the groups. The study results showed that the percentage increment of mean SOD activity was the highest in CmdEx group (+14.69%) compared to Ex: -3.87%, C: -6.82% and Cmd: -6.06%.

### Aerobic Capacity

The mean of aerobic capacity in all groups at pre- and post tests are presented in Table 4. There was a significant simple effect of intervention on aerobic capacity between CmdEx ($F=2.973$, $p<0.05$) and C group respectively. Furthermore, there were statistically significant main effect of time on aerobic capacity between pre- and post tests for Ex and CmdEx ($F=29.527$, $p<0.001$) groups. In post test, there were significant greater values of aerobic capacity as compared to the pre test values in Ex and CmdEx groups. The present results showed that the percentage increment of mean aerobic capacity was the highest in Ex group (+10.26%) compared to CmdEx (+9.43%), C (+3.20%) and Cmd (+2.55%) groups.

## DISCUSSION

One of the notable finding in the present study is that there were significant increases ($p<0.01$) in serum alkaline phospahtase (ALP) in Cmd, Ex and CmdEx groups (Table 3), implying Cmd alone, Ex alone and combined Cmd with Ex may give beneficial effect on bone health by stimulating the bone formation. The finding of increased in serum ALP as result of exercise in the present study was in an agreement with the another study conducted by Lau, et al.,[14] which found that serum ALP was significantly increased in Ex group and the percentage increment was the highest (+12.81%) in the Ex group compared to the other groups.

Another notable findings of the present study is that the percentage of serum osteocalcin concentrations was the highest in CmdEx group (+7.90%) compared to Ex (+2.25%), C (+1.27%) and Cmd (-1.21%) groups (Table 3). This finding indicates that CmdEx may have greater potential in increasing bone formation compared to other groups.

Regarding combined effects of exercise and nutritional supplementation on bone formation marker in humans, the finding of the present study with increased serum ALP in Ex and CmdEx was in consistent with Ooi, et al.[15] which reported that bone formation marker which is serum ALP was significantly increased in young female participants after 6 weeks of consuming honey combined with 6 weeks of aerobic dance exercise. The present finding is also consistent with the finding of an animal study conducted by Gala, et al.[21] Gala, et al.[21] reported that bone formation marker of ALP in ovariectomized rats which performed exercise alone and ovariectomized rats with combined exercise program and diet supplemented with calcium showed increased in ALP compared to ovariectomized control rats group. In another previous study conducted by Wagner, et al.[22] it was reported that serum bone alkaline phosphatase was significantly increased after taking calcium during weight loss training program. In that study, the participants consumed 800 mg of calcium per day and exercise 3 times per week for 12 weeks. A previous study conducted by Tartibian, et al.[23] also reported that serum ALP and serum procollagen type 1 C-terminal (PICP) (bone formation markers) was significantly increased in exercise alone and combined exercise, calcium and vitamin D supplementation groups. In that study, the participants exercised 30 to 45 minutes with 70%-80% maximum intensity for 9 weeks. Additionally, participants in the combined exercise and supplements group consumed 1000 mg of calcium and 200 IU of vitamin D per day. The findings of these previous studies were similar with the present finding which showed that whether exercise alone or exercise combined with nutritional supplementation may elicit beneficial effects on bone formation.

The finding of the present study showed that serum ALP was significantly increased in Cmd alone group. The present finding is inconsistent with Lau, et al.,[18] findings. They found that there were no statistically significant different on serum ALP in Cmd alone group. The discrepancy between the finding

of Lau, et al.,[18] and the present study may be due to the variation of gender. In the present study, the participants are young females while in the previous study, the participants are young males. These study findings have confirmed our speculation that gender may play a role in affecting bone metabolism based on the fact that there is difference in bone mineral density between males and females. In the present study, it was also observed that serum ALP increased non-statistically to similar extent to other experimental groups, nevertheless there were no statistically significant differences among the groups.

Regarding effects of exercise on bone resorption in the present study, it was found that serum C-terminal telopeptide of type 1 collagen (1CTP), a bone resorption marker was significantly decreased in exercise alone (Ex) group (Table 3), this study finding showed that by performing one hour of aerobic dance exercise three times per week, not only increase in bone formation marker of serum ALP as mentioned earlier could be observed, decrease in bone resorption marker of serum 1CTP could also be observed. Collectively, the present findings imply that physical activity or exercises may elicit beneficial effects to bone health by stimulating bone formation and decrease bone resorption. Nevertheless, the absence of statistically significant changes in serum 1CTP in combined exercise and supplementation group needs further investigation, and this observation may imply that effects of exercise alone on bone reportion marker can be different from exercise combined with supplementation.

Several previous studies have showed similar finding with reduction in bone resorption marker as results of exercise as in the present study. For instance, Eliakim, et al.,[24] reported a significant decreased in urinary N-terminal telopeptide cross-links, a bone resorption marker after 5 weeks of endurance-type training consist of running, aerobic dance, competitive sports and occasional weight lifting in the participants. It was reported in another study by Brahm, et al.,[25] that there was a significant decreased in 1CTP, a bone resorption marker in marathon runners compared to the sedentary control group. In an animal study conducted by Ooi, et al.,[13] it was found that serum 1CTP levels were significantly lower in rats, which received exercise loads of 40 jumps per week for 24 weeks compared to the sedentary control rats.

In the present study, no statistically significant differences were found in serum total calcium among all the experimental groups (Table 3). Recently, Marhasiyah, et al.,[17] found that there was significant greater serum total calcium in post test compared to the pre test in honey supplementation alone (H) group. Their finding is inconsistent with the present finding, in which chocolate malt consumption alone did not show any significant increase of serum total calcium in Cmd group. Comparison between Marhasiyah, et al.,[17] and the present study showed that honey drink may have greater potential in increasing serum total calcium than chocolate malt drink. The other factor which may cause differences in the finding of Marhasiyah, et al.[17] and the present study can be variation of the age of the participants recruited, where the present study recruited young females with age range between 19-25 years old while previous study by Marhasiyah, et al.[17] recruited adult women with age range between 25-40 years old. These findings have confirmed our speculation that age can play a role in affecting bone metabolism, based on the fact that there is difference in bone mineral density between young and older populations.

Miyazaki, et al.,[26] found that GPx was significantly increased among all the participants who performed running at 80% of maximal heart rate. Their finding is in agreement with the findings of the present study. During exercise, aerobic metabolic rate can increase up to 10 folds. As a result, it stimulates the enhancement leakage of oxygen from the mitochondria to the cytosol.[27] This reaction gives rise to reactive oxygen species (ROS) which can induce damage to the cells in our body. An increase in ROS during exercise has been considered to be an oxidative stress. That is the reason why antioxidant enzymes such as GPx rise during exercise. They act as scavenger to prevent ROS from damaging the body cells.[28] The mechanism stated above is parallel with the present finding in which GPx activities were significantly increased after 8 weeks of exercise.

In the present study, we found that GPx activity was significantly increased in the combined exercise with chocolate malt drink consumption group. In a previous study conducted by Kan, et al.,[29] it was found that the GPx activity was significantly increased with the supplementation of flavonoids after performing an exhaustive swimming exercise in mice. Tauler, et al.,[30] also found that GPx activities were significantly increased after taking antioxidant diet supplementation combined with exercise training in amateur trained male athletes. In another previous study conducted by Tessier et al.,[31] it was found that the erythrocyte GPx activity level were significantly increased with 10 weeks of endurance training program combined with supplementation of selenium, which act as antioxidant. The present and previous study findings indicate that exercise combined with antioxidant diet supplementation may enhance the level of antioxidant enzyme activities.

The present finding showed that there was no statistically significant difference in SOD activity level among all the experimental groups but the highest increment was observed in the combined group (+14.94%). Several previous studies showed different finding with the present finding. For instance, Kan, et al.,[29] found that SOD activity level was significantly increased with supplementation of polyphenols and flavonoids rich which is Ixora parviflora extract after an exhaustive swimming in mice. Miyazaki, et al.,[26] also found that SOD activity was significantly increased after 12 weeks of intense training in young males. The inconsistent finding between previous and present studies may be due to the variation in the duration of intervention period and types of antioxidant diet consumption among the studies.

The absence of statistically significant changes in SOD activities in the present finding was consistent with the several

other previous studies. For instance, Fadillioglu, et al.,[27] found that there was no significant difference before and after exercise program in plasma and erythrocyte SOD levels among all the groups. Similarly, SOD activity level was not statistically increased after running over 5 kilometers in sedentary men.[32] In another previous study conducted by Tauler, et al.,[30] it was found that there was no statistically significant difference of erythrocyte and lymphocyte SOD activity level in combined of supplemented selenium and exercise training group. The present and previous studies findings may indicate that either exercise alone or exercise combined with antioxidant diet supplement may not give beneficial effect to elevate SOD activity level. Nevertheless, the present finding of the percentage changes of SOD (+14.69%) were the highest in CmdEx group compared to other groups may imply that CmdEx may have greater potential in enhancing antioxidant enzyme activity compared to other groups.

The exercise intensity in the present study was considered moderate to high based on the observation that the exercise heart rate of the participants ranged from 140 bpm to 170 bpm throughout the exercise sessions, which represent 70% to 85% heart rate maximum. The present study showed that participants' aerobic capacity was significantly increased after 8 weeks of study period in exercise and combined groups (Table 4). Other than that, it was also found that there was significant difference between the combined group compared to the control group respectively after 8 weeks of study period. Similar finding between the previous study and the present study have been reported. Donnelly, et al.,[33] found that there was a significant increased in maximal oxygen consumption in the participants across 18 months of exercise. Besides, Rognmo, et al.,[34] also found that the peak oxygen uptake ($VO_{2peak}$) was increased significantly before and after training in high and moderate intensity throughout 10 weeks of training. Women with Systemic Lupus Erythematosus (SLE) who underwent 12 weeks of cardiovascular training program showed significant increased in maximum oxygen consumption compared to the sedentary control group. These findings imply that exercise may improve aerobic capacity in human.[35]

To date, studies of combined exercise and nutritional supplementation on aerobic capacity are lacking, this made the comparison between the present findings with previous related study difficult. More future studies regarding combination of exercise and nutritional supplementation on aerobic capacity are warranted.

## CONCLUSION

As a conclusion, the present study found that either aerobic dance exercise alone or combination of aerobic dance exercise with chocolate malt drink consumption give more beneficial effects on bone health, antioxidant enzyme activities and aerobic capacity compared to the consumption of chocolate malt drink alone and sedentary without chocolate malt drink consumption in young females. Therefore, both consumption of chocolate malt drink with 45 g of MILO® diluted in 300 ml of plain water combined with 3 days per week aerobic dance exercise, and aerobic dance exercise performed at 3 times per week have potential to be proposed for formulating guidelines in planning exercise and nutrition promotion program for the maintenance of bone health, enhancing antioxidant enzyme activities and increasing cardiorespiratory endurance in young females.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

## ACKNOWLEDGEMENTS

We wish to extend our sincere gratitude to all the participants who have participated in this study. We also want to express our appreciation to Mdm. Jamaayah bt. Meor Osman, Mdm. Norlida bt Azalan, Mdm. Hafizah bt. Hamzah, Mdm Parimalah Velo and Mdm. Nor Aini bt. Sudin from Sports Science Unit, Universiti Sains Malaysia for their technical assistance.

## REFERENCES

1. Ooi FK, Singh R, Singh HJ. Jumping exercise and bone health: Beneficial effects of jumping exercise on bone health. Verlag Dr. Muller, Germany; 2009.

2. Nestlé Malaysia Ltd. Company, 10 Reasons to have a MILO. http://www.milo.com.my/10-reasons-to-have-a-milo.html Accessed February 2, 2013.

3. Baucer W, TÜrler-Inderbitzin S. Cocoa and malt. NutriPro Beverages-NESTLE Professional Nutrition Magazine; 2008.

4. Hegarty VM, May HM, Khaw KT. Tea drinking and bone mineral density in older women. *Am J Clin Nutr*. 2007; 71: 1003-1007.

5. Prentice A. Diet, nutrition and the prevention of osteoporosis. *Public Health Nutr*. 2004; 7: 227-243. doi: 10.1079/PHN2003590

6. Robins SP, New SA, Campbell MK. Dietary influences on bone mass and bone metabolism: further evidence of a positive link between fruit and vegetable intake and bone health? *Am J Clin Nutr*. 2000; 71: 142-151.

7. Rizzoli R, Bonjour JP, Ammann P, Chevalley T. Protein intake and bone disorders in the elderly. *Joint Bone Spine*. 2006; 68: 383-392. doi: 10.1016/S1297-319X(01)00295-0

8. Wan Y, Vinson JA, Etherton TD. Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglan-

din concentrations in humans. *Am J Clin Nutr*. 2001; 74: 596-602.

9. Engler MB, Engler MM. The vasculoprotective effects of flavonoids-rich cocoa and chocolate. *Nutr Res*. 2004; 24: 695-706. doi: 10.1016/j.nutres.2004.05.001

10. Ismail A, Jalil AMM, Hamid M, Kamaruddin SHS, Pei CP. Effects of cocoa extract on glucometabolism, oxidative stress and antioxidant enzymes in obese-diabetic (Ob-db) rats. *J Agricult Food Chem*. 2008; 56: 7877-7884. doi: 10.1021/jf8015915

11. Noori S, Nasir K, Mahboob T. Effects of cocoa powder on oxidant/ antioxidant status in liver, heart and kidney tissues of rats. *J Anim Plant Sci*. 2009; 19: 174-178.

12. Ooi FK, Singh R, Singh HJ, Umemura Y. Minimum level of jumping exercise required to maintain exercise-induced bone gains in female rats. *Osteoporos Int.* 2009; 20: 963-972. doi: 10.1007/s00198-008-0760-6

13. Ooi FK, Singh R, Singh HJ. Changes in bone turnover markers and bone mass with reducing levels of jumping exercise regimens in female rats. *Asian J Sports Med*. 2012; 3: 225-232.

14. Somayeh ST, Ooi FK, Krasilshchikov O, Sulaiman SA. Effect of a combination of jumping exercise and honey supplementation on the mass, strength and physical dimensions of bones in young female rats. *J Api Product ApiMed Sci*. 2011; 3: 26-32. doi: 10.3896/IBRA.4.03.1.05

15. Ooi FK, Ismail N, Abdullah MY. Effects of combined aerobic dance exercise and honey supplementation on bone turnover markers in young females. *Asian J Exerc Sport Sci*. 2011; 8: 1-11.

16. Ooi FK, Azlina A, Abdullah MY. Combined effects of a circuit training programme and honey supplementation on bone metabolism markers in young males. Abstract book of the 16th National Conference on Medical and Health Sciences, Kota Bharu, Malaysia; 2011.

17. Marhasiyah R, Ooi FK, Wan Zuraida. Effects of combined aerobic dance exercise and honey supplementation on bone metabolism in women. Proceedings of the 9th International Sports Science Conference, Kota Bharu, Malaysia; 2013.

18. Lau SJ, Ooi FK. Changes in blood bone turnover markers following combined circuit training programme and chocolate malt drink supplementation in young male. *MR Int JAppl Health Sci.* 2014; 1: 30-38.

19. Karlsson MK , Johnell O, Obrant KJ. Bone mineral density in professional ballet dancers. *Bone Miner.* 1993; 21: 163-169.

20. Matthews BL, Bennell KL, McKay HA. Dancing for bone health: a 3-year longitudinal study of bone mineral accrual across puberty in female non-elite dancers and controls. *Osteoporosis Int.* 2006; 17: 1043-1054. doi: 10.1007/s00198-006-0093-2

21. Gala J, Díaz-Curiel M, Piedra C, Calero J. Short- and long-term effects of calcium and exercise on bone mineral density in ovariectomized rats. *J Nutr.* 2001; 86: 521-527. doi: 10.1079/BJN2001428

22. Wagner G, Kindrick S, Hertzler S, DiSilvestro RA. Effects of various forms of calcium on body weight and bone turnover markers in women participating in a weight loss program. *J Am Coll Nutr.* 2007; 26: 456-461.

23. Tartibian B, Motabsaee N, Tolouei-Azar J. The influence of nine-week intensive aerobic exercises, calcium and vitamin D supplementation on the metabolic response of bone formation biomarkers. *Zahedan J Res Med Sci*. 2013; 15: 45-50.

24. Eliakim A, Raisz LG, Brasel JO, Cooper DM. Evidence for increased bone formation following a brief endurance-type training intervention in adolescent males. *J Bone Miner Res*. 1997; 12: 1708-1713. doi: 10.1359/jbmr.1997.12.10.1708

25. Brahm H, Ström H, Piehl-Aulin K, Ljunghall S. Bone metabolism in endurance trained athletes: A comparison to population-based controls based on DXA, SXA, quantitative ultrasound, and biochemical markers. *Calcif. Tissue Int*. 1997; 61: 448-454. doi: 10.1007/s002239900366

26. Miyazaki H, Oh-ishi S, Ookawara T, et al. Strenous endurance training in humans reduces oxidative stress following exhausting exercise. *J Appl Physiol*. 2001; 84: 1-6.

27. Fadillioglu E, Kaya B, Uz E, Emre MH, Unal S. Effects of moderate exercise on mild depressive mood, antioxidants and lipid peroxidation. *Bulletin Clin Psychop*. 2000; 10: 194-199.

28. Daud DM, Karim AAH, Mohamad N, Hamid NAA, Ngah WZW. Effect of exercise intensity on antioxidant enzymatic activities in sedentary adults. *Malaysian J Biochem Mol Biol*. 2006; 13: 37-47.

29. Kan NW, Huang WC, Huang CY, et al. Hepatoprotective effects of Ixora parviflora extract aginst exhaustive exercise-induced oxidative stress in mice. *Open Access Mol.* 2013; 18: 10721-10732. doi: 10.3390/molecules180910721

30. Tauler P, Aguiló A, Fuentespina, Tur JA, Pons A. Response of blood cell antioxidant enzyme defences to antioxidant diet supplementation and to intense exercise. *Eur J Nutr*. 2005; 45: 187-195. doi: 10.1007/s00394-005-0582-7

31. Tessier F, Margaritis I, Richard MJ, Moynot C, Marconnet P. Selenium and training effects on the glutathione system and aerobic performance. *Med Sci Sports Exer.* 1995; 27: 390-396.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-111

32. Ohno H, Yahata T, Sato Y, Yamamura K, Taniguchi N. Physical training and fasting erythrocyte activities of free radical scavenging enzyme systems in sedentary men. *J Appl Physiol.* 1998; 57: 173-176. doi:10.1007/BF00640658

33. Donnelly JE, Jacobsen DJ, Heelan KS, Seip R, Smith S. The effects of 18 months of intermittent vs continuous exercise on aerobic capacity, body weight and composition, and metabolic fitness in previously sedentary, moderately obese females. *Int J Obes Relat Metab Disord*. 2000; 24: 566-572.

34. Rognmo O, Hetland E, Helgerud J, Hoff J, SlØrdahl SA. High intensity aerobic interval exercise is superior to moderate intensity exercise for increasing aerobic capacity in patients with coronary artery disease. *Eur J Cardiovasc Prevention Rehab*. 2004; 11: 216-222. doi: 10.1097/01.hjr.0000131677.96762.0c

35. Tench CM, McCarthy J, McCurdie I, White PD, D'Cruz DP. Fatigue in systemic lupus erythematosus: a randomized controlled trial of exercise. *Br Soc Rheumatol*. 2003; 42: 1050-1054. doi: 10.1093/rheumatology/keg289

SPORTS AND EXERCISE MEDICINE

Openventio PUBLISHERS

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-112

Research

*Corresponding author:
**Marco Aurélio Nemitalla Added**
Professor
Department of Physical Therapy
Universidade de Guarulhos
Rua Apeninos, 930, 01221-020
São Paulo-SP, Brazil
**E-mail:** marcoadded@terra.com.br

*Volume 1 : Issue 3*
*Article Ref. #: 1000SEMOJ1112*

***Article History:***
**Received:** June 6th, 2015
**Accepted:** July 28th, 2015
**Published:** July 29th, 2015

***Citation:***
Added MAN, de Araújo SA, Alcaide AR, et al. Influence of manipulation in the peripheral nervous system tests: a randomized controlled trial. *Sport Exerc Med Open J*. 2015; 1(3): 81-85.



# Influence of Manipulation in the Peripheral Nervous System Tests: A Randomized Controlled Trial

**Marco Aurélio Nemitalla Added[1*], Samir Asbahan de Araújo[2], Alexandre Ribero Alcaide[1], Alexandre Sabbag da Silva[1,4], Cristiano Baldan[3], Diego Galace de Freitas[2,3], Aline Gonçalves Zonta[5], Everton Luiz dos Santos[1], Caroline Galatti Moura Coelho[1], Gustavo Lacreta Toledo Colonezi[2] and André Nogueira Ferraz de Carvalho e Silva[2]**

*[1]Professor, Universidade de Guarulhos - UnG, Guarulhos, SP, Brazil*

*[2]Staff Physical Therapist, Irmandade da Santa Casa de Misericórdia de São Paulo, Rehabilitation Service, São Paulo, SP, Brazil*

*[3]Professor, Universidade Paulista – UNIP, São Paulo, SP, Brazil*

*[4]Professor, Centro Universitário Sant´Anna, São Paulo, SP, Brazil*

*[5]Student, Universidade Gurulhos - UnG, Guarulhos, SP, Brazil*

## ABSTRACT

**Background:** Chronic cervical pain is a serious public health issue that affects a large part of the world population and results in huge costs for the society. The chronic cervical pain is associated to problems in the peripheral nervous system. Clinical practice presents multiple techniques conventionally used to ameliorate chronic cervical pain. The spinal manipulation is a technique that can increase the range of motion of the peripheral nervous system.
**Objective:** To determine the efficacy of the cervical and thoracic manipulation in the peripheral nervous system tests.
**Methods:** The present study is a randomized controlled trial, with blinded assessor. The patient population consisted of 128 patients with chronic cervical pain and also tested positive (pain in motion) to the neural system. They were randomly divided into four groups: group A (manipulation of the sixth cervical vertebra), group B (manipulaton of the sixth thoracic vertebra), group C (manipulation of the sixth cervical and sixth thoracic vertebra) and group D (without intervention). These patients underwent an assessment before and after spinal manipulation by the blinded assessor.
**Results:** After the manipulation, an improvement of the ragion of motion was observed on the group that the sixth cervical vertebra was handled: left median nerve range of motion (p=0.04) and right (p=0.04) and left ulnar nerve (p=0.03) and right (p=0.04). Also was observed a statistically significant result when associate manipulation of the sixth cervical vertebra and sixth thoracic vertebra: left median nerve (p=0.01) and the right side (p=0.03).
**Conclusion:** Isolated manipulation of the sixth cervical vertebra or together with the manipulation of the sixth thoracic vertebra in individuals with chronic neck pain appears to be an effective resource in improving range of motion in patients that tested positive to the peripheral nervous system tests.

**KEYWORDS:** Chronic cervical pain; Manipulation; Peripheral nervous system.

**ABBREVIATIONS:** PNST: Peripheral Nervous System Tests; SD: Standard Deviation.

## INTRODUCTION

Chronic cervical pain is a major public health problem that affects approximately 18% of the world population.[1-3] Treatment guidelines claim that manual therapy techniques such as spinal manipulation are essential for rehabilitation.[4,5] The manipulation of cervical spine

is highly recommended in cases of cervical pain and stiffness, tension headache and migraine. The technical purpose aims to increase the range of motion, consequently relieve pain by the vertebral repositioning.[6,7] Besides being effective in cervical pain complaints, vertebral repositioning is are also indicated in cases of radiculopathy.[1,8-13]

One of the manual therapy technique which promotes the mobilization of nerve roots and acts to test and treat the peripheral nervous system, is the neurodynamic mobilization.[14,15] The neural tissue has been pointed as a source radicular symptoms.[16] The provocative tests of this tissue are used to identify whether the neural tissue is the cause of pain or not.[16,17] These tests consist of a series of passive movements in the affected limb, in order to assess tissue neural mechanics.[18] The test is considered to be positive when the symptoms are reproduced during application.[19] According to some researchers, the neurodynamic mobilizations do not promote additional benefits when compared with other interventions.[20,21] A systematic review shows limited evidence for the use of neurodynamic mobilizations in the treatment of radicular conditions involving the upperlimbs.[22] The effects of neurodynamic mobilizations on physiological mechanisms are still unknown in literature and more research regarding these effects may account for its therapeutic effectiveness.[23] Even though the association of techniques for treatment of spine disorders is backed by evidence, there are no studies relating the use of manipulation and neurodynamic mobilization in subjects with chronic neck pain.[24-26] It is believed that spinal manipulation can influence greatly on the answer of the peripheral nervous system tests, thus the objective of this study is to evaluate the influence of spinal manipulation on the Peripheral Nervous System Tests (PNST).[27,28]

## METHODS

### Study Design

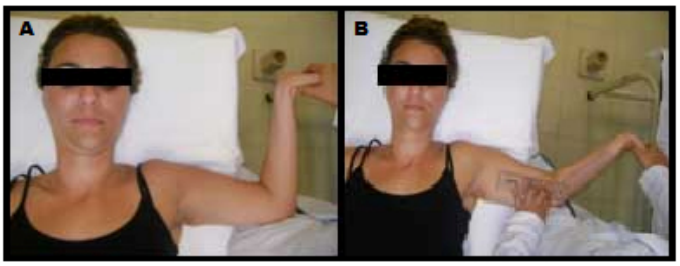
This study is a randomized controlled trial with blinded evaluator. Consent procedures were approved by the Ethics and Research Committee of the Irmandade da Santa Casa de Misericordia de Sao Paulo, Brazil (Protocol No. 159/10).

### Sample Calculation

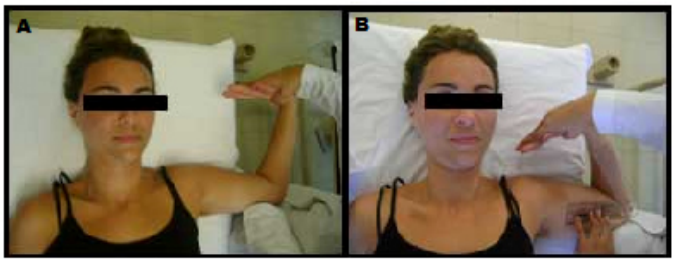
The sample size calculation for this study was based on a difference of 2 points in the end pain and a difference of 5° on the measurement of the amplitude of movement through goniometry. Using the statistical power of 80%, 5% alpha, and with possible sample loss of 10%, we reached the total of 32 individuals per group, amounting 128 participants.

### Participants

The patient population was composed of males and females aged between 18 to 40 years old, with cervical pain over 12 weeks and positive response (Figures 1 and 2) to peripheral nervous system testing (PNST). Subjects who submitted positive response to the Dekleyn test, underwent spinal manipulation on the previous week, with pain and stiffness in the cervical spine or upper limbs, tension headache, migraine, obese, hypertensive patients, with cervical and/or thoracic osteoarthritis or osteoporosis, chest pain, prior accident in the head or neck, neurologic deficit, Radiculopathy and severe diseases of spine (tumor, fracture and infections), were excluded from the stud.

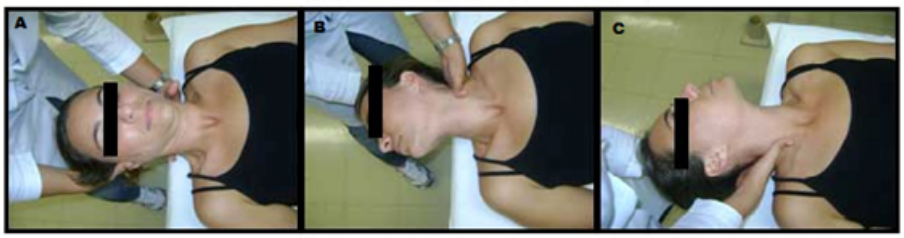



A. Initial position of the test. B. Final Position of the test, with goniometry measurement.
**Figure 1:** TPNS for median nerve.




A. Initial position of the test. B. Final Position of the test, with goniometry measurement.
**Figure 2:** TPNS for ulnar nerve.

The Dekleyn test was performed with the patient supine, with the spine off the stretcher, hyperextended and rotate, for about 1 minute on each side (left and right)[17] (Figure 3). This test aims to evaluate the integrity of the basilar artery and insure that it is secure to perform cervical spinal manipulation. The test is considered to be positive when there is the presence of vertigo, nystagmus, dizziness, tinnitus, visual changes, nausea or fainting, indicating insufficiency of the basilar artery, ending the cervical spinal manipulation.[17]




A. Initial position of the Dekleyn test. B. Extension more right inclination C. Extension more right inclination.
**Figure 3:** Dekleyn test.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-112

The characteristics of the sample (age, gender, weight, high, simptons duration, dominant member, medicantion use, physical activity practice, smoker and visual analogic scale) was collected in baseline.

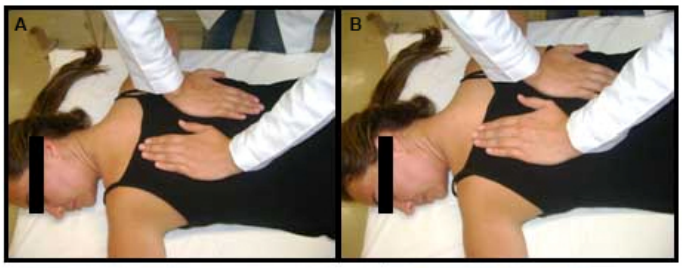
### Evaluation of Peripheral Nervous System Tests

The evaluation of the individuals previously randomized, was performed by blinded evaluator. The evaluator made the PNST to check the involvement of nerve roots of the median nerve and ulnar in positive cases was measured to range of motion by means of a goniometer, where the patient was supine, with the member tested in 90° shoulder abduction and 90° of flexion of elbow, with the fulcrum of the goniometer positioned at the medial epicondyle of the volunteers.[17] During the PNST for the median nerve, the volunteer was initially held supine with the shoulder to be tested with abduction of 90° and rotated sideways, with elbow 90° flexion, forearm supinated, wrist and fingers held in extension.[17] Below elbow extension was held until the maximum amplitude allowed by the patient and measured the range of motion (Figure 1).[17] In PNST for the ulnar nerve, the volunteer was kept in the same position of the median nerve, but with forearm pronated. Below elbow flexion was carried out to the maximum extent permitted by the patient and measured the range of motion (Figure 2).[17] Prior to randomization of patients and collecting data, we conducted a pilot study with 10 volunteers (5 men and 5 women), in order to test the reliability of the evaluators about the positioning of the goniometer and handling proposed in the study. The procedures were reassessed after a week. The evaluators presented an intraclass correlation coefficient of 0.87 for the positioning of the goniometer and 0.88 for handling.

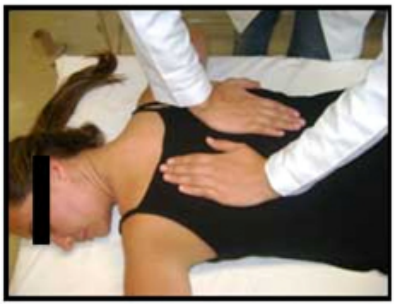
### Randomization

Patients were randomized into four groups through browns envelopes done by a second evaluator who was the only person aware of which group the patient was allocated andthe handling proposal. Blinded evaluator and patients were unaware of the group allocation.

Group A underwent the sixth cervical vertebra manipulation (C6). The patient is positioned supine, with head slightly off the gurney and the therapist conducted a rotation with hyperextension of the segment to be manipulated, by performing a move posterior-anterior (Figure 4).Group B underwent manipulation of the sixth thoracic vertebra (T6). The patient was placed in ventral decubitus, with upper limbs abductees and relaxed for body-worn operation, the therapist positioned both hands parallel to the inferior angle of the scapula region performing cranio-rotational flow pressure ("screw movement") for implementation of proposal handling (Figure 5). Group C held the combination of groups A and B, manipulation of the vertebras C6 and T6, as described earlier. Group D received placebo manipulation, the patient was positioned in ventral decubitus, upper limbs abductees and relaxed by their sides, the therapist positioned both hands parallel to the inferior angle of the scapula and has not achieved any pressure (Figure 6). After manipulation, patients were reassessed as regards PNST (ulnar nerve and median nerve) goniometry by the blinded assessor. The data collected were sent to statistical analysis.



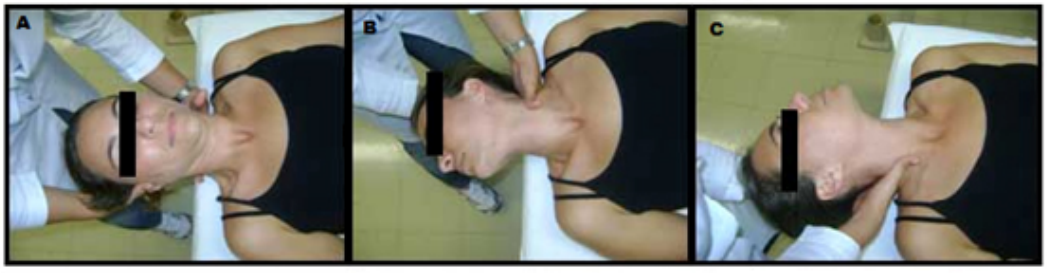

A. Position initial. B. Position final.
**Figure 5:** Toracic trust.



The patient position.
**Figure 6:** Sham trust.

### Statistical Analysis

For statistical analysis, SPSS 19 and Excel Office 2010 were used. The level of reliability was established at $p<0.05$. The Wilcoxon test was used to compare the results between periods in each group for the variables measured. The analysis colleted




A. Initial position of the neck manipulation. B. Position of the right trust. C. Position of the left trust.
**Figure 4:** Neck manipulation.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-112

by a blinded assessor.

## RESULTS

Between the period of April 2011 and December 2012, 182 patient residents in the city of São Paulo were evaluated by the rehabilitation of the Irmandade da Santa Casa de Misericordia de São Paulo. Among the volunteers evaluated, 54 did not comply any of the inclusion criteria and were excluded from the study. An exclusion criterion was: neural test negative (n=19), positive Dekleyn test (n=13) tendinopathy of the supraspinatus muscle (n=8), tendinopathy of the wrist flexors (n=7), previous surgeries in the column (n=6), neurofibromatosis (n=1). Table 1 presents the characteristics of patients who participated in the study. Overall this study consisted mostly of women, age of 32 years, weighing 74.5 kg and with an average duration of 15 months of neck pain. As for the dominant side, 82.7% of the population was right-handed and 17.3% were left-handed. There was a significant difference (p=0:03) for the dominant side.

After interventions were complete, the intra-group comparison analysis for the median nerve and ulnar nerve right and left. When analyzed the right median nerve, Group A (p=0.043), handling C6; and Group C (p=0.028); manipulation of theC6 vertebra associated with the manipulation of T6, showed to be significant for the gain of movement amplitude (Table 2). In the analysis of the left median nerve, as well as the right, the Group A (p=0.03) and Group C (p=0.01), showed significant values for the gain of range of motion. Regarding the variable for PNST right and left ulnar nerve in intra-group comparison, there was a statistically significant difference only in Group A, handling C6, on both sides, right (p=0.04) and left (p=0.03) (Table 3).

## DISCUSSION

The objective of this study was to analyze the effects of the manipulation on the peripheral nervous system through PNST of median nerve and ulnar nerve in order to investigate a possible improvement on the range of motion. The choice of C6 and T6 segment for the realization of the manipulation was made according to a study conducted by Butler,[17] where they assume the points of reduced vertebral mobility during the flexion movement are the segments of C6, T6 and L4. As the objective of this study was to evaluate the influence of the manipulation in the tests of the peripheral nervous system of the upper limb, only the segment of C6 and T6 were chosen to be tested.

As long as these segments have reduced mobility, end up being more susceptible to form points of tension and develop patologias.[13] This rationale confirms the exposed in the study of Christensen and Buswel,[13] where it was reported that the cervical Radiculopathy is most prevalent in the seventh nerve root of cervical spine, between C6 and C7 (60%) and in the sixth nerve root of cervical spine, between C5 and C6 (25%). In the variables measured for both the median nerve and ulnar nerve, the groups (C6-handling) and C (C6-handling and T6) feature significant results when compared with the other groups. This may be explained by the fact that 81.2% of the population of the study consisted of right-handed volunteers; but also the methodology established for the study was to initiate the assessment through the peripheral nervous system testing by Member of the volunteer, causing the nerve introduce greater tour tested in neural tube defects when it was tested on the left Member.

But the choice of a control group had aimed to minimize this bias and evaluate if the results achieved were due the manipulations performed or just the fact of having stretched the nerves during the assessment and reassessment of peripheral nervous system tests. As a result, it is possible observe that the control group did not show any improvement in the reassessment of the peripheral nervous system tests. It suggests that handling the C6 vertebra in association with the manipulation of the T6 vertebra, presents better results when compared with the placebo or with only the manipulation of T6 vertebra, since these vertebral regions are considered to have reduced levels of mobility, which can limit the movement of the nerve in the neural tube.

## CONCLUSION

Manipulation of the C6 vertebra isolated or associated with manipulation of the T6 vertebra appears to be an effective resource for increasing the range of motion of the median nerve and ulnar nerve. More studies with similar objectives should be carried out, to verify the effectiveness of spinal manipulation on nerve tests of the peripheral nervous system.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. Haneline MT. Chiropractic manipulation and acute neck pain: a review of the evidence. *J Manipulative Physiol Ther.* 2005; 28(7): 520-525. doi: 10.1016/j.jmpt.2005.07.010

2. Vernon H, Humphreys K, Hagino C. Chronic mechanical neck pain in adults treated by manual therapy: a systematic review of change scores in randomized clinical trials. *J Manipulative Physiol Ther*. 2007; 30(3): 215-226. doi: 10.1016/j.jmpt.2007.01.014

3. Gay RE, Madson TJ, Cieslak KR. Comparison of the neck disability index and the neck Bournemouth questionnaire in a sample of patients with chronic uncomplicated neck pain. *J Manipulative PhysiolTher.* 2007; 30(4): 259-269. doi: 10.1016/j.jmpt.2007.03.009

4. Di Fabio RP. Manipulation of the cervical spine: risks and benefits. *Phys Ther.* 1999; 79: 1.

5. Di Fabio RP. Efficacy of manual therapy. *Phys Ther.* 1992;

72: 853- 864

6. Whalen WM. Resolution of cervical radiculopathy in a woman after chiropractic manipulation. J *Chiropractic Med.* 2008; 7: 17-23. doi: 10.1016/j.jcme.2007.10.002

7. Alcantara J, Plaugher G, Thornton RE, Salem C. Chiropractic care of a patient with vertebral subluxations and unsuccessful surgery of the cervical spine. *J Manipulative Physiol Ther*. 2001; 24(7): 477-482. doi: 10.1016/S0161-4754(01)40866-9

8. Murphy DR, Hurwitz EL, Gregory A, Clary R. A nonsurgical approach to the management of patients with cervical radiculopathy; a prospective observational cohort study. *J Manipulative Physiol Ther.* 2006; 29(4): 279-287.

9. Murphy DR. Herniated disc with radiculopathy following cervical manipulation: nonsurgical management. *Spine J.* 2006; 6(4): 459-463. doi: 10.1016/j.spinee.2006.01.019

10. Tatarek NE. Variation in the human cervical neural canal. Spine J. 2005; 5(6): 623-631. doi: 10.1016/j.spinee.2005.07.009

11. BenEliyahu DJ. Magnetic resonance imaging and clinical follow-up. Study of 27 patients receiving chiropractic care for cervical and lumbar disc herniation. *J Manipulative Physiol Ther.* 1996; 19(9): 597-606.

12. Strunk RG, Hondras MA. A feasibility study assessing manual therapies to different regions of the spine for patients with subacute or chronic neck pain. *J Chiropractic Med.* 2008; 7: 1-8. doi: 10.1016/j.jcme.2007.10.004

13. Christensen KD, Buswell K. Chiropractic outcomes managing radiculopathy in a hospital setting: a retrospective review of 162 patients. *J Chiropractic Med.* 2008; 7: 115-125. doi: 10.1016/j.jcm.2008.05.001

14. Rubenstein SM, Leboeuf-Yde C, Knol DL, Koekkoek TE, Pfeifle CE, van Tulder MW. The benefits outweigh the risks for patients undergoing chiropractic care for neck pain: a prospective, multicenter, cohort study. *J Manipulative Physiol Ther.* 2007; 30(6): 408-418. doi: 10.1016/j.jmpt.2007.04.013

15. Lee KP, Carlini WG, McCormick GF, Albers GW. Neurologic complications following chiropractic manipulation: a survey of California neurologists. *Neurology.* 2005; 45(6): 1213-1215.

16. Peñas CF, Joshua A, Cleland, Peter H, Cerro LP, Iglesias JG. Repeated applications of thoracic spine thrust manipulation do not lead to tolerance in patients presenting with acute mechanical neck pain: a secondary analysis. *J Man Manip Ther.* 2009; 17(3): 154-162.

17. Butler D. Adverse mechanical tension in the nervous system: a model for assessment and treatment. *Australian J Physio.* 1989; 35(4).

18. Coppieters M, Stappaerts K, Janssens K, Jull G. Reliability of detecting onset of pain" and "submaximal pain" during neural tissue provocation testing of the upper quadrant. *Physiother Res Int.* 2002; 7: 146-156.

19. Walsh J, Flatley M, Johnston N, Bennett K. Slump Test: Sensory Responses in Asymptomatic Subjects. *J Man Manip Ther.* 2007; 15(4): 231-238.

20. Scrimshaw SV, Maher CG. Randomized controlled trial of neural mobilization after spinal surgery. *Spine J.* 2001; 26: 2647-2652.

21. Tal-Akabi A, Rushton A. An investigation to compare the effectiveness of carpal bone mobilization and neurodynamic mobilization as methods of treatment for carpal tunnel syndrome. *Man Ther.* 2000; 5: 214-222. doi: 10.1054/math.2000.0355

22. Ellis RF, Hing WA. Neural mobilization: a systematic review of randomized controlled trials with an analysis of therapeutic efficacy. *J Man Manip Ther.* 2008; 16: 8-22.

23. Beneciuk JM, Bishop MD, George SZ. Effects of upper extremity neural mobilization on thermal pain sensitivity: a sham-controlled study in asymptomatic participants. *JOSPT.* 2009; 39(6): 428-438. doi: 10.2519/jospt.2009.2954

24. Boyles RE, Walker MJ, Young BA, Strunce JB, Wainner RS. The addition of cervical thrust manipulations to a manual physical therapy approach in patients treated for mechanical neck pain: a secondary analysis. *JOSPT.* 2010; 40 (3): 133-140. doi: 10.2519/jospt.2010.3106

25. Cleland JA, Whitman JM, Fritz JM, Palmer JA. Manual physical therapy, cervical traction and strengthening exercises in patients with cervical radiculopathy: a case series. *JOSPT.* 2005; 35 (12): 802-811.

26. Added MA, Costa LO, Fukuda TY, et al. Efficacy of adding the Kinesio Taping method to guideline-endorsed conventional physiotherapy in patients with chronic nonspecific low back pain: a randomized controlled trial. *BMC Musculoskelet Disord.* 14: 301.

27. Licht PB, Christensen HW, Hoilund-Carlsen PE. Is there a role for premanipulative testing before cervical manipulation? *J Manipulative Physiol Ther.* 2000; 23(3): 175-179. doi: 10.1016/S0161-4754(00)90247-1

28. Cleland JA, Flynn TW, Childs JD, Eberhart S. The audible pop from thoracic spine thrust manipulation and its relation to short-term outcomes in patients with neck pain. *J Man Manip Ther.* 2007; 15(3): 143-154

SPORTS AND EXERCISE MEDICINE

Openventio PUBLISHERS

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-113

*Short Communication*

***Corresponding author:***
**Moataz Eltoukhy, PhD**
Assistant Professor
Department of Kinesiology and Sport
School of Education and Human Development
1507 Levante Avenue
Max Orovitz Building, Room 132
Coral Gables, Florida 33146, USA
**E-mail:** meltoukhy@miami.edu

***Volume 1 : Issue 3***
***Article Ref. #: 1000SEMOJ1113***

***Article History:***
**Received:** April 19th, 2015
**Accepted:** July 28th, 2015
**Published:** July 31st, 2015

***Citation:***
Eltoukhy M, Kuenze C. Moving toward clinic-based motion analysis: kinect® camera as an example. *Sport Exerc Med Open J*. 2015; 1(3): 86-88.



# Moving Toward Clinic-Based Motion Analysis: Kinect® Camera as an Example

**Moataz Eltoukhy* and Christopher Kuenze**

*Department of Kinesiology and Sport Sciences, University of Miami, Coral Gables, FL, USA*

## INTRODUCTION

Lower extremity injury and the associated rehabilitation that follows represent an enormous source of annual health care costs as well as time lost from activity in the physically active population.[1,2] Currently, assessment tools such as the Landing Error Scoring System (LESS) have been described in the literature and adopted by clinicians as valuable tools used to assess knee injury risk.[3] Unfortunately, while these tools have been shown to be valid, they rely on subjective criteria to assess patient performance, which may be affected by clinician experience. Cost effective, quantifiable assessment of lower extremity movement in the clinical setting represents a tremendous improvement in the standard of injury risk evaluation as well as the ability to track patient outcomes.

In the laboratory setting, camera-based three dimensional motion analysis, which are considered the gold standard for dynamic movement assessment, are commonly utilized to assess lower extremity and trunk kinematics associated with lower extremity injury. Cost effective and clinician friendly motion analysis technology that allow for valid and sensitive real time assessment of lower extremity movement patterns are an essential next step toward optimized patient care. Providing clinicians access to a low cost, user friendly motion analysis system that can be easily integrated in to clinical practice may provide a valuable objective tool to assess knee joint injury risk as well track patient progress and outcomes throughout the rehabilitation process. The Microsoft Kinect® camera system (Microsoft Corp. Redmond, WA) is a widely available and cost effective video game system accessory that utilizes multiple cameras to measure 3 dimensional joint kinematics.[4,5]

## METHODS

We conducted a descriptive laboratory study to use the Microsoft Kinect® camera system to assess lower extremity biomechanics indicative of injury risk in Men's and Women's Division I basketball athletes (Table 1). The Microsoft Kinect® camera setup is shown in Figure 1.

| | Male | Female | |
|---|---|---|---|
| | Mean±SD | Mean±SD | p-value |
| Age | 19.4±1.6 | 19.3±1.6 | 0.88 |
| Height (cm) | 195.5±10.2 | 177.1±7.6 | <0.001 |
| Weight (Kg) | 93.4±13.4 | 71.5±7.6 | <0.001 |
| BMI | 24.3±1.9 | 22.8±1.4 | 0.03 |

**Table 1:** Participant demographics.

Subjects were 18-24 years old, and they participates in NCAA Division I Basketball. Subjects were excluded if he/she has any lower extremity surgery in the past 6 months. Data collected was in the form of Means and SD calculated for each group, and the groups were compared using independent sample t-tests, Alpha level: $P \leq 0.05$.

Each participant performed three sessions of Single Leg Hop Performance, Dynamic Balance Assessment using Star Excursion Balance Test,[6] and Lower Extremity Jump Landing

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-113

Biomechanics (Participants completed a drop-jump landing task from a 30 cm-high box with a landing target placed on the floor at a distance equal to 50% of his height). All data was processed using an in-house custom LabVIEW program (National Instruments Corp, Austin, TX).

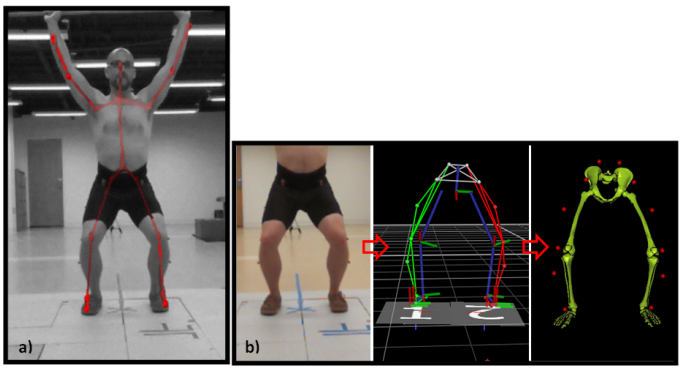



**Figure 1:** a) Kinect automatic body segments and joints recognition, b) lower extremity biomechanical model developed to calculate the joints' kinematics using the BTS system.

## RESULTS AND DISCUSSION

Results obtained from the study is shown in Figures 2-4 below. On the other hand, Table 2 summarizes the hip and knee kinematics during jump landing.

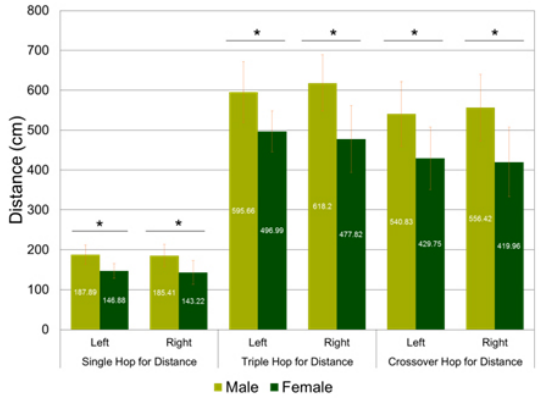



**Figure 2:** Between sex comparison of non-normalized single leg hop distances. Significant difference indicates with *.

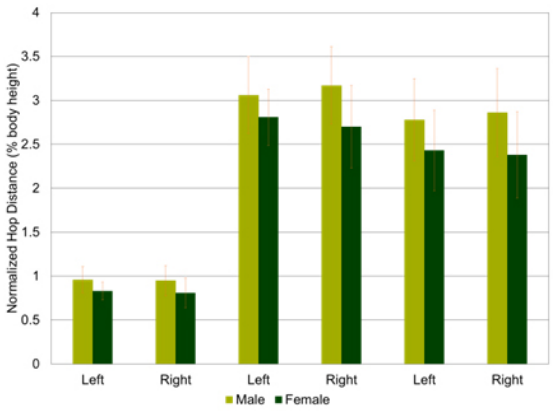



**Figure 3:** Between sex comparison of normalized single leg hop distances. Significant difference indicates with *.

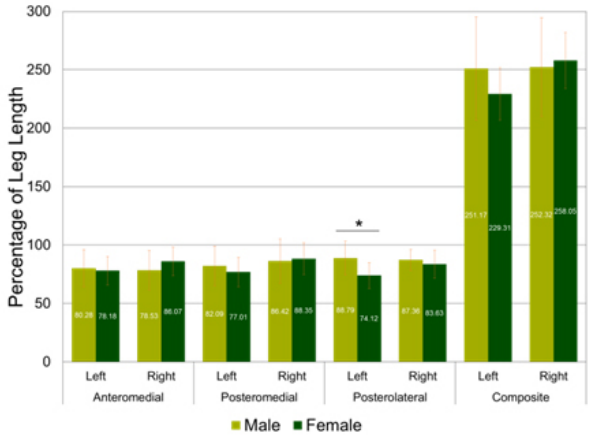



**Figure 4:** Star excursion balance test results. Significant difference indicates with *.

| | | Male | Female | |
|---|---|---|---|---|
| | | Mean±SD | Mean±SD | p-value |
| AKnee Flexion (°) | Left | 71.7±12.4 | 76.4±16.0 | 0.60 |
| | Right | 73.2±15.3 | 71.6±15.8 | 0.85 |
| AKnee Valgus (°) | Left | 6.2±4.2 | 2.1±0.5 | 0.02 |
| | Right | 5.5±5.4 | 2.0±1.1 | 0.11 |
| Hip Flexion (°) | Left | 85.1±14.1 | 71.5±16.0 | 0.16 |
| | Right | 84.2±17.2 | 71.9±17.5 | 0.23 |
| Hip Adduction (°) | Left | 23.6±6.2 | 14.6±4.4 | 0.01 |
| | Right | 21.9±3.5 | 13.3±5.5 | 0.01 |

**Table 2:** Hip and Knee Kinematics during a jump landing task.

As shown above, for the Single Leg Hop Performance; the non-normalized hop values showed that male participants jumped significantly further which is expected due to longer legs/greater body size. For the normalized hop values, which improves comparability between sexes, yet, males continue to outperform woman overall. As far as sex differences; males generated greater lower extremity power which may explain better performance on hop tests.

On the other hand, Dynamic Balance Assessment results similar findings between the sexes. Males were better in the posterolateral direction which is similar to previous studies with recreationally active participants.

Finally, in the Lower Extremity Jump Landing Biomechanics results, it was found that men displayed higher knee valgus and hip adduction. This pattern of injury may indicate high ACL injury risk.

Based on our findings, it was concluded that the Microsoft Kinect® was suitable for collecting joint kinematic data during a functional task. Also, future research should assess the ability of the Kinect camera system to measure knee and hip kinematics during different tasks such as walking gait or stair climbing.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-113

**REFERENCES**

1. Luc B, Gribble PA, Pietrosimone BG. Osteoarthritis prevalence following anterior cruciate ligament reconstruction: a systematic review and numbers-needed-to-treat analysis. *J Athl Train.* 2014; 49(6): 806-819. doi: 10.4085/1062-6050-49.3.35

2. Nwachukwu BU, Schairer WW, Bernstein JL, Dodwell ER, Marx RG, Allen AA. Cost-Effectiveness analyses in orthopaedic sports medicine: a systematic review. *Am J Sports Med.* 2014. doi: 10.1177/0363546514544684

3. Padua DA, Marshall SW, Boling MC, Thigpen CA, Garrett WE, Jr., Beutler AI. The landing error scoring system (less) is a valid and reliable clinical assessment tool of jump-landing biomechanics: The JUMP-ACL study. *Am J Sports Med.* 2009; 37(10): 1996-2002. doi: 10.1177/0363546509343200

4. Pfister A, West AM, Bronner S, Noah JA. Comparative abilities of Microsoft Kinect and Vicon 3D motion capture for gait analysis. *J Med Eng Technol.* 2014; 38(5): 274-280. doi: 10.3109/03091902.2014.909540

5. Stone EE, Butler M, McRuer A, Gray A, Marks J, Skubic M. Evaluation of the Microsoft Kinect for screening ACL injury. *Conf Proc IEEE Eng Med Biol Soc.* 2013; 2013: 4152-4155. doi: 10.1109/EMBC.2013.6610459

6. Gribble PA, Hertel J, Plisky P. Using the star excursion balance test to assess dynamic postural-control deficits and outcomes in lower extremity injury: a literature and systematic review. *J Athl Train*. 2012; 47(3): 339-357. doi: 10.4085/1062-6050-47.3.08

SPORTS AND EXERCISE MEDICINE

Openventio PUBLISHERS

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-114

Review

*Corresponding author:
**Arturo A. Arce-Esquivel, MD, PhD**
Department of Health and Kinesiology
College of Nursing and Health Studies
The University of Texas at Tyler
3900 University Blvd.
Tyler, TX 75799, USA
Tel. (903) 565-5838
Fax: (903) 566-7065
**E-mail:** aarce@uttyler.edu

***Volume 1 : Issue 3***
***Article Ref. #: 1000SEMOJ1114***

***Article History:***
**Received:** July 7th, 2015
**Accepted:** July 30th, 2015
**Published:** July 31st, 2015

***Citation:***
Arce-Esquivel AA, Ballard JE. Effects of resistance training on bone and muscle mass in older women: a review. *Sport Exerc Med Open J*. 2015; 1(3): 89-96.



# Effects of Resistance Training on Bone and Muscle Mass in Older Women: A Review

**Arturo A. Arce-Esquivel* and Joyce E. Ballard**

*Department of Health and Kinesiology, The University of Texas at Tyler, Tyler, TX 75799, USA*

**ABSTRACT**

Aging is associated with declines of different physiological systems. These reductions are directly related to loss of mobility in older individuals, reducing the independence and quality of life for this population. Regular physical activity (e.g, resistance training, RT), has been shown to decrease mortality and age-related morbidity in older adults, including women. A fracture is closely related to the typical decline in bone mass (i.e, osteoporosis) especially in postmenopausal women. In fact, up to 30% of postmenopausal females have osteoporosis. In postmenopausal women the rate of bone mineral density loss progressively increases with age. In addition, decreases in muscle tissue (i.e, sarcopneia) may begin to occur before the fourth decade and gradually worsens through the later stages of adulthood. Sarcopenia, is characterized by low muscle mass, low muscle strength, and low physical performance, which can lead to disability, risk of falls and fractures, and death. Further, sarcopenia occurs to a greater degree in older women than men. Menopause is characterized by important changes in hormonal status and these changes have a significant effect on body composition (i.e, bone mass density, muscle mass, and body fat). Importantly, RT is effective in increasing bone and muscle mass and improving measurements of physical performance. Thus, this review is intended to summarize the effects of RT on bone and muscle mass in older postmenopausal women.

**KEY WORDS:** Exercise; Postmenopausal women; Osteoporosis; Sarcopenia; Aging.

**ABBREVIATIONS:** ADLs: Activities of Daily Living; QOL: Quality of Life; RT: Resistance Training; ACSM: American College of Sports Medicine; BMD: Bone Mineral Density; WBV: Whole Body Vibration.

## INTRODUCTION

The aging process is associated with declines of different physiological systems, including the neuromuscular system, through the loss of strength, power and muscle mass,[1] as well as the cardiovascular system, which presents through a reduction in Activities of Daily Living (ADLs). These reductions are directly related to loss of mobility in older individuals, reducing the independence and Quality of Life (QOL) for this population.[2] On the other hand, regular physical activity, including Resistance Training (RT), is a keystone intervention to counteract many age-associated diseases, to increase functional independence, and have a positive effect on several health outcomes in the elderly.[3,4] Moreover, regular exercise has been shown to decrease mortality and age-related morbidity in older adults.[5] Although the positive benefits of aerobic exercise are widely accepted, the importance of RT especially in the older female population has not been as well recognized or implemented. Indeed, the addition of RT guidelines to the 1998 American College of Sports Medicine (ACSM) position statement was the result of overwhelming evidence of the health and functional benefits associated with this type of training.[6] The 2011 ACSM guidelines[7] updates the scientific evidence published since the 1998 Position Stand in regards to RT.[6] Further, ACSM currently recognizes that weight-bearing exercise confers beneficial effects for bone health across the age spectrum.[8] Not only does RT significantly impact strength and endurance, it contributes to the maintenance of functional ability (i.e, ADLs), and is an important factor in preventing osteoporosis, sarcopenia and

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-114

disabilities.[6-10] RT is particularly effective in increasing muscle mass and strength and improving several measurements of physical performance.[11] Furthermore, integrating balance training and RT into regular physical activity programs appears particularly efficient in reducing the rate of falls in older people, and in improving the overall QOL.[12]

It is well-known that the risk of fractures is closely related to the typical decline in bone mass (i.e, osteoporosis) during the aging process, in both women and men.[13] Since osteoporosis is a major public health concern, the prevention of osteoporosis is a national health initiative by the Surgeon General's office. Certainly, the Surgeon General predicts that, by 2020, there will be 13.9 million individuals with osteoporosis (more than 75% of these will be women) and 47.5 million with low bone mass (64% women).[14] Further, the World Health Organization reports 30% of postmenopausal females have osteoporosis.[15] Notably, osteoporosis is more prevalent among women with an accelerated rate of bone loss for 4 to 8 years following menopause due to estrogen withdrawal.[16,17] These women also experience a decline in physical activity.[18] In their early 50s,women lose up to 5% of bone mass in the first few years after menopause, followed by 2-3% annual loss thereafter, while men lose approximately 1-2% of Bone Mineral Density (BMD) per year, starting from a higher baseline.[19] For instance, in postmenopausal women the rate of bone mineral density (BMD) loss progressively increased with age: -0.6%, -1.1% and -2.1% per year for the 60-69, 70-79 and ≥ 80 years age groups, respectively.[20] Importantly, RT seems to be a powerful stimulus to improve and maintain BMD during the aging process.[21]

Correspondingly, it has been reported that muscle strength declines by 15% per decade after age 50 and 30% per decade after age 70.[22] Indeed, decreases in muscle tissue quantity and quality may begin to occur before the fourth decade and gradually worsens through the later stages of adulthood.[23] Muscle cross-sectional area and single-fiber atrophy account for some of the variability in strength loss among elderly persons, and this atrophy appears to be exaggerated in Type II fiber.[24] Sarcopenia, frequently observed in the elderly, is characterized by low muscle mass, low muscle strength, and low physical performance, which can lead to disability, risk of falls and fractures, and death.[25,26] In sarcopenic or frail patients, RT is capable of improving lean tissue mass, muscle strength and several physical performance measures; such as, time up and go, stair-climbing power, aerobic capacity, gait speed and 6-minute walking distance. All of these performance measures require increased muscle mass or strength.[27,28] Moreover, Peterson at el, reported that there is a strong association between RT and increases in lean body mass among adults >50 years of age (n=1328).[29] These investigators revealed that following 20 weeks of RT, both men and women experienced an approximate 1-kg (2.2-lb) increase in lean body mass. Interestingly, that finding is in contrast to the nearly 0.2-kg (0.44-lb) annual decline that might occur through sedentary lifestyles beyond 50 years of age.[30] Additionally, sarcopenia occurs to a greater degree in older women than men. For instance, results from the Framingham disability study demonstrated that 45% of women older than 65 years and 65% older than 75 years could not lift 10 pounds.[31] Further, it has been reported that there is an accelerated decline in muscle mass that has been shown to occur after the 5th decade, or around the years of menopause.[32] Across-sectional study reported a decline in muscle mass of 0.6% per year after menopause.[33] Menopause is characterized by important changes in hormonal status and these changes have a significant effect on bone mass density and body fat distribution.[34] Strong evidence supports the hypothesis that the decline in estrogen levels with menopause may play a role in muscle mass loss in postmenopausal women.[35] In truth, estrogen has a direct anabolic action on the skeletal muscle that contains estrogenic receptors.[36] Notably, RT is an important stimulator of anabolic hormones. For instance, estradiol responses to RT appear to depend on the exercise intensity and stage of the menstrual cycle,[37] and occur mainly during the workout.[38] Furthermore, RT can result in 25 to 100%, or more, strength gains in older adults through muscle hypertrophy and, presumably, increased motor unit recruitment.[22,39] These improvements in muscle strength appear to be proportional to the intensity of the RT exercise.[11] Nevertheless, only 27% of the US population is estimated to participate in leisure-time RT exercise, and these rates are substantially less for individuals over the age of 50 years.[40] Thus, the main purpose of this review is to discuss the effects of RT on bone and muscle mass in older postmenopausal women, as a starting point for developing future intervention programs that help to maintain a healthy bone and muscle mass and a higher QOL.

## EFFECTS OF RESISTANCE TRAINING ON BONE MASS

The studies that evaluated the role of RT on BMD in postmenopausal women have obtained mixed results. Some authors described increased mineralization,[41,42] others reported attenuated demineralization,[41,43] while still others indicated no effect[44,45] following RT. Nevertheless, RT is still one of the most recurrent types of exercise applied in order to improve bone mass in elderly individuals; especially in preventing bone loss among postmenopausal women.[21,46] Previous studies have reported that RT is able to prevent bone demineralization in older women.[47,48] For instance, the study by de Matos, et al.[48] reported that 12 weeks of weight training, among postmenopausal women with osteoporosis or osteopenia, did not significantly improve BMD (1.17%) at the lumbar spine in the exercise group (n=30). However, the control group (n=29) showed a significant loss in the spine BMD (2.26%). Further, Bocalini, et al.[47] evaluated the effects of RT on the BMD of postmenopausal women without Hormone Replacement Therapy (HRT). These investigators reported that following 24 weeks of training, untrained women exhibited a significant percentage decrease in BMD at the lumbar spine and femoral neck. Interestingly, BMD was maintained in trained women at both sites. Another 6-month exercise program study (n=125, 52-72 years old)[49] reported no significant change

in the BMD of the femoral neck, lumbar spine, and ultra-distal and proximal radius; but they did note an increase in the density of the cortical component of the ultra-distal radius. Clearly, these previous studies seem to indicate that site-specific moderate physical exercises have very little effect on bone mass.

On the other hand, long-term RT programs (i.e, >1 year) reported significant effects of RT in postmenopausal women. Certainly, Nelson, et al.[41] revealed that 1 year of high-intensity RT was able to enhance BMD by 1% at the femoral neck and lumbar spine; while in the control group, women lost 2.5% and 1.8% at these sites, respectively. Another 1 year RT study of 56 postmenopausal women[50] reported that bone mass, in the exercise group, increased significantly at the trochanteric and intertrochanteric hip site, at Ward's triangle and at the ultra-distal radial site compared with the control group. This study concluded that postmenopausal bone mass can be significantly increased by RT, particularly when high-load low repetitions were used.[50] Finally, it has been reported that a significant effect of RT in postmenopausal women (mean age, 60 +/- 5 years) over 2 years at the clinically important intertrochanter hip site (+1.1%).[46] Remarkably, the maximum change in BMD occurred in the first year of the intervention. There was lative decline in the rate of change during the second year; however, BMD remained more than a 3% difference between the exercise and control group after 2 years.

Importantly, exercise intensity and duration, and continuous training are key components when prescribing RT programs. In regards to the exercise intensity, the majority of studies have determined that high intensity RT programs (70% to 90% of one repetition maximum; 1RM) had an osteogenic effect on the BMD in postmenopausal women by either increasing or preventing further bone loss as compared to the control group.[8,50-52] Really, it has been reported that the skeleton adapts to the increasing load applied by progressive RT in postmenopausal women by increasing BMD.[50] Similarly, the study by Nelson et al,[41] established that high intensity (i.e, 80% of 1RM) RT had a positive effect on the femoral neck BMD and lumbar BMD in postmenopausal women. Further, regular high intensity RT seems to be appropriate exercise therapy in maintaining lumbar spine BMD among postmenopausal women although the inclusion of other weight bearing activities may also be necessary to best augment hip BMD without other therapeutic agents.[52] Remarkably, for BMD improvement, the magnitude of the stimuli seems to be more important than the frequency of the stimuli.[53,54] Accordingly, RT has been more effective in increasing or maintaining BMD when compared to running, an already known osteogenic factor.[55] This positive effect occurs especially in anatomical sites where both activities produce mechanical stress, such as in the femur neck. Noticeably, the previous evidence appears to indicate that greater improvements could be achieved through RT of high-loading intensities with 3 sessions per week. It is imperative to emphasize that bone mass undergoes a never-ceasing process of formation and resorption and responds to the constantly changing mechanical forces impacting on its surfaces.[56] In regards to exercise duration, RT programs that last over 12 months appear to be responsible for a positive effect on bone mass. Although significant effects on BMD can be observed after 4 or 6 months in some locations of the body; the efficacy of the RT seems to be greater when the exercise programs lengthens for at least 1 year. Definitely, RT should last 12 to 18 months to ensure the training effect on BMD can be measured in an equilibrium state.[57] Further, when comparing pre- and postmenopausal women, the later require longer periods of intervention and higher loads because they are in a period of accelerated bone loss.[58] Lastly, the RT effects appear to disappear after the training is finished, as BMD decreased after the completion of the program.[56,59] For instance, Sinaki, et al.[59] reported changes in BMD continued 8 years after cessation of the 2-year RT program. The back exercise group had a loss in BMD, but the loss was significantly less in the back exercise group than in the control group. Clearly, RT helps prevent bone loss among postmenopausal women. It seems that the increased mechanical stress on the bone, provided by RT, is the causal factor of osteogenesis.[41] Furthermore, RT studies suggested that muscle contraction can also increase BMD by stimulating tissue remodeling,[45] bone formation,[60] or even augmentation of bone formation associated with an inhibition of reabsorption.[61]

Interestingly, it has been reported that high-frequency mechanical strain (i.e, vibration loading) might also have potential for preventing and treating osteoporosis. Really, Rubin, et al.[62] provided evidence in an animal model that low-risk, high-frequency mechanical accelerations may have a strong osteogenic effect. These investigators observed a dramatic increase of the quality and quantity of trabecular bone in sheep when exposed to low-level, high-frequency mechanical stimuli. Moreover, a high-frequency loading regimen applied to ovariectomized rats was effective in preventing early post-ovariectomy bone loss.[63] Further, Whole Body Vibration (WBV) training uses high frequency mechanical stimuli, which are generated by a vibrating platform and transmitted through the body where they load the bone and stimulate sensory receptors. Based on this background, a 6-month randomized controlled trial was aimed to study the effect of WBV on hip density in postmenopausal women.[64] The WBV participants (n=25, mean age=65 years) trained 3 times per week, performing static and dynamic knee-extensor exercises on a vibration platform, which mechanically loaded the bone and evoked reflexive muscle contractions. Importantly, no vibration-related side effects were observed; and BMD of the hip increased significantly (+0.93%) after vibration training.

In summary, the majority of studies cited previously seemed to indicate that RT promotes high-intensity loading force that are effective in increasing BMD in postmenopausal women. Thus, RT should be recommended as an adjunct lifestyle approach to osteoporosis prevention or in combination with other treatments in this group of women.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-114

**EFFECTS OF RESISTANCE TRAINING ON MUSCLE MASS**

Physical activity guidelines for older adults, men and women, have been developed byACSM and American Heart Association.[65] Indeed, muscle-strengthening activity should be performed, at least, 2 or more non-consecutive days per week, using a single set of 8-10 resistance exercises for the whole body, and at a moderate to high level of effort that allows 10-15 repetitions.[65] Additionally, RT is considered to be a safe and effective method for increasing strength and lean muscle tissue, and attenuating age-related muscle loss.[66-68] Interestingly, a 2004 systematic review of randomized controlled trials (n=28) on postmenopausal women (2646 participants, aged 50 to 65 years), reported that only 11 studies focused on muscular strength or endurance.[69] Additionally, a 12-week exercise program was conducted to determine the effects of RT and detraining on muscle mass in postmenopausal women (aged>50 years).[70] The intervention consisted of 3 sets of 10 repetitions; 3 times a week. The results showed that RT was sufficient to enhance strength of postmenopausal women. In addition, a 4-week detraining period had an adverse effect on muscle strength; however, the strength was greater in the RT group compared to the baseline values and the control group. The study by Charett, et al.[71] was aimed at determining whether increases in muscle strength were associated with changes in cross-sectional fiber area in older women (mean age=69 years). These investigators reported that the cross-sectional area of type II muscle fibers significantly increased in the exercise group (20.1 +/- 6.8%), after 12 weeks, compared with baseline. In contrast, no significant change in type II fiber area was observed in the controls.[71] A 16-week RT program promoted an increase on muscle mass and muscle strength in sedentary postmenopausal women (n=22, mean age=58 years).[72] The program targeted upper and lower body, 3 times per week in three series of 8-12 repetitions (60-80% 1RM). The exercise group gained 1.8 kg of muscle mass (10%) than the control group. Similar results were reported by Bonganha et al,[73] after a 16-week RT program. Postmenopausal women were randomized into exercise (n=16) and control (n=16) groups. RT was performed 3 times per week at 70 to 85% of 1RM. Their findings demonstrated that RT promoted a significant increase in muscle strength for leg press, bench press and curl. Moreover, after a 21-week progressive RT, performed twice a week,[74] maximal force increased by 37% and 1-RM by 29% of the leg extensors in older women (aged 64 years). Similarly, the cross-sectional area of the quadriceps increased after training. Holviala, et al.[75] studied the effects of 21-week heavy RT, twice a week, in 48 postmenopausal women (mean age=59 years). The study reported large increases in maximal and explosive strength, and in walking speed, as well as an improvement in dynamic balance test performance. These results indicate that total heavy RT may be applied in rehabilitation or preventive exercise protocols to improve balance capabilities in aging women. Additionally, studies reported similar results. For instance, Humphries, et al.[76] examining the effects of 24-week high intensity RT on muscular strength among postmenopausal women (n=34), either taking HRT or not taking HRT. RT was performed twice weekly (60-90% 1RM). Maximal bench press and squat strength improved significantly (25% and 37%, respectively), directly depending on the time and intensity of training.

On the other hand, long-term programs (i.e, >1 year) have also reported significant effects of RT on muscle mass. Indeed, Sipila et al.[77] examined the effects of a 12-month HRT combined with RT in postmenopausal women. The participants (n=80, aged 50-57 years) were randomized into four different groups; (a) RT only (2 sessions per week); (b) HRT only; (c) RT combined with HRT (2 sessions per week); and (d) control group. The results showed that those performing RT combined with HRT or receiving HRT alone significantly increased quadriceps cross-sectional area (+7.1% and +6.3%, respectively) compared to RT only group (+2.2%) or the controls (+0.7%). These investigators concluded that, in postmenopausal women, muscle performance, muscle mass and muscle composition are improved by HRT. Importantly, the beneficial effects of HRT combined with high-impact RT may exceed those of HRT alone.[77] Likewise, Teixeira, et al.[78] studied the impact of a 1-yr RT on body composition and muscle strength in postmenopausal women (40-66 years). Participants who were already users or non-users of HRT were randomly assigned to exercise (n=117; 60-75 min per day, 3 days per week) or non-exercise (n=116) groups. Significant gains in lean body mass were observed for women who exercised, regardless of HRT status, whereas women who did not exercise lost lean mass. The exercise group showed a mean increase of 0.9 kg in DXA-measured muscle mass that depended on the volume of training. Clearly, the results demonstrated that RT had a significant and positive impact on the total and regional body composition of postmenopausal women, independent of HRT.[78]

Lastly, Fjeldstad, et al.[79] studied the effects of 8-month RT with and without WBV on body composition in sedentary postmenopausal women. Participants (aged 60-75 years) were assigned to RT only (n=22), WBV+RT (n=21) or non-exercising control (n=12) groups. RT (3 sets 10 repetitions 80% 1RM) was performed using isotonic weight training equipment, and the WBV was done with the use of the power plate; both 3 times per week. Both, the RT and WBV+RT groups showed significant increases in arm bone free lean tissue mass, and in trunk bone free lean tissue mass. Likewise, in healthy postmenopausal women (n=25, mean age=65 years) a 24-week WBV program, 3 times per week, is feasible and able to modify muscle strength.[80] Indeed, WBV improved isometric and dynamic muscle strength (+15% and + 16%, respectively) compared to control subjects.

In summary, RT seems to be a safe and effective intervention for reversing the loss of muscle function and the deterioration of muscle structure by increasing muscle mass and strength in postmenopausal women. In addition, RT improves the functional status and levels of physical activity inpost menopausal women bringing about gains in speed, balance and gen-

eral spontaneous activities. Thus, RT should be recommended to counteract sarcopenia and muscle weakness in postmenopausal women.

## CONCLUSION

High intensity RT which places heavy loads on the skeleton during a training session, is effective at increasing BMD, muscle strength and muscle mass in postmenopausal women compared with control groups. The gradual decrease in strength has a potential for leading to disability and functional impairment in ADLs. In addition, the increased incidence of falls and hip fractures is feasibly the result of age-related atrophy in muscle mass. Indeed, sarcopenia, frequently observed in the elderly, contributes significantly to decreased QOL. Whenever physical exercise is stopped (i.e, detraining), the body may lose its beneficial adaptations, which is a response to diminished physiological demand. Postmenopausal women should continue to train and minimize detraining periods, as increased physical activity levels are essential for the protection of neuromuscular function, bone and muscle mass, and functional performance. Thus, progressive RT exercise programs should be targeted to obtain long lasting effects on bone and muscle mass.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

A.A.A.E.: Conception and drafting of the manuscript. J.E.B.: Drafting of the manuscript. The authors were involved in revising the manuscript, providing intellectual content, and approving the final version.

## REREFERNCES

1. Aagaard P, Magnusson PS, Larsson B, Kjaer M, Krustrup P. Mechanical muscle function, morphology, and fiber type in lifelong trained elderly. *Med Sci Sports Exerc.* 2007; 39(11): 1989-1996. doi: 10.1249/mss.0b013e31814fb402

2. Lauretani F1, Russo CR, Bandinelli S, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol (1985).* 2003; 95(5): 1851-1860. doi: 10.1152/japplphysiol.00246.2003

3. American College of Sports, Chodzko-Zajko M, Proctor WJ, et al. American College of Sports Medicine position stand. Exercise and physical activity for older adults. *Med Sci Sports Exerc.* 2009; 41(7): 1510-1530. doi: 10.1249/MSS.0b013e3181a0c95c

4. Sattelmair JR, Pertman JH, Forman DE. Effects of physical activity on cardiovascular and noncardiovascular outcomes in older adults. *Clin Geriatr Med.* 2009; 25(4): 677-702. doi: 10.1016/j.cger.2009.07.004

5. Vita AJ, Terry RB, Hubert HB, Fries JF. Aging, health risks, and cumulative disability. *N Engl J Med.* 1998; 338(15): 1035-1041. doi: 10.1056/NEJM199804093381506

6. ACSM-a. American College of Sports Medicine Position Stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. *Med Sci Sports Exerc.* 1998; 30(6): 975-991.

7. Garber CE, Blissmer B, Deschenes MR, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc.* 2011; 43(7): 1334-1359. doi: 10.1249/MSS.0b013e318213fefb

8. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR, American College of Sports M. (American College of Sports Medicine Position Stand: physical activity and bone health. *Med Sci Sports Exerc.* 2004; 36(11): 1985-1996.

9. Feigenbaum MS, Pollock ML. Prescription of resistance training for health and disease. *Med Sci Sports Exerc.* 1999; 31(1): 38-45.

10. Hurley BF, Roth SM. Strength training in the elderly: effects on risk factors for age-related diseases. *Sports Med.* 2000; 30(4): 249-268.

11. Mangione KK, Miller AH, Naughton IV. Cochrane review: Improving physical function and performance with progressive resistance strength training in older adults. *Phys Ther.* 2010; 90(12): 1711-1715. doi: 10.2522/ptj.20100270

12. Clemson L, Fiatarone Singh MA, Bundy A, et al. Integration of balance and strength training into daily life activity to reduce rate of falls in older people (the LiFE study): randomised parallel trial. *Bmj.* 2012; 345: e4547. doi: 10.1136/bmj.e4547

13. Nguyen ND, Pongchaiyakul C, Center JR, Eisman JA, Nguyen TV. Abdominal fat and hip fracture risk in the elderly: the Dubbo Osteoporosis Epidemiology Study. *BMC Musculoskelet Disord.* 2005; 6: 11. doi: 10.1186/1471-2474-6-11

14. US Department of Health and Human Services. Bone Health and Osteoporosis: A Report of the Surgeon General. 2004.

15. Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO Study Group. *Osteoporos Int.* 1994; 4(6): 368-381.

16. Al-Azzawi F, Palacios S. Hormonal changes during menopause. *Maturitas.* 2009; 63(2): 135-137. doi: 10.1016/j.maturi-

tas.2009.03.009

17. Khosla S, Riggs BL. Pathophysiology of age-related bone loss and osteoporosis. *Endocrinol Metab Clin North Am.* 2005; 34(4): 1015-1030.

18. Slingerland AS, van Lenthe FJ, Jukema JW, et al. Aging, retirement, and changes in physical activity: prospective cohort findings from the GLOBE study. *Am J Epidemiol.* 2007; 165(12): 1356-1363. doi: 10.1093/aje/kwm053

19. Looker AC, Orwoll ES, Johnston CC Jr, et al. Prevalence of low femoral bone density in older U.S. adults from NHANES III. *J Bone Miner Res.* 1997; 12(11): 1761-1768. doi: 10.1359/jbmr.1997.12.11.1761

20. Nguyen TV, Sambrook PN, Eisman JA. Bone loss, physical activity, and weight change in elderly women: the Dubbo Osteoporosis Epidemiology Study. *J Bone Miner Res.* 1998; 13(9): 1458-1467. doi: 10.1359/jbmr.1998.13.9.1458

21. Zehnacker CH, Bemis-Dougherty A. Effect of weighted exercises on bone mineral density in post menopausal women. A systematic review. *J Geriatr Phys Ther.* 2007; 30(2): 79-88.

22. ACSM. American College of Sports Medicine Position Stand. Exercise and physical activity for older adults. *Med Sci Sports Exerc.* 1998; 30(6): 992-1008.

23. Lexell J. Ageing and human muscle: observations from Sweden. *Can J Appl Physiol.* 1993; 18(1): 2-18.

24. Trappe TA, Lindquist DM, Carrithers JA. Muscle-specific atrophy of the quadriceps femoris with aging. *J Appl Physiol* 1985. 2001; 90(6): 2070-2074.

25. Fried LP, Tangen CM, Walston J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci.* 2001; 56(3): M146-M156.

26. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc.* 2002; 50(5): 889-896.

27. Liu CJ, Latham NK. Progressive resistance strength training for improving physical function in older adults. Cochrane Database Syst Rev. 2009; (3): CD002759. doi: 10.1002/14651858.CD002759.pub2

28. Peterson MT, Henry CA. Hedgehog signaling and laminin play unique and synergistic roles in muscle development. *Developmental Dynamics.* 2010; 239(3): 905-913.

29. Peterson MD, Sen A, Gordon PM. Influence of resistance exercise on lean body mass in aging adults: a meta-analysis. *Med Sci Sports Exerc.* 2011; 43(2): 249-258. doi: 10.1249/MSS.0b013e3181eb6265

30. Melton LJ 3rd, Khosla S, Crowson CS, O'Connor MK, O'Fallon WM, Riggs BL. Epidemiology of sarcopenia. *J Am Geriatr Soc.* 2000; 48(6): 625-630.

31. Jette AM, Branch LG. The Framingham Disability Study: II. Physical disability among the aging. *Am J Public Health.* 1981; 71(11): 1211-1216.

32. Aloia JF, McGowan DM, Vaswani AN, Ross P, Cohn SH. Relationship of menopause to skeletal and muscle mass. *Am J Clin Nutr.* 1991; 53(6): 1378-1383.

33. Rolland, YM, Perry HM 3rd, Patrick P, Banks WA, Morley JE. Loss of appendicular muscle mass and loss of muscle strength in young postmenopausal women. *J Gerontol A Biol Sci Med Sci.* 2007; 62(3): 330-335.

34. Carr MC. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab.* 2003; *88*(6): 2404-2411. doi: 10.1210/jc.2003-030242

35. Maltais ML, Desroches J, Dionne IJ. Changes in muscle mass and strength after menopause. *J Musculoskelet Neuronal Interact.* 2009; 9(4): 186-197.

36. Lemoine S, Granier P, Tiffoche C, Rannou-Bekono F, Thieulant ML, Delamarche P. Estrogen receptor alpha mRNA in human skeletal muscles. *Med Sci Sports Exerc.* 2003; 35(3): 439-443. doi: 10.1249/01.MSS.0000053654.14410.78

37. Sipila S, Poutamo J. Muscle performance, sex hormones and training in peri-menopausal and post-menopausal women. *Scand J Med Sci Sports.* 2003; *13*(1): 19-25.

38. Hakkinen K, Pakarinen A, Kraemer WJ, Newton RU, Alen M. Basal concentrations and acute responses of serum hormones and strength development during heavy resistance training in middle-aged and elderly men and women. *J Gerontol A Biol Sci Med Sci.* 2000; 55(2): B95-B105.

39. Kamen G, Knight CA. Training-related adaptations in motor unit discharge rate in young and older adults. *J Gerontol A Biol Sci Med Sci.* 2004; 59(12): 1334-1338.

40. Centers for Disease and Prevention. QuickStats: percentage of adults aged 18 years who engaged in leisure-time strengthening activities,* by age group and sex-National Health Interview Survey, United States, 2008. *MMWR Morb Mortal Wkly Rep.* 2009; 58(34): 955.

41. Nelson ME, Fiatarone MA, Morganti CM, Trice I, Green-

berg RA, Evans WJ. Effects of high-intensity strength training on multiple risk factors for osteoporotic fractures. A randomized controlled trial. *Jama.* 1994; 272(24): 1909-1914.

42. Notelovitz M, Martin D, Tesar R, et al. Estrogen therapy and variable-resistance weight training increase bone mineral in surgically menopausal women. *J Bone Miner Res.* 1991; 6(6): 583-590. doi: 10.1002/jbmr.5650060609

43. Ryan AS, Treuth MS, Hunter GR, Elahi D. Resistive training maintains bone mineral density in postmenopausal women. *Calcif Tissue Int.* 1998; 62(4): 295-299.

44. McCartney N, Hicks AL, Martin J, Webber CE. Long-term resistance training in the elderly: effects on dynamic strength, exercise capacity, muscle, and bone. *J Gerontol A Biol Sci Med Sci.* 1995; 50(2): B97-B104.

45. Pruitt LA, Jackson RD, Bartels RL, Lehnhard HJ. Weight-training effects on bone mineral density in early postmenopausal women. *J Bone Miner Res.* 1992; 7(2): 179-185. doi: 10.1002/jbmr.5650070209

46. Kerr D, Ackland T, Maslen B, Morton A, Prince R. Resistance training over 2 years increases bone mass in calcium-replete postmenopausal women. *J Bone Miner Res.* 2001; 16(1): 175-181. doi: 10.1359/jbmr.2001.16.1.175

47. Bocalini DS, Serra AJ, dos Santos L, Murad N, Levy RF. Strength training preserves the bone mineral density of postmenopausal women without hormone replacement therapy. *J Aging Health.* 2009; 21(3): 519-527. doi: 10.1177/0898264309332839

48. de Matos O, Lopes da Silva DJ, Martinez de Oliveira J, Castelo-Branco C. Effect of specific exercise training on bone mineral density in women with postmenopausal osteopenia or osteoporosis. *Gynecol Endocrinol.* 2009; 25(9): 616-620. doi: 10.1080/09513590903015593

49. Adami S, Gatti D, Braga V, Bianchini D, Rossini M. Site-specific effects of strength training on bone structure and geometry of ultradistal radius in postmenopausal women. *J Bone Miner Res.* 1999; 14(1): 120-124. doi: 10.1359/jbmr.1999.14.1.120

50. Kerr D, Morton A, Dick I, Prince R. Exercise effects on bone mass in postmenopausal women are site-specific and load-dependent. *J Bone Miner Res.* 1996; 11(2): 218-225. doi: 10.1002/jbmr.5650110211

51. Bemben DA, Fetters NL, Bemben MG, Nabavi N, Koh ET. Musculoskeletal responses to high- and low-intensity resistance training in early postmenopausal women. *Med Sci Sports Exerc.* 2000; 32(11): 1949-1957.

52. Martyn-St James M, Carroll S. High-intensity resistance training and postmenopausal bone loss: a meta-analysis. *Osteoporos Int.* 2006; 17(8): 1225-1240. doi: 10.1007/s00198-006-0083-4

53. Burrows M, Nevill AM, Bird S, Simpson D. Physiological factors associated with low bone mineral density in female endurance runners. *Br J Sports Med.* 2003; 37(1): 67-71.

54. Creighton DL, Morgan AL, Boardley D, Brolinson PG. Weight-bearing exercise and markers of bone turnover in female athletes. *J Appl Physiol (1985).* 2001; 90(2): 565-570.

55. Gremion G, Rizzoli R, Slosman D, Theintz G, Bonjour JP. Oligo-amenorrheic long-distance runners may lose more bone in spine than in femur. *Med Sci Sports Exerc.* 2001; 33(1): 15-21.

56. Hartard M, Haber P, Ilieva D, Preisinger E, Seidl G, Huber J. Systematic strength training as a model of therapeutic intervention. A controlled trial in postmenopausal women with osteopenia. *Am J Phys Med Rehabil.* 1996; 75(1): 21-28.

57. Smidt GL, Lin SY, O'Dwyer KD, Blanpied PR. The effect of high-intensity trunk exercise on bone mineral density of postmenopausal women. *Spine (Phila Pa 1976).* 1992; 17(3): 280-285.

58. Snow CM, Shaw, JM, Winters KM, Witzke KA. Long-term exercise using weighted vests prevents hip bone loss in postmenopausal women. *J Gerontol A Biol Sci Med Sci.* 2000; 55(9): M489-M491.

59. Sinaki M, Itoi E, Wahner HW, et al. Stronger back muscles reduce the incidence of vertebral fractures: a prospective 10 year follow-up of postmenopausal women. *Bone.* 2002; 30(6): 836-841.

60. Menkes A, Mazel S, Redmond RA, et al. Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. *J Appl Physiol (1985).* 1993; 74(5): 2478-2484.

61. Andreoli A, Monteleone M, Van Loan M, Promenzio L, Tarantino U, De Lorenzo A. Effects of different sports on bone density and muscle mass in highly trained athletes. *Med Sci Sports Exerc.* 2001; 33(4), 507-511.

62. Rubin C, Turner AS, Muller R, et al. Quantity and quality of trabecular bone in the femur are enhanced by a strongly anabolic, noninvasive mechanical intervention. *J Bone Miner Res. 2002;* 17(2): 349-357. doi: 10.1359/jbmr.2002.17.2.349

63. Flieger J, Karachalios T, Khaldi L, Raptou P, Lyritis G. Mechanical stimulation in the form of vibration prevents postmenopausal bone loss in ovariectomized rats. *Calcif Tissue Int.* 1998; 63(6): 510-514.

64. Verschueren SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. *J Bone Miner Res.* 2004; 19(3): 352-359. doi: 10.1359/JBMR.0301245

65. Nelson ME, Rejeski WJ, Blair SN, et al. Physical activity and public health in older adults: recommendation from the American College of Sports Medicine and the American Heart Association. *Circulation.* 2007; 116(9): 1094-1105. doi: 10.1161/CIRCULATIONAHA.107.185650

66. Reeves ND, Narici MV, Maganaris CN.). Effect of resistance training on skeletal muscle-specific force in elderly humans. *J Appl Physiol (1985).* 2004; 96(3): 885-892. doi: 10.1152/japplphysiol.00688.2003

67. Roth SM, Ferrell RF, Hurley BF. Strength training for the prevention and treatment of sarcopenia. *J Nutr Health Aging.* 2000; 4(3): 143-155.

68. Vincent KR, Braith RW, Feldman RA, et al. Resistance exercise and physical performance in adults aged 60 to 83. *J Am Geriatr Soc.* 2002; 50(6): 1100-1107.

69. Asikainen TM, Kukkonen-Harjula K, Miilunpalo S. Exercise for health for early postmenopausal women: a systematic review of randomised controlled trials. *Sports Med.* 2004; 34(11): 753-778.

70. Delshad M, Ghanbarian A, Mehrabi Y, Sarvghadi F, Ebrahim K. Effect of Strength Training and Short-term Detraining on Muscle Mass in Women Aged Over 50 Years Old. *Int J Prev Med.* 2013; 4(12): 1386-1394.

71. Charette SL, McEvoy L, Pyka G, et al. Muscle hypertrophy response to resistance training in older women. *J Appl Physiol (1985).* 1991; 70(5): 1912-1916.

72. Orsatti FL, Nahas EA, Maesta N, Nahas-Neto J, Burini RC. Plasma hormones, muscle mass and strength in resistance-trained postmenopausal women. *Maturitas.* 2008; 59(4): 394-404. doi: 10.1016/j.maturitas.2008.04.002

73. Bonganha V, Modeneze DM, Madruga VA, Vilarta R.Effects of resistance training (RT) on body composition, muscle strength and quality of life (QoL) in postmenopausal life. *Arch Gerontol Geriatr.* 2012; 54(2): 361-365. doi: 10.1016/j.archger.2011.04.006

74. Hakkinen K, Pakarinen A, Kraemer WJ, Hakkinen A, Valkeinen H, Alen M. Selective muscle hypertrophy, changes in EMG and force, and serum hormones during strength training in older women. *J Appl Physiol (1985).* 2001; 91(2): 569-580.

75. Holviala J, Sallinen J, Kraemer W, Alen M. Effects of strength training on muscle strength characteristics, functional capabilities, and balance in middle-aged and older women. *The Journal of Strength and Conditioning Research.* 2006; 20(2): 336-344.

76. Humphries B, Newton RU, Bronks R, et al. Effect of exercise intensity on bone density, strength, and calcium turnover in older women. *Med Sci Sports Exerc.* 2000; 32(6): 1043-1050.

77. Sipila S, Taaffe, DR, Cheng S, Puolakka J, Toivanen J, Suominen H. Effects of hormone replacement therapy and high-impact physical exercise on skeletal muscle in post-menopausal women: a randomized placebo-controlled study. *Clin Sci (Lond). 2001;* 101(2): 147-157.

78. Teixeira PJ, Going SB, Houtkooper LB, et al. Resistance training in postmenopausal women with and without hormone therapy. *Med Sci Sports Exerc.* 2003; 35(4): 555-562. doi: 10.1249/01.MSS.0000058437.17262.11

79. Fjeldstad C, Palmer IJ, Bemben MG, Bemben DA. Whole-body vibration augments resistance training effects on body composition in postmenopausal women. *Maturitas.* 2009; 63(1): 79-83. doi: 10.1016/j.maturitas.2009.03.013

80. Verschueren SM, Roelants M, Delecluse C, Swinnen S, Vanderschueren D, Boonen S. Effect of 6-month whole body vibration training on hip density, muscle strength, and postural control in postmenopausal women: a randomized controlled pilot study. *J Bone Miner Res.* 2004; 19(3): 352-359. doi: 10.1359/JBMR.0301245

SPORTS AND EXERCISE MEDICINE

Openventio PUBLISHERS

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-115

Research

*Corresponding author:
**Giorgio Radetti**
Department of Paediatrics
Regional Hospital
*via* L. Boehler 5
39100 Bolzano, Italy
**Tel.** +39-0471/908651
**Fax:** +39-0471/909730
**E-mail:** giorgio.radetti@asbz.it

*Volume 1 : Issue 3*
*Article Ref. #: 1000SEMOJ1115*

***Article History:***
**Received:** May 5th, 2015
**Accepted:** July 31st, 2015
**Published:** July 31st, 2015

***Citation:***
Grossgasteiger S, Lupi F, Cappa M, et al. Different sports in adolescence: effect on lipid profile, glucose metabolism, body composition, bone density, bone quality, bone markers, vascular function and structure, pituitary and hypothalamic antibodies. *Sport Exerc Med Open J*. 2015; 1(3): 97-104.



# Different Sports in Adolescence: Effect on Lipid Profile, Glucose Metabolism, Body Composition, Bone Density, Bone Quality, Bone Markers, Vascular Function and Structure, Pituitary and Hypothalamic Antibodies

**Simone Grossgasteiger[1], Fiorenzo Lupi[2], Marco Cappa[3], Davide Gatti[4], Flavio Egger[5], Annamaria De Bellis[6] and Giorgio Radetti[2*]**

*[1]Department of Sports Medicine, General Hospital Bolzano, Territorial Area (G.,S.), Italy*
*[2]Department of Paediatrics, Regional Hospital, via L. Boehler 5, Bolzano 39100, Italy*
*[3]Ospedale Bambin Gesù, UOC of Endocrinology and Diabetology, Bambino Gesù Children's Hospital, IRCCS, Rome Roma (C.M), Italy*
*[4]Department of Rheumatology, University of Verona (G.,D.), Italy*
*[5]Department of Internal Medicine, General Hospital Bolzano (E.F), Italy*
*[6]Division of Endocrinology and Metabolism, Department of Cardiothoracic and Respiratory Sciences, Second University of Naples (A,D.B.), Italy*

## ABSTRACT

**Introduction:** Sports can positively influence body health, however expose the athletes also to risks of repetitive trauma, in particular brain trauma.
**Aim of the study:** To evaluate the effects of different sports on glucose metabolism, lipid profile, insulin resistance (HOMA-r) and sensitivity (QUICKI), body composition, bone mass and mineralization (DEXA), bone quality (SOS and BTT), bone metabolism (PiNP, CTX, COMP, DKK1 and sclerostin) vascular structure and function (IMT and FMD) and on the presence of anti-pituitary and anti-hypothalamus antibodies. A health score was also calculated.
**Patients:** We evaluated 80 athletes practicing alpine skiing (#19), ice-hockey (#21), rugby (#20) and swimming (#20).
**Results:** Swimmers showed the highest triglycerides values ($p<0.05$). Glucose was more elevated in the rugby players ($p<0.05$) than in skiers and swimmers and insulin also higher in swimmers ($p<0.05$) than in rugby and hockey players. HOMA-r and QUICKI were significantly higher and lower respectively in swimmers ($p<0.05$) compared to the hockey players. DKK1 was significantly higher in the hockey players ($p<0.05$) compared to skiers and swimmers. FMD was significantly higher in hockey ($p<0.05$) and rugby players ($p<0.05$) compared to swimmers. Bone quality (BTT) was better in the skiers ($p<0.05$) compared to the hockey players. Anti-hypothalamus antibodies were not different among groups, while anti-pituitary antibody were significantly more frequent in the hockey players compared to swimmers ($p<0.05$). The health score was more advantageous in the skiers group.
**Conclusion:** Different sports exert distinct effects. Altogether, alpine skiing seems to produce the most benefits, in particular, because of the positive effects on bone and cardiovascular function.

**KEYWORDS:** Sport; Insulin sensitivity; Body composition; Bone mineralization; Bone ultrasound; Children; Anti pituitary and hypothalamus antibody; FMD; IMT.

**ABBREVIATIONS:** TBI: Traumatic Brain Injury; AHA: Anti-hypothalamus antibodies; APA: Anti-pituitary antibodies; COMP: Cartilage Oligomeric Matrix Protein; BAP: Bone-specific

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-115

alkaline phosphatase; FMD: Flow Mediated Dilation; IMT: Intima-media thickness; SOS: Speed-of-sound; BTT: Bone-transmission-time; BMI: Body Mass Index; SDS: Standard Deviation Scores; QUICKI: Quantitative-insulin-sensitivity-check-index; HOMA-r: Homeostasis-model-assessment-for-insulin-resistance; AI: Atherogenic Index; CVs: Coefficients of variation; FITC: Fluorescein isothiocyanate; DEXA: Dual Energy X-ray Absorptiometry; BMC: Bone Mineral Content; BMD: Bone Mineral Density.

## INTRODUCTION

Physical activity is known to exert many positive effects on body composition and therefore it is strongly recommended to children and adolescents. Sports require many physical, technical and mental abilities, and different physical activities may have an important role in body growth, metabolism regulation and bone architecture. In particular, one the most successful measure to prevent osteopenia in old age is building bones as strong as possible during adolescence when bone growth is high. Within competitive sports, studies support the greater beneficial effects on bone mass and bone geometric of disciplines that include impact activities, in which body weight plays a significant role, in comparison to non impact activities, such as swimming. Following regular training, i.e. more than two hours three times a week, a beneficial influence can be observed on the lipid profile, insulin sensitivity, cardiovascular system, muscle, bone mass and density.[1-3] To our knowledge, however, it is not known which is the most healthiest sport and furthermore how each sport influences the different parameters. On the other hand contact sports which imply tackles such as rugby or hockey expose the athlete to the risk of brain trauma which may cause different hypothalamic and pituitary hormonal deficits.[4-6] Hypopituitarism following Traumatic Brain Injury (TBI) was described almost one century ago. The most common cause of TBI being motor vehicle accidents falls, child abuse, violence and sports injuries. The association of hypopituitarism due to TBI with the presence of Anti-hypothalamus antibodies (AHA) and Anti-pituitary antibodies (APA) was found after long-term follow up.[7,8]

The aim of this study was therefore to investigate the effects of four different sports; ski, swimming, rugby and hockey in a group of young athletes training intensively. We evaluated in particular about the lipid profile, insulin sensitivity, bone health and mineralization, the cardiovascular risk factors and the presence of anti-hypothalamus and anti-pituitary antibodies.

## STUDY PROTOCOL

We evaluated 80 athletes practicing alpine skiing (#19), ice-hockey (#21), rugby (#20) and swimming (#20) after an appropriate approval by the Ethics Committee of the hospital institutional board was obtained together with an informed written consent from the parents. Mean chronological age was 12.7±0.9, 11.2±0.8, 12.9±0.6 and 12.7±1.03 years respectively. The clinical characteristics of the subjects are reported in table 1. The children were admitted to the ward in the morning following an overnight fast of more than 11 hours. The auxological data was recorded and a detailed history was obtained concerning the duration (years) and the intensity of training (hours/week). A positive history for migraine was considered as an exclusion factor, because it might influence the evaluation of vascular reactivity,[9] however none of the subjects were affected. A blood sample was obtained between 08.30 and 09.00 by venipuncture, for assessment of glucose, insulin, total and HDL cholesterol, triglycerides and Cartilage Oligomeric Matrix Protein (COMP).

COMP is a glycoprotein found in articular cartilage that helps to stabilize and align type II collagen molecules[10] and used as marker of cartilage degeneration.[10,11] Serum samples were stored at -80 ºC until being assayed for parathormone (PTH 1–84), 25-hydroxyvitamin D (25OHD), serum carboxy-terminal telopeptide of collagen-1 (CTX) as a marker of bone resorption, intact procollagen-1 N-terminal propeptide (P1NP) and Bone-specific alkaline phosphatase (BAP) as markers of bone formation, sclerostin and Dickkopf-1 (DKK1) as inhibitors of Wnt/β-catenin signalling. At the same time also the presence of antibodies against the pituitary and the hypothalamus were assessed.

The endothelial function was evaluated as Flow Mediated Dilation of the brachial artery (FMD) and the Intima-media thickness (IMT) by B-mode ultrasound at the level of the common carotid artery. On the same day, after blood sample collection, bone health [SOS (Speed-of-sound) and BTT (Bone-

| | Number | Chronological age (yrs) | h-SDS | BMI-SDS | W/Ht | Years of training | hrs/week | Fat% | Lean% |
|---|---|---|---|---|---|---|---|---|---|
| Ski | 19 | 12.7±0.9§ | 0.8±0.8 | -0.1±0.7 | 0.4±0.0 | 5.2±2.1 | 7.7±2.2§ | 20.4±5.98 | 76.1±5,74 |
| Hockey | 21 | 11.2±0.8 | 0.6±1.0 | -0.4±0.7 | 0.4±0.7 | 5.5±1.2 | 5 | 19.9±5.12 | 77.6±4.9 |
| Rugby | 20 | 12.9±0.6ß | 1.3±0.8 | -0.2±0.7 | - | 5.6±1.0 | 7.3±1.0ß | 18,3±5.19 | 76.9±6.8 |
| Swimming | 20 | 12.7±1.0& | 1.1±1.2 | -0.2±0.6 | 0.4±0.0 | 4.6±1.7 | 8.6±1.8&# | 18.9±5.06 | 77.7±4.9 |

&for the differences between swimmers and hockey players
#for the differences between swimmers and rugby players
§for the differences between skiers and hockey players
ßfor the differences between rugby players and hockey players
p<0.05 for all differences

**Table 1:** Clinical data in the four groups of athletes.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-115

transmission-time)] was determined by bone ultrasound, while bone mass and mineralization were assessed by DEXA.

## METHODS

For reasons of homogeneity, height and Body Mass Index (BMI) were expressed as Standard Deviation Scores (SDS), according to Cacciari.[12] Pubertal status was also recorded according to Tanner.[13] Waist circumference was measured midway between the lowest rib and the top of the iliac crest after gentle expiration and the waist to height ratio (WHtR), which is also considered as an index of cardiovascular risk[14] was calculated. Insulin sensitivity was evaluated with QUICKI ((Quantitative-insulin-sensitivity-check-index)=1/[log(I0)+log(G0)], where I0 is the fasting insulin and G0 the fasting glucose.[15] Insulin resistance was also calculated with the HOMA-r (Homeostasis-model-assessment-for-insulin-resistance) = (fasting insulin mIU/L X fasting glucose mg/dl) / 405.[16] The Atherogenic Index (AI), an index of severe cardiovascular risk, was calculated by the ratio Total/HDL-C.[17]

### Laboratory

Serum glucose was measured with automatic analyzers, using a hexokinase catalyzed-glucose oxidase method. Serum insulin was measured with an immunoradiometric assay (Immulite 2000 Insulin, DPC, LA, CA), with an intra- and inter-assay C.V. of 8.3% and 8.6% respectively. Total and high-density-lipoprotein cholesterol and triglycerides were measured enzymatically by an automatic photometric method (Olympus Diagnostica Gmbh, Lismeehan, O'Callaghan's Mills Co. Clare, Ireland). P1NP, PTH, 25OHD, BAP and CTX were measured using the IDS-iSYS Multi Discipline automated analyser (IDS iSYS, Immunodiagnostics System, Boldon, UK). All assays are based on chemiluminescence immunoassay technology except for the spectrophotometric IDS-iSYS Ostase BAP assay. Intra-assay Coefficients of variation (CVs) in our laboratory were 3% for P1NP, 8%, for 25OHD, 2% for BAP, 3% for CTX and 4.5% for PTH. Inter-assay CVs were 5% for P1NP, 12% for 25OHD and 7% for BAP, CTX and PTH.

For the detection of serum COMP an ELISA kit was used (EuroDiagnostica Wieslab, Sweden), with an intra-assay CV of 7% and an inter-assay CV of 8%. Serum DKK1 and sclerostin were measured by ELISA (Biomedica Medizinprodukte GmbH and Co. KG, Wien, Austria) with sensitivities of 0.38 pmol/L and 2.6 pmol/L respectively, intra-assay CVs of 8 and 5% and inter-assay CVs of 10 and 4%, respectively.

Anti-pituitary (APA) and anti-hypothalamus antibody (AHA) were evaluated in sera of the athletes and in 20 sex/age-matched normal controls. APA and AHA were detected by simple indirect immunofluorescence method on cryostat sections of young baboon pituitary gland and young baboon hypothalamus supplied by Halifax spa (Polverara, Pordenone, Italy) and Bio-medis srl (Roma,Italy), respectively, as previously described.[18] In particular, Fluorescein isothiocyanate (FITC) conjugated goat antihuman Ig was used to detect the presence of APA and AHA; they were considered positive starting at dilution >1:8.

### Endothelial Function and Intima Media Thickness

Endothelial function was assessed in a non invasive way, by the same operator, using high-resolution ultrasound to measure brachial artery responses to reactive hyperaemia, according to the recently published guidelines.[19] The dilatation of the artery caused by the increased blood flow is known as flow-mediated dilatation (FMD). A 12 MHz linear phased array transducer and a high-resolution ultrasonic vessel wall tracking system were used to image the dominant arm brachial artery longitudinally, just above the antecubital fossa. Brachial artery diameter was measured using electronic calipers from the anterior to the posterior m-line at a fixed distance from an anatomic marker. Images were acquired at baseline, during hand hyperaemia, i.e. 90 sec after deflation of a wrist cuff inflated to suprasystolic pressure (at least 50 mm Hg above systolic pressure) for 5 min for measurement of FMD. FMD was calculated as there was as an increase in arterial diameter during hyperaemia compared with the diameter at rest.

IMT was assessed by the same operator, using high resolution-mode real time ultrasound with a 12 MHz linear array transducer. IMT was defined by the lumen-intima and media-adventitia interfaces of the far wall. Three measurements were taken on each side and the mean of right and left taken as the overall average IMT.

Bone mineralization and body composition were determined by Dual Energy X-ray Absorptiometry (DEXA) (GE-Lunar, Madison, WI, USA). From the whole body scan, fat mass, lean tissue and Bone Mineral Content (BMC) were determined. Bone Mineral Density (BMD) total (BMD tot), BMD at the L2-L4 level (BMD L2-L4) and BMD at the femoral neck (BMD FN) were also calculated.

**Quantitative bone ultrasound:** The device used (DBM Sonic Bone Profiler, IGEA, Carpi, Italy) is based on the transmission of an ultrasound wave through the distal end of the first phalangeal diaphysis of the last four fingers of the not dominant hand. Two 12 mm diameter transducers on a high precision (0.02 mm) caliper, which measures the distance between the two probes, are positioned on the lateral and medial surface of each finger. The device calculates the Amplitude Dependent Speed of Sound (Ad-SOS, in m/s) through the phalanx by measuring the width of the finger divided by the time of flight, defined as the time from emitted pulse to received signal considering the signal which reaches a predetermined minimum amplitude value (2 mV) for the first time. Moreover, the device calculates the bone transmission time (BTT, in m/s) as the difference between the time when the first peak of the signal received attains its maximum and the

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-115

time that would be measured if no bone but only soft tissue were present between the transducers. The results were expressed as SDS according to Baroncelli.[20] The intra- and inter-coefficient of variation were calculated to be 0.55% and 0.83% for Ad-SoS, and 0.81% and 1.47% for BTT, respectively.[20] Quantitative bone ultrasound, which is influenced by bone density, architecture and elasticity has been previously shown to be a non invasive method of estimating bone quality.[20-22]

**Health score:** In an attempt to summarize in a unique score the results of the different findings we assigned, according to the results of the ANOVA evaluation, a plus or a minus for each parameter resulted significantly positive or negative compared to the other groups. Then an arithmetic sum was calculated and a "health score" was obtained for each group. To our knowledge a similar score has not been previously obtained and therefore there was no data for comparison

## STATISTICAL ANALYSIS

Quantitative data was normally distributed and is expressed as mean±SD. All DEXA measures, which strongly depend from bone size, were corrected by the height of the subjects. Differences among groups were evaluated by ANOVA with the Bonferroni correction as post-hoc test. Simple correlation analysis was used to investigate the association between cardiovascular, bone markers and insulin sensitivity/resistance with the auxological and clinical parameters and a logistic regression was used to verify differences in anti-pituitary and anti-hypothalamus antibodies among groups. A P value of less than 0.05 indicates statistical significance. The StatView® statistical software (SAS Institute Inc. Cary, NC, 27513, USA) was used for these analyses.

## RESULTS

### Clinical Data

The groups of hockey players were significantly younger compared to the other groups; they trained less frequently during the week but the duration of training (years) was similar to the other groups. Height SDS, BMI SDS and the WHtR were not different among groups. There was also no difference in body composition among groups (Table 1).

### Biochemical Data

No difference was observed among groups regarding total-cholesterol, HDL-cholesterol and atherogenic index. Rugby players had significantly higher triglycerides serum levels compared to skiers ($p<0.05$) while swimmers had significantly higher triglycerides ($p<0.05$) compared to skiers and hockey players. Glucose was mildly more elevated in the rugby players ($p<0.05$) compared to skiers and swimmers and insulin was significantly higher in swimmers ($p<0.05$) than in rugby and hockey players. Homa-r and QUICKI were significantly higher and lower respectively in swimmers ($p<0.5$) compared to the hockey players (Table 2).

### PTH, 25OHD and Bone Markers

These could be evaluated in only three groups. There was no difference in the 25OHD3 and PTH serum levels. Bone neoformation (P1NP) and bone resorbtion (ctx) markers were also similar. Sclerostin was not different while DKK1 was significantly higher in the hockey players ($p<0.05$) compared to skiers and swimmers. COMB was also similar in the three groups (Table 3).

### Cardiovascular Function

IMT was not different between skiers, hockey players and swimmers, while FMD was significantly higher in hockey ($p<0.05$) and rugby players ($p<0.05$) compared to swimmers.

### Bone Assessment

Total bone area was significantly smaller in the group

| | Total cholesterol (mg/dl) | HDL cholesterol (mg/dl) | AI | Tryglicerides (mg/dl) | Glycaemia (mg/dl) | Insulin (μU/ml) | HOMA-r | QUICKI |
|---|---|---|---|---|---|---|---|---|
| Ski | 169±26 | 65±12 | 0.39±0.07 | 53±15 | 76±7 | 9.6±3.4 | 1.8±0.7 | 0.35±0.02 |
| Hockey | 168±30 | 59±15 | 0.35±0.07 | 60±28 | 80±5 | 7.7±3.5 | 1.5±0.7 | 0.37±0.03[&] |
| Rugby | 171±16 | 65±12 | 0.38±0.07 | 80±18[$] | 83±6[$#] | 8.5±4.4 | 2.0±0.1 | 0.35±0.05 |
| Swimming | 179±22 | 64±12 | 0.36±0.06 | 88±42[*&] | 76±8 | 12.7±4.3[&#] | 2.3±0.7[&] | 0.34±0.02 |

[*]for the differences between swimmers and skiers
[&]for the differences between swimmers and hockey players
[$]for the differences between rugby players and skiers
[#]for the differences between swimmers and rugby players
$p<0.05$ for all differences

**Table 2:** Biochemical data in the four groups of athletes.

of hockey players ($p<0.05$) compared to swimmers and rugby players. However, this parameter strongly depends from the size of the subject and even if we tried to normalize the data for the height of the subjects, we think that the younger age and the pubertal status of the hockey players might have played a role. The same was found also for the spine area ($p<0.05$) and the leg area ($p<0.05$). On the contrary, total BMD and leg BMD were not different among groups but spine BMD was again lower in the hockey player ($p<0.05$), reflecting a reduced size. Bone quality, evaluate as BTT, which is a more reliable parameter since it is independent from the soft tissue surrounding the bone and the size of the bone, could be assessed in the skiers and hockey players only; it was significantly better in the formers ($p<0.05$) (Table 4).

### Anti-pituitary and Anti-hypothalamus Antibody

As can be seen in figure 1 no difference was found among skiers, hockey players and swimmers regarding the anti-hypothalamus antibody while anti-pituitary antibody were found significantly more frequent in the hockey players than in swimmers ($p<0.05$).

### Health Score

Alpine skiers seem to perform better in particular because of the positive effect on bone status and cardiovascular function.

### Correlations

A significant positive correlation was found between the intensity of training (hrs/week) with BTT ($r=0.37$; $p<0.05$), percentage of lean mass with SOS ($r=0.45$; $p<0.001$) and percentage of fat with SOS ($r= -0.47$; $p<0.001$) and BTT ($r= -0.32$; $p<0.05$).

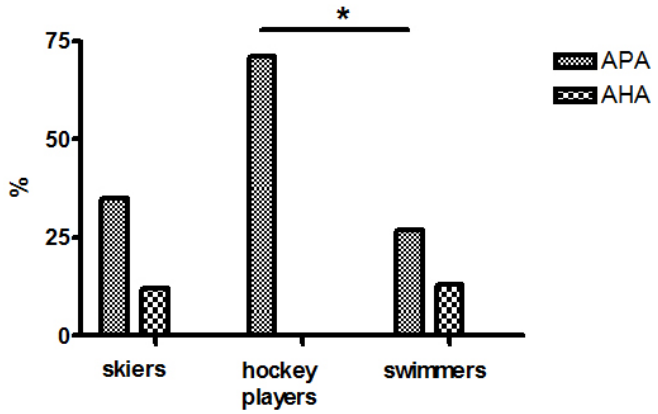



**Figure 1:** Anti-pituitary (APA) and anti-hypothalamus antibody (AHA) in the three groups of athletes.

## DISCUSSION

The aim of this study was to verify the influence of 4 different sports on several parameters including anthropometric measurements, biochemical indices, bone metabolism,

| | 25OHD3 ng/ml | P1NP ng/ml | PTH pg/ml | CTX ng/ml | DKK1 pmol/L | Sclerostin pmol/L | COMP µg/ml |
|---|---|---|---|---|---|---|---|
| Ski | 33±6.67 | 792.95±283.92 | 28.35±15.71 | 0.57±0.37 | 26.75±9.84 | 39.31±26.89 | 1.88±0.45 |
| Hockey | 30.79±11.67 | 828.16±290.47 | 25.38±15.14 | 0.51±0.32 | 33.36±6.36[&§] | 40.79±11.10 | 1.95±0.39 |
| Rugby | - | - | - | - | - | - | - |
| Swimming | 35.88±9.63 | 692.12±433.07 | 18.05±7.40 | 0.57±0.36 | 25.35±7.06 | 45.68±75.18 | 1.72±0.40 |

[&]for the differences between swimmers and hockey players
[§]for the differences between skiers and hockey players
$p<0.05$ for all differences

**Table 3:** Bone and cartilage markers.

| | IMT mm | FMD % | Area tot/h | BMD tot/h | Spine area/h | Spine BMD/h | Leg area/h | BMDleg/h | SOS SDS | BTT SDS |
|---|---|---|---|---|---|---|---|---|---|---|
| Ski | 0.48±0.12 | 13.63±3.5 | 11.38±1.01[§] | 0.01±0 | 0.57±0.06[§] | 0.03±0.01[§] | 2.15±0.19[§] | 0.01±0 | 1.14±1.02 | 0.55±0.85[§] |
| Hockey | 0.48±0.05 | 15.35±4.91[&] | 9.97±0.82 | 0.01±0 | 0.48±0.06 | 0.02±0.01 | 1.76±0.28 | 0.01±0 | 0.63±0.89 | -0.28±0.60 |
| Rugby | - | 14.44±2.19[#] | 10.95±0.99[ß] | 0.01±0 | 0.54±0.05[ß] | - | 2.01±0.31[ß] | 0.01±0 | - | - |
| Swimming | 0.45±0.05 | 11.24±2.47 | 10.80±0.96[&] | 0.01±0 | 0.53±0.09 | 0.03±0.01[&] | 1.99±0.21[&] | 0.01±0 | - | - |

[&]for the differences between swimmers and hockey players
[#]for the differences between swimmers and rugby players
[§]for the differences between skiers and hockey players
[ß]for the differences between rugby players and hockey players
$p<0.05$ for all differences

**Table 4:** Vascular structure and function, bone mineralization and bone quality in the four groups of athletes.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-115

mineralization and bone quality, cardiovascular function and the presence of anti-pituitary and anti-hypothalamus antibody. Altogether we found several differences between groups; however we could not identify a unifying trend which would identify the healthiest sports.

As to the specific findings, no difference was found in auxological parameters and body composition, while some dissimilarity were present in the lipid profile and in the glucose metabolism, swimmers showing in particular, significantly decreased insulin sensitivity. Bone markers were also not different, apart from DKK1 which was more elevated in the hockey players. An increased DDK1, which negatively influences bone metabolism by inhibiting the Wnt/β-catenin signaling, might underpin the less favorable ultrasound finding in the hockey players.

As to the vascular function, hockey and rugby players showed a greater FMD compared to swimmers, suggesting that in our athletes a combination of aerobic and resistance training results in an even better endothelial function than with aerobic activity only.

Bone mass and mineralization was significantly lower in hockey player compared to the other groups, even after correction for the height of the subjects. This sounds quite unexpected, however we think that the results are biased by the fact that these athletes were younger in comparison with the other and therefore differences in the pubertal status surely have played a role. It is well known in fact that testosterone stimulates bone expansion together with growth hormone leading eventually to an increased bone size. Nevertheless BTT, which reflects bone quality and which is independent from bone size, was also significantly reduced compared to skiers.

Athletes are more likely to sustain joint injuries compared with the average individual. Such joint injuries may cause joint instability and degeneration of the articular cartilage.[20,23] We wondered therefore whether an initial derangement of the cartilage might be detected already at this early age by a serum marker. We evaluated COMP therefore. When articular cartilage is broken down, COMP is released into the circulation, which makes it a useful marker of cartilage degeneration.[10,11] Circulating levels of COMP are elevated in patients with radiographically apparent OA and increase as the OA burden increases.[18,24] COMP was also shown useful in evaluation and monitoring the impact of joint tissue damage as joint bleed,[25] as femoroacetabular impingement in athletes.[26]

In this study work we could not find any differences among groups in serum COMP. In particular, we did not find any differences, even between skiers, hockey and rugby players, who frequently suffer from knee injuries, and swimmers, who never have physical contact and furthermore exercise in absence of gravity. However there are several limitations to the COMP evaluation in current study. No specific reference values for COMP are available for younger subject, so we can only compare different populations. We evaluated only a single time point in our subjects and did not assess whether COMP levels change over time or immediately after a single sport event. Finally a limitation in the use of any circulating biomarker of cartilage breakdown is the inability to identify specific joints where they originated.

At the end, we tried to evaluate cumulatively the results for each sport creating a health score. As reported in table 5, it seems that ski should be considered the healthiest sport, mostly due to its positive effects on lipid profile and bone status.

| | ski | hockey | Rugby | swimming |
|---|---|---|---|---|
| auxology | 0 | 0 | 0 | 0 |
| Lipid profile | + | + | - | - |
| Glucose homeostasis | 0 | + + + | - + | - - - |
| Bone metabolism | + | - | 0 | + |
| Bone mineralization | + | - | 0 | + |
| Bone quality | + | - | 0 | 0 |
| Cardiovascular function | 0 | + | + | - |
| Anti pituitary/hypothalamus Abs | 0 | - | 0 | + |
| Total score | +4 | +1 | 0 | -2 |

The total score represents the arithmetic sum of the single points.

**Table 5:** Health score. A plus is assigned for every parameters positively different from the other groups and a minus when negatively different (ANOVA+Bonferroni).

One of the most interesting finding of the study however is the high level of anti-pituitary antibodies in the groups of hockey players who obviously are more at risk for chronic repetitive head trauma. No difference was found instead regarding the anti-hypothalamus antibodies. These findings are strongly in agreement with those of a recently published paper reporting, in a five years prospective investigation, a significant increase of anti-pituitary antibodies even after mild brain trauma.[8] The appearance of these antibodies has been suggested to be the consequence of the head trauma triggering an ongoing cascade of vascular and histopathological alterations involving mediators of inflammatory process, thus favoring the immune system activation which can contribute to late pituitary dysfunction.[7,8] The presence of these antibodies is in fact considered a reliable prognostic factor for future pituitary dysfunction even in subjects with mild brain trauma;[8] these antibodies therefore might identify those athletes at risk of developing pituitary dysfunction in the future. The evaluation of the pituitary function was out of the scope of the study and therefore we have no data concerning this point. Our athletes were actually in optimal physical condition, having passed through a regular medical investigation in order

to obtain the license to compete, however we cannot exclude that in the future they might suffer from some endocrine disorder. It is our intention therefore to follow them up regularly in order to detect promptly any abnormalities.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

## ACKNOWLEDGMENT

We are indebted with Dr. Antonio Fanolla for his help in the statistical analysis and we thank Caterina Fraccarollo for the biochemical assays.

## REFERENCES

1. Daskalopoulou SS, Cooke AB, Gomez YH et al. Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects. *European journal of endocrinology*. 2014; 171(3): 343-352. doi: 10.1530/EJE-14-0204

2. Gordon B, Chen S, Durstine JL. The effects of exercise training on the traditional lipid profile and beyond. *Current sports medicine reports*. 2014; 13(4): 253-259. doi: 10.1249/JSR.0000000000000073

3. Venckunas T, Lionikas A, Marcinkeviciene JE, Raugaliene R, Alekrinskis A, Stasiulis A. Echocardiographic parameters in athletes of different sports. *Journal of sports science & medicine*. 2008; 7(1): 151-156.

4. Aimaretti G, Ambrosio MR, Di Somma C, et al. Traumatic brain injury and subarachnoid haemorrhage are conditions at high risk for hypopituitarism: screening study at 3 months after the brain injury. *Clinical endocrinology*. 2004; 61(3): 320-326. doi: 10.1111/j.1365-2265.2004.02094.x

5. Aimaretti G, Ambrosio MR, Di Somma C, et al. Residual pituitary function after brain injury-induced hypopituitarism: a prospective 12-month study. *The Journal of clinical endocrinology and metabolism*. 2005; 90(11): 6085-6092. doi: 10.1210/jc.2005-0504

6. Kozlowski Moreau O, Yollin E, Merlen E, Daveluy W, Rousseaux M. Lasting pituitary hormone deficiency after traumatic brain injury. *Journal of neurotrauma*. 2012; 29(1): 81-89. doi: 10.1089/neu.2011.2048

7. Tanriverdi F, De Bellis A, Battaglia M, et al. Investigation of antihypothalamus and antipituitary antibodies in amateur boxers: is chronic repetitive head trauma-induced pituitary dysfunction associated with autoimmunity? *European journal of endocrinology / European Federation of Endocrine Societies*. 2010; 162(5): 861-867. doi: 10.1530/EJE-09-1024

8. Tanriverdi F, De Bellis A, Ulutabanca H, et al. A five year prospective investigation of anterior pituitary function after traumatic brain injury: is hypopituitarism long-term after head trauma associated with autoimmunity? *Journal of neurotrauma*. 2013; 30(16): 1426-1433. doi: 10.1089/neu.2012.2752

9. Tunis MM, Wolff HG. Studies on headache; long-term observations of the reactivity of the cranial arteries in subjects with vascular headache of the migraine type. *A.M.A. archives of neurology and psychiatry*. 1953; 70(5): 551-557.

10. Garvican ER, Vaughan-Thomas A, Clegg PD, Innes JF. Biomarkers of cartilage turnover. Part 2: non-collagenous markers. *Veterinary journal*. 2010; 185(1): 43-49. doi: 10.1016/j.tvjl.2010.04.012

11. Neidhart M, Hauser N, Paulsson M, DiCesare PE, Michel BA, Hauselmann HJ. Small fragments of cartilage oligomeric matrix protein in synovial fluid and serum as markers for cartilage degradation. *British journal of rheumatology*. 1997; 36(11): 1151-1160. doi: 10.1093/rheumatology/36.11.1151

12. Cacciari E, Milani S, Balsamo A, et al. Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). *Journal of endocrinological investigation*. 2006;29(7): 581-593. doi: 10.1007/BF03344156

13. JM T. Growth at adolescence. In. Oxford: Blackwell Scientific; 1962.

14. Maffeis C, Banzato C, Talamini G. Obesity study group of the italian society of pediatric e, diabetology. waist-to-height ratio, a useful index to identify high metabolic risk in overweight children. *The Journal of pediatrics*. 2008; 152(2): 207-213.

15. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *The Journal of clinical endocrinology and metabolism*. 2000; 85(7): 2402-2410.

16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985; 28(7): 412-419. doi: 10.1007/BF00280883

17. Castelli WP. Lipids, risk factors and ischaemic heart disease. *Atherosclerosis*. 1996; 124: S1-9. doi: 10.1016/0021-9150(96)05851-0

18. Clark AG, Jordan JM, Vilim V, et al. Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: the johnston county osteoarthritis project.

ISSN 2379-6375 Open Journal http://dx.doi.org/10.17140/SEMOJ-1-115

*Arthritis and rheumatism*. 1999; 42(11): 2356-2364. doi: 10.1002/1529-0131(199911)42:11<2356::AID-ANR14>3.0.CO;2-R

19. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force. *Journal of the American College of Cardiology*. 2002; 39(2): 257-265. doi: 10.1016/S0735-1097(01)01746-6

20. Baroncelli GI, Federico G, Bertelloni S, et al. Assessment of bone quality by quantitative ultrasound of proximal phalanges of the hand and fracture rate in children and adolescents with bone and mineral disorders. *Pediatric research*. 2003; 54(1): 125-136. doi: 10.1203/01.PDR.0000069845.27657.EB

21. Reginster JY, Dethor M, Pirenne H, Dewe W, Albert A. Reproducibility and diagnostic sensitivity of ultrasonometry of the phalanges to assess osteoporosis. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. 1998; 63(1): 21-28. doi: 10.1016/S0020-7292(98)00113-1

22. Wuster C, Albanese C, De Aloysio D, et al. Phalangeal osteosonogrammetry study: age-related changes, diagnostic sensitivity, and discrimination power. The Phalangeal Osteosonogrammetry Study Group. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2000; 15(8): 1603-1614. doi: 10.1359/jbmr.2000.15.8.1603

23. Mow V RM. Articular cartilage: biomechanics. In: BJ Woo SL-Y, ed. Injury and repair of the musculoskeletal soft tissues. Park Ridge, IL: American Academy of Orthopedic surgeons; 1988: 427-63.

24. Conrozier T, Saxne T, Fan CS, et al. Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein in hip osteoarthritis: a one year prospective study. *Annals of the rheumatic diseases*. 1998; 57(9): 527-32.

25. van Vulpen LF, van Meegeren ME, Roosendaal G, et al. Biochemical markers of joint tissue damage increase shortly after a joint bleed; an explorative human and canine in *vivo* study. *Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society*. 2015: 23(1): 63-69. doi: 10.1016/j.joca.2014.09.008

26. Bedi A, Lynch EB, Sibilsky Enselman ER, et al. Elevation in circulating biomarkers of cartilage damage and inflammation in athletes with femoroacetabular impingement. *The American journal of sports medicine*. 2013; 41(11): 2585-2590. doi: 10.1177/0363546513499308