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Effect of Combination Therapy of Fatty Acids, Calcium, Vitamin D and Boron with Regular Physical Activity on Cardiovascular Risk Factors in Rat

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Abstract: The effect of consumption of fatty acids and selected nutrients, along with regular physical activity, on cardiovascular risk factors in rats was investigated.
Male rats were divided into the seven groups: Group 1: regular food and drinking water, Group 2: same as Group. 1 + physical activity (whole body vibration; WBV), Group 3: same as Group. 2 + calcium, vitamin D, boron, Group 4: same as Group. 3 + canola oil, Group 5: same as Group. 3 + sunflower oil, Group 6: same as Group. 3 + mix of sunflower oil and canola oil, Group 7: same as Group. 3 + coconut oil. Rats were treated for 8 weeks, and analysis of the frozen plasmas was performed. A- Analysis between the treatment groups and control revealed that vibration training in Group 2 increased body weight (P = 0.04), plasma creatin kinase (CK), (P = 0.02), and estradiol (E2), (P = 0.03). Rats in Group 5 consumed less food and plasma levels of cholesterol and LDL-cholesterol (LDL-C) increased significantly (P = 0.02) in Group 6 and in Group 7 (p<0.05). B- Analysis of data among Group 4 - 7 (the oil consuming groups) and Group 3 revealed significant differences in cholesterol (Chol), LDL-C, HDL-cholesterol (HDL-C), triglycerides (TG), C- reactive protein (hs-CRP), estradiol (E2), atherogenic index (AI), and risk factor (RF), (p<0.05). In addition, plasma levels of testosterone (T) and free testosterone (FT) in Group 7 had a remarkable but non-significant increase. As a result of vibration training, a similar trend was observed for vitamin D in Group 2-7. The findings show that WBV is effective in improving health status by influencing cardiovascular disease (CVD) risk factors. Moreover, canola oil and sunflower oil, separately, showed beneficial impacts on CVD risk factors; whereas their combination had negative impacts on lipid profile. Coconut oil revealed to be efficient to provide health benefits in terms of CVD treatments.

Key words: cardiovascular disease, fatty acids, calcium, boron, vitamin D, vibration

1 INTRODUCTION

Cardiovascular disease (CVD) causes cardiac arrest and heart failure and is a leading cause of disability and death worldwide. Clearly, physical activity has long been considered the cornerstone of interventions and has been shown to be extremely important in reducing the burden of this disease.

Physical activity as an effective strategy, as recommended in general practice, can be used to increase lean mass and bone mass while decreasing fat mass, and is also beneficial in improving health status of the individuals[1)]. Recently, whole body vibration (WBV) has been regarded as an exercise training method with a potential for improving body composition[2)]. It is suggested that a WBV training program as a non-pharmacological supportive treatment option appears to be an efficient alternative treatment for chronic disease conditions, such as bone disorders and cardio-respiratory fitness. In mice, reduced adipogenesis and

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factors associated with the onset of type II diabetes (e.g., triglycerides and nonesterified free fatty acid) has been reported[3]. During exercise and recovery in young men, energy expenditure increased significantly with vibration and exercise training[4]. Energy expenditure, carbohydrate and fat oxidation rates, and oxygen consumption in a similar study group were increased by vibration[5].

The findings suggest that WBV training improved isometric and dynamic muscle, muscle strength and cardiorespiratory fitness[2, 6, 7], and was reported to reduce body fat accumulation and serum leptin concentration[8].

The pathogenesis of coronary heart disease is of multifactorial origin and numerous clinical trials have evaluated the use of dietary factors such as nutrients and fatty acids in the prevention of coronary heart disease. The studies have yielded conflicting results. Among dietary factors, evidence exists that boron may have antioxidant and anti-inflammatory properties. It may also reduce tissue damage from inflammation by hastening the destruction of reactive oxygen species by increasing activities of key antioxidant enzymes[9]. It might be interesting to study further the above effects simultaneously with alterations in steroid hormone concentrations as a result of boron supplementation trials.

Calcium intake above the recommended daily amount may reduce all cause mortality, with no consistent inverse associations observed with CVD mortality[10]. Cardiovascular mortality was decreased by 23% in men in the highest compared with the lowest tertiles of calcium intake, although this reduction was not statistically significant ($p = 0.064$)[10].

A reassessment of the role of calcium supplements in the management of osteoporosis is warranted, because it is reported to be associated with an increased risk of myocardial infarction[11]. Moreover, several studies are providing evidence that the protective effect of vitamin D on the heart is now well established and recognized as important for cardiovascular health and its deficiency as a potential risk factor for several cardiovascular disease processes[12, 13].

Furthermore, the effects of dietary oils on lipid composition, in particular the benefits and protective effect of omega-3 fatty acid intake on cardiovascular health have been extensively investigated and it has been suggested that they confer benefit in patients with known coronary heart disease[14, 15]. Also, many biological functions are dependent on intake of n-6 fatty acids, like n-3 fatty acids, and have long been known to reduce serum total and low-density lipoprotein cholesterol. After doubling its intake, coronary heart disease mortality fell by 50% over a period of several decades[16]. In fact, a balance of these two fatty acids keeps the individuals healthy and an imbalance is thought to contribute to inflammation that increases the risk of developing diseases like type II diabetes and heart disease[17].

High intakes of *trans* fatty acids[18] and high intakes of saturated fat are associated with elevated serum cholesterol concentrations and contribute to a sizeable proportion of coronary heart disease (CHD) events[19]. Although the evidence indicates that a reduction in dietary saturated fat is associated with improved cardiovascular health, a recent document of a meta-analysis reported that the intake of saturated fat was not associated with an increased risk of CHD, stroke, or CVD[20]. According to the data, among the different sources of saturated fat, coconut oil or fat is not a predictor for CHD compared with the intakes of animal foods[19]. It is generally believed to be better than partially hydrogenated trans fats, and possibly animal fats, and contains an unusual blend of short and medium chain fatty acids[21], antioxidant properties due to phenolic compounds[22]. It appears that its dietetic supplementation does not cause dyslipidemia and seems to promote a reduction in abdominal obesity[23].

Since the effects of vibration exercise on the plasma parameters have rarely been studied, therefore the effects of a combination therapy including the simultaneous impacts of a regular physical activity program [whole body vibration (WBV)], consumption of different fatty acids (polyunsaturated, monounsaturated and saturated fatty acids), plus selected nutrients (calcium, vitamin D, and boron) on the cardiovascular risk factors in rats were investigated and presented in this work.

2 MATERIALS AND METHODS

2.1 Animals

The research was approved by the University research and ethics committee. Male Wistar rats weighing 140-180 g were obtained from the Animal House of Physiology Group-Baqiyattallah University of Medical Sciences. Eight rats in each group (Control vs. six treatment groups) were randomly kept in plastic cages (four rats per cage) in a controlled environment with a 12-h light/dark cycle and a constant temperature (22℃) and humidity (55-65%), with free access to food and water. Animals were weighed and provided with clean cages weekly. Food intake was determined three times per week.

2.2 Vibration training

Vibration training started after a week of acclimatization and animals were placed in a compartment attached to a vibration platform (China). The vibration intervention for the groups consisted of five minutes cycle in day 1, followed by extra five minutes cycle each time for the next 4 sessions (5×5 minute cycles) and increased to 30 minutes in day 10-21 (3×10 minute cycles) of vertical sinusoidal whole body vibration. A total of 11 vibration sessions were recorded in the first three weeks. It gradually increased to

45 minutes until day 24 (with three sessions per week); followed by an increase up to 60 minutes per set for the next 20 sessions until the end of 8 weeks of the experiment. Each training session was performed between 8.30-10.00 A.M.

After each vibration cycle, the animals were given 1-2 minute rest break between cycles. The vibration was performed at mode 1 with an amplitude of 1-10 and a frequency of 10-50 Hz. The speed of mode 1 in each cycle increased gradually and then decreased with the same trend within each specific time period. The control animals remained in their cages and were placed over the vibration platform without vibration treatment.

2.3 Diets and animal treatments

Rats in all groups were fed with standard chow from Pars Animal Food Co. (Tehran, Iran) and water ad libitum throughout the study. According to the manufacturer, it contained 650 mg Ca/100 g food and 80 I.U vitamin D3/100 g food. The boron content was not analyzed, but it is reported to be kept at 70 ug/kg of the food[24]. Food and water were provided in an identical manner for each group, except for the test lipid or fat source, which was added as a commercial oil (w/w; 5%): canola oil (Co), sunflower oil (AF), mix of sunflower oil and canola oil (AC), and coconut oil (N), added to the diet of the groups 4-7, respectively. Commercial canola oil (as a rich source of monounsaturated fatty acids) and sunflower oil (as a rich source of polyunsaturated fatty acids) were manufactured by Khoramshahr oil Co., (Varamin, Iran). Commercial coconut oil (C.B.C pure white coconut oil, as a rich source of saturated fatty acids) from Sime Darby Edible, Singapore was purchased from local market. Rats in the groups 3-7 were supplemented with 100 mg Ca/d, 40 I.U vitamin D3, and 1 mg B/d in their water, daily. Boric acid (Merck- Germany) was used as the source of boron and Ca^{+} vitamin D3 tablets (Darou paksh Co., Tehran- Iran) was used as the source of Ca and vitamin D3. Overall, the food and water provided 210 mg Ca, 55 I.U vitamin D3, and 1 mg B/rat/day. Fresh Food and water was provided three times per week and the consumption was monitored and recorded.

Procedure is detailed as follow:

Group 1 (control): regular food and drinking water

Group 2 (Sport; Sp): same as Group. 1 + physical activity (whole body vibration; WBV)

Group 3 (SPM): same as Group. 2 + calcium, vitamin D3, and boron

Group 4 (Co): same as Group. 3 + canola oil

Group 5 (AF): same as Group. 3 + sunflower oil

Group 6 (AC): same as Group. 3 + mix of sunflower oil and canola oil

Group 7 (N): same as Group. 3 + coconut oil.

2.4 Sampling

After weeks of the training program, rats from all groups were weighed after 12 h fasting and anesthetized for the collection of blood by cardiac puncture with a syringe and needle. Rats were restrained from food for 12 h but had access to drinking water. Some parameters such as steroid hormones are subject to circadian rhythm, therefore blood samples were collected at the peak time in the afternoon between 14.0-16.0 P.M. All animals were euthanized immediately after blood sampling. Plasma samples were separated immediately, frozen and stored until analysis.

2.5 Plasma analysis

Commercially available assay kits were used to determine the blood parameter levels.

Plasma lipid concentrations were measured enzymatically by commercially kits (Chol and TG by Greiner, Bahlingen, Germany; LDL by Pars Azmun, Tehran, Iran; and HDL by Randox, Antrim, UK).

Risk factor (RF) or a predictor of CHD risk was simply obtained as the TC/HDL-C ratio, and atherogenic index (AI) as the LDL/HDL ratio.

Plasma creatin kinase (CK) was measured by CK-NAC, Photometric Test using kit from Pars Azmun, Tehran, Iran.

The assays for plasma total testosterone (T), free testosterone (FT), and estradiol (E2) were performed by ELISA methods using reagent kits (Diagnostics Biochem Canada Inc., Ontario, Canada).

Plasma 25-Hydroxy vitamin D was measured by EIA method using reagent kit obtained from Immunodiagnostic System Ltd (IDS Ltd), Boldon, UK.

High-sensitivity C-reactive protein (hs-CRP) was measured by ELISA method using Kit from Biovendor Research and Diagnostics, Heidelberg, Germany.

2.6 Statistical analysis

Data are expressed as mean ± SD and the Statistical Package for the Social Sciences [(SPSS 17.0), New York: McGraw-Hill] was used to perform all comparisons. Analysis of variance (ANOVA) was used to evaluate the effects of training and treatments between the groups (determined by LSD test). A P-value of less than 0.05 was considered significant for the differences.

3 RESULTS

3.1 Feeding

The rats in the vibration groups adjusted to the vibration, and tolerated and acclimated well to the vibration training with no signs of stress after one week. Furthermore, no major differences was observed in the amount of food and water intake between the control and the treatment groups, both groups continued to consume food and

water normally (Table 1) and stayed healthy throughout the study; except for the lower amount of food intake in the AF group (15.5 g/d).

3.2 Body weight

The vibrated rats weighed more than the control group at the end of the study; except in the AF group, which showed lower body weight. They weighed approximately 15% less (Table 1).

3.3 Fatty acids intake

Table 2 compares the type and the amount of fatty acids available from various dietary oil sources with the relevant fatty acid ratio provided by the test fats.

3.4 Supplementation and plasma risk factors

The influence of different treatments on the selected risk factors is listed in Table 3. The comparison between the vibrated group (Gr. 2) with the control indicates that significant differences were only observed in higher plasma levels of creatin kinase, estradiol, and IL-6. The mean of vitamin D level was 15% higher; hsCRP level was 11% lower and IL-6 level was 32% higher in the vibration group.

The highest concentrations of cholesterol and LDL-C were present in groups 6 and 7; lower LDL-C in group 2; lower HDL-C in group 3; higher triglycerides in groups 5-7; lower AI and RF in group 2; and a high AI and RF in group 6, fed a mix of sunflower oil and canola oil. No significant change with AI and RF was noted in group 7 fed coconut oil. Significant increases of estradiol concentrations were present in groups 2 and 7. The higher levels of testosterone and free testosterone concentrations were observed when rat diet was supplemented with coconut oil (Gr. 7). The same trend was observed for the concentrations of vitamin D in the groups 2-6.

3.5 Comparisons on the no oil vs. the test oil groups

Table 4 is derived from Table 3 and shows the comparisons made between group 3 (SPM, receiving no oil) as the control group with the test oil groups (groups 4-7). The aim of presenting this table was to highlight the influence of oils on the study parameters. Significant differences were found in the plasma levels of cholesterol, LDL-C in groups 6 and 7; triglycerides (TG), C- reactive protein (hs-CRP), and HDL-cholesterol (HDL-C) in group 7; and atherogenic index (AI), and risk factor (RF) in group 6 were observed. The mix of two supplemented oils in group 6 created a hyperlipidemic state for plasma levels of choles-

Table 1 Food consumption, body weight of control and treatment groups.

	Control (1)	Sp (2)	SpM (3)	Co (4)	Af (5)	AC (6)	N (7)
Food Intake (g/d)	17.1	17.8	17.8	16.6	15.5	16.3	16.7
Body Weight (gr): 1st day	145.0 ± 10.0	149.0 ± 6.0	143.0 ± 8.0	151.0 ± 15.0	152.0 ± 14.0	155.0 ± 5.0	160.0 ± 12.0
4 weeks	229.0 ± 27.0	242.0 ± 34.0	230.0 ± 11.0	240.0 ± 22.0	227.0 ± 20.0	243.0 ± 15.0	253.0 ± 24.0
8 weeks	280.0 ± 31.0	306.0 ± 41.0	285.0 ± 21.0	296.0 ± 37.0	272.0 ± 24.0	293.0 ± 26.0	304.0 ± 29.0
Difference in Body Weight (gr)*	135.0 ± 21.0	157.0 ± 36.0	142.0 ± 13.0	145.0 ± 24.0	120.0 ± 11.0	137.0 ± 21.0	144.0 ± 18.0

* = Difference Between 1st day & 8 weeks

Table 2 Fatty acid composition of added oils in the experimental diets (mg/d).

Fatty Acid Content \ oil	Canola(4)	Sunflower(5)	Canola+ Sunflower(6)	Coconut(7)
total saturated(mg)	60	80	71	**713**
total monounsaturated(mg)	**523**	150	**332**	48
total polyunsaturated(mg)	232	**509**	**380**	15
Saturated: Monounsaturated: polyunsaturated Ratio	1:8.7:3.8	1:1.9:6.36	1:4.67:5.35	47.5:3.8:1

Table 3 Effects of exercise training and dietary supplementation on plasma parameters in the groups.

Variable \ Group	Control (1)	Sp (2)	SpM (3)	Co (4)	Af (5)	AC (6)	N (7)
Food Intake (g/day)	17.1	17.8	17.8	16.6	15.5	16.3	16.7
Diff in Body Wt (gr)	135.0 ± 21.0	157.0 ± 36.0[a]	142.0 ± 13.0	145.0 ± 24.0	120.0 ± 11.0[b]	137.0 ± 21.0	144.0 ± 18.0
Chol (mg/dl)	53.0 ± 7.0	52.0 ± 4.0	52.0 ± 7.5	58.0 ± 12.0	59.0 ± 11.0	67.0 ± 8.0[c]	68.0 ± 10.0[d]
LDL-c (mg/dl)	21.0 ± 4.0	18.0 ± 5.0[e]	23.0 ± 5.0	25.0 ± 8.0	26.0 ± 8.0	33.0 ± 5.0[f]	30.0 ± 6.0[c]
HDL-c (mg/dl)	17.0 ± 3.0	17.0 ± 3.0	14.0 ± 3.0[g]	16.0 ± 3.0	16.0 ± 3.0	16.0 ± 4.0	18.0 ± 3.0
TG (mg/dl)	73.0 ± 11.0	85.0 ± 14.0	74.0 ± 16.0	84.0 ± 14.0	87.0 ± 17.0[h]	87.0 ± 15.0[h]	92.0 ± 15.0[i]
hs-CRP (μg/ml)	321.0 ± 42.0	285.0 ± 77.0	308.0 ± 91.0	285.0 ± 33.0	297.0 ± 34.0	305.0 ± 25.0	358.0 ± 106.0[j]
CK (u/l)	188.0 ± 20.0	240.0 ± 37.0[kl]	220.0 ± 30.0[k]	209.0 ± 27.0	193.0 ± 37.0	218.0 ± 40.0	189.0 ± 27.0
T (ng/ml)	1.80 ± 0.40	1.80 ± 0.63	1.83 ± 0.77	2.04 ± 0.58	1.86 ± 0.65	1.76 ± 0.51	2.15 ± 0.75
FreeT (pg/ml)	0.44 ± 0.19	0.40 ± 0.17	0.38 ± 0.15	0.42 ± 0.16	0.39 ± 0.16	0.44 ± 0.24	0.52 ± 0.27
E2 (pg/ml)	8.35 ± 1.30	9.82 ± 2.2[m]	8.60 ± 0.90	8.30 ± 1.50	8.70 ± 0.94	8.80 ± 0.99	10.0 ± 0.88[n]
VitD (nmol/l)	91.0 ± 19.0	108.0 ± 22.0	107.0 ± 15.0	108.0 ± 15.0	109.0 ± 22.0	107.0 ± 20.0	87.0 ± 22.0
AI	1.30 ± 0.28	1.02 ± 0.31[o]	1.60 ± 0.40	1.60 ± 0.42	1.60 ± 0.55	2.10 ± 0.50[p]	1.70 ± 0.23
RF	3.20 ± 0.42	3.03 ± 0.48[o]	3.70 ± 0.69	3.80 ± 0.60	3.80 ± 0.97	4.30 ± 0.91[a]	3.80 ± 0.45

a) Statistically significant with 1
b) Statistically significant with 2,3,4,7
c) 〃 〃 1,2,3
d) 〃 〃 1,2,3,4
e) 〃 〃 4,5,6,7
f) 〃 〃 1,2,3,4,5
g) 〃 〃 7
h) 〃 〃 1
i) 〃 〃 1,3
j) 〃 〃 2,4
k) 〃 〃 1,7
l) 〃 〃 4,5
m) 〃 〃 1,4
n) 〃 〃 1,3,4,5
o) 〃 〃 3,4,5,6,7
p) 〃 〃 all
Non - significantly elevated

terol and LDL-C which may be due to the variation of the n-6: n-3 ratio. The estradiol concentration significantly increased and the testosterone and free testosterone concentrations were higher in group 7.

4 DISCUSSION

In its most basic form, exercise is any type of physical exertion performed in an effort to improve the health, shape the body and boost performance. Recently, whole body vibration has been proposed as a potential alternative, or adjuvant, to exercise[8]. A comprehensive review on the potential effects of WBV on several physiological systems is presented by Prisby *et al.* (2008)[2]. Findings from human studies confirm the effectiveness of WBV in improving health status such as improving pain and fatigue in women with fibromyalgia[25], reducing the risk of bone fracture more than walking[26], increasing the serum levels of testosterone and growth hormone[27], and representing no stressful stimulus for the neuroendocrine and neuromuscular systems[27, 28]. Further, it would be well-advised to determine the influence of WBV on CVD risk factors. In our study, the rats were healthy and treated well with the vibration, and showed no signs of distress. The WBV group (Gr. 2) weighed approximately 14% more than control group after 8 weeks (weight gain: 135.0 vs 157.0 g), while both groups consumed similar amounts of food (17.1 vs 17.8 g/d). Although the mechanism responsible for the increased body weight was not studied, it is assumed that the observed body weight gain may be the result of higher muscle mass and bone mass and/or the result of higher blood flow leading to the alteration on the peripheral vascular and tissue perfusion. Maddalozzo et al. (2008) reported that mature female vibrated rats weighed 10% less, with less body fat and serum leptin concentrations[8]. Interestingly, in the AF group (Gr. 5) lower body weight was recorded despite added 5% sunflower oil in the diet. In a study, Roelants *et al.* (2004) reported a significant increase in fat-free mass and strength with whole-body vibration[6].

This effect of sunflower oil seems to be mediated by a reduction of appetite or an increase in the feeling of satiety or partially by hormonal variations. Additionally, rats in this group consumed 12% less food.

A lower body weight observed in the group 3 (SPM) compared to that in the group 2 (SP) could be attributed to the supplementation of vitamin D, calcium and boron, and these findings require further clarification. The cholesterol

Table 4 Comparison of the effects of exercise training and dietary supplementation on plasma parameters in the vibration group and the test oil groups.

Variable \ Group	Control (1) (sham)	SpM (3)	Co (4)	Af (5)	AC (6)	N (7)
Food Intake(g/day)	17.1	17.8	16.6	15.5	16.3	16.7
Diff in Body Wt (gr)	135.0 ± 21.0	142.0 ± 13.0	145.0 ± 24.0	120.0 ± 11.0[a]	137.0 ± 21.0	144.0 ± 18.0
Chol(mg/dl)	53.0 ± 7.0	52.0 ± 7.5[b]	58.0 ± 12.0[c]	59.0 ± 11.0	67.0 ± 8.0	68.0 ± 10.0
LDL-c(mg/dl)	21.0 ± 4.0	23.0 ± 5.0	25.0 ± 8.0	26.0 ± 8.0	33.0 ± 5.0[d]	30.0 ± 6.0[e]
HDL-c(mg/dl)	17.0 ± 3.0	14.0 ± 3.0	16.0 ± 3.0	16.0 ± 3.0	16.0 ± 4.0	18.0 ± 3.0[f]
TG(mg/dl)	73.0 ± 11.0	74.0 ± 16.0	84.0 ± 14.0	87.0 ± 17.0	87.0 ± 15.0	92.0 ± 15.0[f]
Hs-CRP(μg/ml)	321.0 ± 42.0	308.0 ± 91.0	285.0 ± 33.0	297.0 ± 34.0	305.0 ± 25.0	358.0 ± 106.0[g]
CK(u/l)	188.0 ± 20.0	220.0 ± 30.0	209.0 ± 27.0	193.0 ± 37.0	218.0 ± 40.0	189.0 ± 27.0
T (ng/ml)	1.80 ± 0.40	1.83 ± 0.77	2.04 ± 0.58	1.86 ± 0.65	1.76 ± 0.51	2.15 ± 0.75
FreeT(pg/ml)	0.44 ± 0.19	0.38 ± 0.15	0.42 ± 0.16	0.39 ± 0.16	0.44 ± 0.24	0.52 ± 0.27
E2(pg/ml)	8.35 ± 1.30	8.60 ± 0.90	8.30 ± 1.50	8.70 ± 0.94	8.80 ± 0.99	10.0 ± 0.88[h]
VitD(nmol/l)	91.0 ± 19.0	107.0 ± 15.0	108.0 ± 15.0	109.0 ± 22.0	107.0 ± 20.0	87.0 ± 22.0
AI	1.30 ± 0.28	1.60 ± 0.40	1.60 ± 0.42	1.60 ± 0.55	2.10 ± 0.50[i]	1.70 ± 0.23[j]
RF	3.20 ± 0.42	3.70 ± 0.69	3.80 ± 0.60	3.80 ± 0.97	4.30 ± 0.91[a]	3.80 ± 0.45

a) Statistically significant with 3, 4, 7
b) Statistically significant with 6,7
c) 〃 〃 7
d) 〃 〃 3, 4, 5
e) 〃 〃 3,5
f) 〃 〃 3
g) 〃 〃 4
h) 〃 〃 3,4,5,6
i) 〃 〃 3,4,5,7
j) 〃 〃 5

levels in the group 6 were significantly higher compared to the groups 1-3, possibly due to the imbalance of the fatty acids in this group leading to the equal ratio of omega-3 and omega-6 (ratio 1:1). A mix of sunflower oil and canola oil contains less saturated fatty acids compared to coconut oil and naturally has no cholesterol, but it exerts the same impacts on increasing the cholesterol level similar to the coconut oil. This suggests that maintaining on the proper ratio of the fatty acids has important nutritional significant, and in particular in the management of the CVD risk factors.

Moreover, the higher cholesterol level seen in group 7 compared to the groups 2-4 indicated that saturated chains in coconut oil are responsible for this increased level. No significant differences were noted in the AI and RF measurements for coconut oil versus the control and SPM groups, suggesting no risk for an unusual blend of short and medium chain fatty acids content in coconut oil[21]. It was reported that supplementation with coconut oil did not cause dyslipidemia[23], and was not a predictor for CHD in a food culture study[19]. The antioxidant properties of virgin coconut oil due to phenolic compounds has been reported[22], but it is recommended not be used on a regular basis in adults.

Among the different dietary oils, canola accounted for lower cholesterol level in these groups, while Miettinen *et al.* (1995) reported that dietary plant sterols, especially sitostanol, present in canola oil are responsible for this effect[29].

A significantly lower LDL-C level was noted in the group 2 (Sp) compared to the groups 4-7. The findings suggest that the test fats increased the LDL-C levels; and a mix of sunflower oil and canola oil induced higher LDL-C, even higher than coconut oil. Similar findings for cholesterol with regard to AI and RF measures were also observed and these indices in the group 6 (AC) were not significant compared to the control and group 3 (SpM).

Lower risk factors were mainly observed with consumption of canola oil and sunflower oil, separately. The HDL-C levels were higher after feeding different oils and the level in coconut group compared to the group 3 (SpM) increased significantly. The triglycerides levels in the groups 5 (AF (and 6 (AC) were significantly higher than the control. Coconut oil consumption resulted to the significantly higher TG in the group 7 compared to the control and group 3 (SpM).

The results suggest that as a result of higher activity in the group 2 (Sp) compared to the control, a higher triglycerides content is released into the plasma to provide the energy source of the activity. Lower levels observed in the group 3 versus the group 2 may be due to the effect of vitamin D, calcium and boron consumption, which deserves

further investigation. A significant increase in hs-CRP levels in the group 7 compared to the groups 2 and 4 might be the result of lower vitamin D levels observed with coconut oil consumption. Similarly, Melamed *et al.*(2008) reported a relation between low vitamin D with high hs-CRP levels[30].

It is reported that the vitamin E content of the sunflower oil enhances the defensive antioxidant systems against oxidative stress and thus reduces the hs-CRP level[31]. Moreover, boron is reported to have antioxidant and anti-inflammatory properties, resulting to lower hs-CRP[32]. The lower levels of hs-CRP as a result of WBV activity was noted in all the groups except the group 7. The mechanism of low hs-CRP due to the physical activity is not confirmed, but changes in secretion of inflammatory cytokines to anti-inflammatory ones like IL-10[33], and lower LDL-C oxidation might be related to this effect.

Whole body vibration was shown to increase creatine kinase(CK)involved in muscle damage and levels in the vibrating group 2(Sp). It is assumed that increases in pro-inflammatory cytokines appear to be the result of an overtraining protocol.

Circulating creatine kinase(CK)levels are often monitored as an indirect biomarker of muscle damage after resistive exercise have been shown to increase after infrequent exercise, typically involving eccentric actions[34]; and both moderate exercise(60% VO(2)max)and intensive exercise(75% VO(2)max)significantly increased IL-6, CK, CRP and white blood cells(WBC), as well[35]. However, consumption of test oils with vitamin D, calcium and boron resulted in lower Ck levels, particularly in coconut group.

The mean testosterone and free testosterone levels was 15% higher in the group 7 which is consistent with the result of Hurtado de catalfo *et al.*(2008). They reported that a coconut oil diet produced a high testicular level of antioxidants, testosterone, 3 beta- or 17 beta-hydroxysteroid dehydrogenase enzymes[36]. The activity of these two key enzymes involved in testosterone biosynthesis is enhanced by coconut oil[37].

A significant higher concentration of estradiol was found in the group 7 in comparison with the groups 3, 4 and 6. The increase in the group 2 compared to the control and group 4 seems to be mediated by the vibration, alone. In general, estradiol concentration showed an increased level in most oil test groups and in particular after coconut consumption. Additionally, estradiol production is reported to be influenced by boron supplementation[32].

The 15% higher level of vitamin D found in all treatment groups except group 7 seems to be the result of vitamin D and boron intake. The reported vitamin D production by boron[38]is consistent with the finding of our study. Vitamin D is best known for its effects on calcium regulation, but lately its beneficial effects on vascular health and blood pressure have been recognized and a low level is associated with higher risk of myocardial infarction[39].

5 CONCLUSION

Since whole-body vibration training is low impact, it may be a particularly good choice for people who have trouble doing traditional weight-bearing exercise.

It is now assumed that evaluation of the effect of WBV on body weight and biochemical parameters requires a long-term study in which body composition consisting of fat, muscle and bone mass, or metabolic factors in normal, overweight and obese human and animal models needs to be determined. The parameters measured under the influence of vibration in this study were mostly within normal levels, indicating that the potential effects of physiological responses of WBV on several physiological systems are without deteriorations. These findings further promote the eliciting of scientific inquiries into this potentially therapeutic aid through further investigations to determine the optimal frequency, duration, amplitude, and appropriate protocols.

Besides physical activity, other risk factors for CVD are well-established and it is known that dietary changes and other lifestyle alterations can lower the risk of developing CVD and can delay its progression in patients with established disease. Healthy diets and more physical activity have been proven to reduce cardiovascular risk significantly. In the current study, a simultaneous combination effect of the known factors consisting of different fatty acids, vitamin D, Calcium and boron plus physical activity has been determined. Overall, dietary oil supplementations appeared to have beneficial effects on most of the analytical parameters. The more evident changes particularly on lipid profile, testosterone and vitamin D were mediated by canola oil consumption. Less food intake was noticed in the sunflower group. Despite the high content of saturated fat in coconut oil, some health benefits were identified, which makes it as a better choice than other saturated fats, if consumed in moderation. However, there is further agreement that more research is needed regarding this issue and its relationship to cardiovascular health.

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