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Effect of Oat Bran Consumption and Brisk Walking Exercise on Immune Functions Parameters in 40 to 50 Years Old Hypercholesterolemic Women

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ABSTRACT

Moderate physical activity and adequate nutritional intake are believed can enhance immune function of an individual. We aimed to investigate the additional beneficial effects of combined oat bran consumption and brisk walking exercise compared to oat bran consumption alone on immune functions parameters in hypercholesterolemic women. Thirty three hypercholesterolemic women participants aged 40-50 years old were recruited and being assigned into three groups, with eleven participants per group (n=11): sedentary without oat bran consumption control (C), oat bran consumption alone (Ob), and combined oat bran consumption and brisk walking exercise (ObEx) groups. Participants in ObEx group performed brisk walking exercise sessions 30 minutes per session, 3 sessions per week for 6 weeks. Participants in Ob group and ObEx group consumed 18 gram of oat bran powder, 7 days per week for 6 weeks. Participants' anthropometry and immune functions parameters which include full blood counts and immunophenotyping measurements were measured at pre- and post tests. Repeated measures ANOVA was performed for statistical analysis. There were significant ($p<0.05$) increases in eosinophil and neutrophil counts in post test compared to pre test in Ob group. There were also significant ($p<0.05$) decreases in values of T cytotoxic ($CD8^+$) and natural killer cells ($CD16^+$) in post-test compared to pre-test in ObEx groups. Six weeks of oat bran consumption alone may have potential to enhance immune functions in 40 to 50 years old hypercholesterolemic women. Nevertheless, future study with larger sample size needs to be carried out to confirm the present study findings.

Keywords: *oat bran, brisk walking, immune functions, hypercholesterolemic women*

INTRODUCTION

One of the major problems in elderly is their tendency to infection is higher, particularly due to immunological insufficiencies (Kuroiwa et al., 2004). According to Nieman (2011), elderly are more susceptible to vaccine failure and many infections, auto-immune disorders, and cancers when compared

with younger adults. Exercise such as brisk walking, jogging, jumping and resistance training is highly recommended to improve immune. Short-duration, moderate-to-vigorous exercise which lasting less than 60 minutes has been considered as a valuable complement to the immune system (Nieman & Wentz, 2019). A previous study on the influence of moderate exercise training on immune function reported that daily brisk walking can reduce the number of sickness days by half over a 12 to 15-week period compared to inactivity (Nieman & Pedersen, 1999).

Besides exercise, immune functions also can be affected by nutritional status of an individual, especially elderly. Insufficient intake or deficiencies in minerals and vitamins have been shown to detrimentally impact immune responses (Shao et al., 2021). It is believed that oat bran is one of the good nutritional supplementation for immune functions. It is known as good source of B-complex vitamins, fat, soluble fiber β-glucan (Butt et al., 2008) and also contains macronutrients and minerals such as protein, magnesium, zinc and iron which are important for immune function (Biogrow Company, 2015). Oat bran has total β-glucan and dietary fibre not less than 5.5 and 16.0% respectively with at least one-third of total dietary fiber is soluble fiber (American Association for Clinical Chemistry, 1989). β-glucan in oat plays an important role in improving immunity and prevention against diseases (Hong et al., 2004; Liang et al., 1998; Volman et al., 2008; Yun et al., 2003). According to Pike and Chandra (1995), supplementation with vitamin and trace elements can plays an important role in the maintenance of normal immune function in the elderly. It has capacity to enhance the immune functions of an organism by elevating immunoglobulin, NK cells, killer T-cells and boost the resistance to infectious and parasitic diseases of an individual (Daou & Zhang, 2012). Table 1 illustrates the nutrition facts of the oat bran powder used in the present study.

Table 1: Nutrition facts of oat bran powder

	Per Serving (1 sachet ≈ 9g)	*Per 100 g*
Energy	114 kJ	1274 kJ
Calories	27 kcal	303 kcal
	0.3 g	3.2 g
Total Fat	0.1 g	1.4 g
Monounsaturated Fat	0.1 g	1.2 g
Polyunsaturated Fat	< 0.1 g	0.6 g
Saturated Fat	0.0 g	0.0 g
Trans Fat		
	2.7 g	30.5 g
Carbohydrate	0.3 g	3.2 g
Total Sugars		
	3.7 g	40.7 g
Total Dietary Fiber	1.8 g	20.0 g
*of which **beta-glucan** soluble fiber*		
	1.7 g	18.6 g
Protein	23 mg	260 mg
Magnesium (Mg)	0.8 mg	8.4 mg
Iron (Fe)	0.5 mg	5.5 mg
Zinc (Zn)	< 1 mg	7 mg
Sodium (Na)		

Adapted from Biogrow Company (Biogrow Company, 2015)

It has been reported that individuals with hypercholesterolemia are associated with immune function impairments. Han et al. (2003) mentioned that hypercholesterolemia contribute to impaired immune response. In their study, it was found that consumption of a low fat diet can enhance the cellular immune response in older adults with elevated low density lipoprotein (LDL) cholesterol levels. Consumption of a low fat diet significantly improved T cell-mediated immune response while it had no effect on B cell

function or production of proinflammatory mediators in older adults with moderate hypercholesterolemia. Furthermore, a previous study on animal by Martens et al. (2008) reported that hypercholesterolemia increases the tuberculosis susceptibility in mice. The mice are more susceptible to tuberculosis due to elevated cholesterol level which delayed the expression of adaptive immunity to tuberculosis.

To date, studies on the effects of oat bran consumption on immune functions are lacking. Additionally, information are also lacking on the additional beneficial effects of combined oat bran consumption and brisk walking exercise compared to oat bran consumption alone on immune functions. Therefore, the present study was proposed to investigate the combined effects of oat bran consumption and brisk walking exercise with moderate intensity on immune functions in hypercholesterolemic women. The findings of the present study can be proposed as a guideline in planning exercise and nutritional programmes.

METHODS

Participants

Thirty three adult women participants were involved in this study. Participants were screened in order to determine the inclusion criteria. Inclusion criteria of participants were physically healthy volunteers who were free from any chronic diseases, hypercholesterolemia with total cholesterols ranged between 5.2 to 7.0 mmol/L, non-smokers and with age between 40 to 50 years old. The exclusion criteria were individuals who had the habit of taking oat bran as daily consumption prior to the study period, engaged in any training programme and exercised more than once per week. This study was approved by the human research ethic committee of Universiti Sains Malaysia (JEPeM Code: USM/JEPeM/15100389).

Participants Grouping

Participants were randomly assigned into three groups with 11 participants per group: sedentary without oat bran consumption control group (C), oat bran consumption alone group (Ob) and combined brisk walking exercise with oat bran consumption group (ObEx). Participants in the control group (C) did not perform brisk walking exercise nor having oat bran consumption for 6 weeks. Meanwhile, participants in oat bran consumption alone group (Ob) consumed 18g of oat bran per day without performing brisk walking exercise for 6 weeks. Participants in combined oat bran consumption with brisk walking exercise consumed 18g of oat bran per day for 6 weeks and performed brisk walking exercise, 30 min per session, 3 sessions per week for 6 weeks.

Brisk Walking Exercise Program

The participants in brisk walking exercise with oat bran consumption (ObEx) group were required to perform brisk walking exercise with 30 minutes per sessions (from 6.00 p.m. to 6.30 p.m.), three sessions per week for six weeks. In each brisk walking exercise session, participants warmed up with stretching activities for five minutes and then followed by brisk walking for 30 minutes, and ended with cooling down with stretching activities for five minutes. The estimated walking distance covered was approximately 2.5 km. The exercise intensity during brisk walking was set at 55% to 70% of the participants' age-predicted heart rate maximum (HRmax) (HRmax=220-age), i.e. the targeted range of exercise heart rate for moderate intensity exercise. Heart rate monitors (Polar watch) were worn by participants throughout the brisk walking sessions. In order to ensure that the exercise intensity was maintained within the targeted range, participants were required to record their post exercise heart rate at the end of the brisk walking session. If the walking pace did not elicit a heart rate within targeted exercise heart rate, the participants were requested to change their pace during the subsequent walking session. The brisk walking programme was carried out at the jogging track in the Health Campus of Universiti Sains Malaysia and under the supervision of the researcher. The attendance of the participants during each brisk walking session was recorded by the researcher in order to ensure that they have complied with the exercise programme. The average attendance of the participants in the exercise group was 99.1 ± 3.0%.

Oat Bran Supplementation

The participants in both oat bran consumption alone group (Ob) and combined brisk walking exercise with oat bran consumption group (ObEx) consumed oat bran supplementation with two sachets of oat bran powder (18g of oat bran powder containing 3.6 grams of β glucan) diluted with plain water per day, 7 days per week for 6 weeks. The participants were required to consume one sachet of oat bran powder which was mixed with 250ml of plain water before breakfast, and another one sachet of oat bran powder which was mixed with 250ml of plain water before lunch or dinner. On the exercise days, the participants in ObEx group were required to consume oat bran one hour before brisk walking exercise.

Measurements Of Anthropometric Parameters

Anthropometric parameters such as body height and body weight were measured. Body height was measured by using a stadiometer (Seca 220, Germany). Body weight was measured by a body composition analyser (Tanita, model TBF – 410). Participants were required to be shoeless and wore minimal clothes during these measurements.

Blood Sample Collection And Analysis

Six ml of blood samples were taken immediately before and after the six weeks of experimental period in the morning after 10 hours overnight fast and drinking plain water was allowed. Blood sample were drawn from the antecubital vein of the participants. Blood taking sessions for participants in ObEx in post test were carried out at 8.30 a.m. the next morning after performing brisk walking exercise, i.e. 14-h post exercise.

Full blood counts of white blood cells, neutrophils, basophils, eosinophils, monocytes and total lymphocytes were analysed by using an automated hematology analyser (Sysmex XS-800i, USA). Meanwhile, the concentration of blood immunophenotyping parameters, i.e. natural killer (NK) cells, total T lymphocyte ($CD3^+$), T helper ($CD4^+$) cells and T cytotoxic ($CD8^+$) cells were analysed by flow cytometer (BD FACS Cantor™ II, Bector Dickinson, USA) by using reagent kit (BD MultiTEST™ IMK Kit, USA).

Statistical Analysis

Data were analysed using the statistical software in the Statistical Package for Social Science (SPSS) Version 22.0. All data are expressed as means and standard deviation (SD). Repeated measure ANOVA was performed to determine the significance of the difference between and within groups. Statistical significance was accepted at $p<0.05$.

Calculation of sample size

The sample size for the present study was determined using PS Power and Sample Size Calculation version 3.0.43. Referencing a prior study by Liew et al. (2013), the study power was set at 80% with a 95% confidence interval. The standard deviation (SD) observed was 0.35 x 10^3 cells/μL of total lymphocyte counts, and the difference in population means was set at 0.45 x 10^3 cells/μL of total lymphocyte counts. The calculated sample size was 11 participants per group. Since this study included three groups, a total of 33 participants were recruited.

RESULTS & DISCUSSION

Participant Physical Characteristics

A total of thirty three participants with mean age: 45±4.0 years, mean body weight: 66.21±13.51 kg and mean body height: 153.74±5.03 cm completed the study. The mean age of the participants in C, Ob and ObEx group was 44.64±4.06, 45.27±4.71 and 45.45±3.50 years respectively. At pre test, the participants' mean body weight in C, Ob and ObEx was 59.83±9.32 kg, 67.69±13.95 kg and 71.10±15.14 kg respectively, meanwhile, the mean body height in C, Ob and ObEx was 153.50±5.90 cm, 153.71±5.39 cm

and 154.00±4.12 cm respectively. There were no significant differences of mean body weight and body height between all the three groups at pre test.

Immune Function Parameters

White blood cell, monocyte, eosinophil and neutrophil counts

Means white blood cell and monocyte counts of all the groups are presented in Table 2. There were no significant differences in these parameters among all the experimental groups at pre test and post test. Similarly, there were also no significant differences in these parameters between pre- and post tests in all the experimental groups.

After 6 weeks of the experimental period, eosinophil counts in Ob group significantly increased ($p=0.042$) compared to pre test value as presented in Figure 1. The percentage increase of eosinophil counts in Ob group was the highest (+45.0%) among the groups. Neutrophil counts in Ob group significantly increased ($p=0.017$) in post test compared to pre test value as presented in Figure 2.

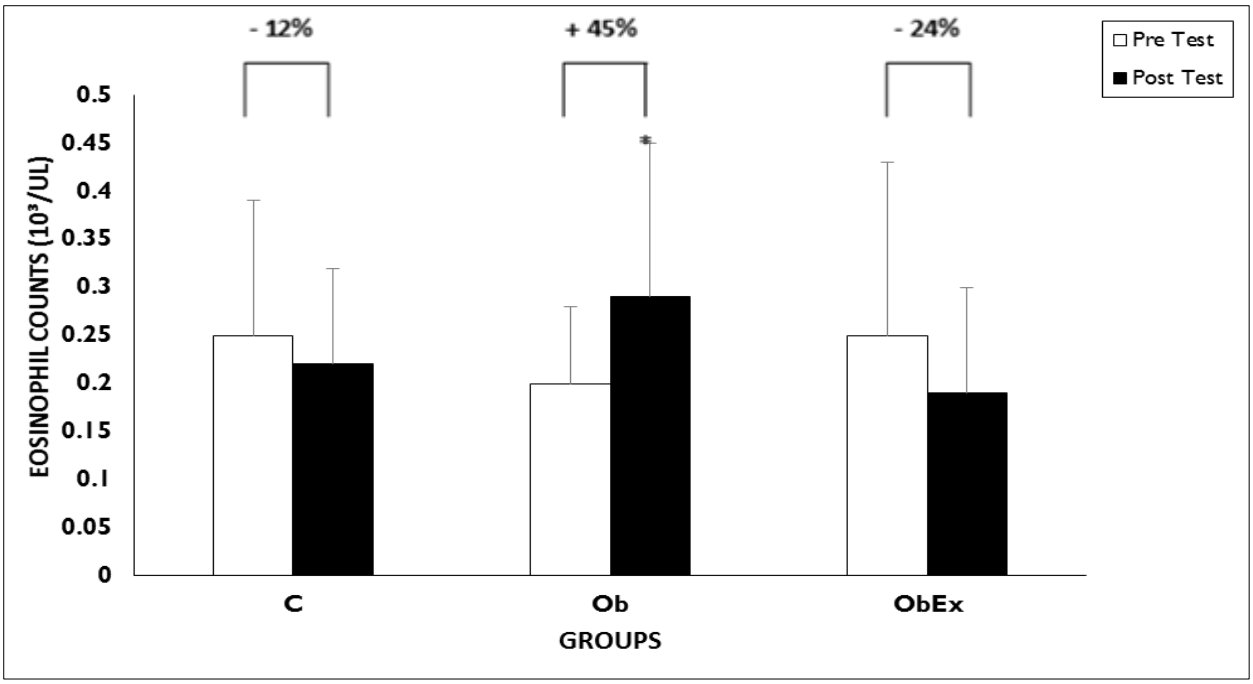
Table 2: Means white blood cell and monocyte counts at pre- and post tests.

Groups	White blood cell counts (10^3/μL) (Mean±SD)			Percent difference compared to pre test (%)	Monocyte counts (10^3/μL) (Mean±SD)			Percent difference compared to pre test (%)
	Pre Test	Post Test	Mean difference between pre- and post tests		Pre Test	Post Test	Mean difference between pre- and post tests	
C	6.52 ± 1.95	6.91 ± 1.84	0.40 ± 1.01	+6.0	0.42 ± 0.11	0.47 ± 0.17	0.05 ± 0.12	+11.9
Ob	6.42 ± 1.47	7.06 ± 1.75	0.63 ± 1.45	+10.0	0.42 ± 0.13	0.46 ± 0.17	0.05 ± 0.17	+9.5
ObEx	6.54 ± 1.70	6.80 ± 2.21	0.25 ± 1.40	+4.0	0.50 ± 0.11	0.48 ± 0.15	-0.01 ± 0.10	-4.0

*, $p<0.05$ significantly different from pre test

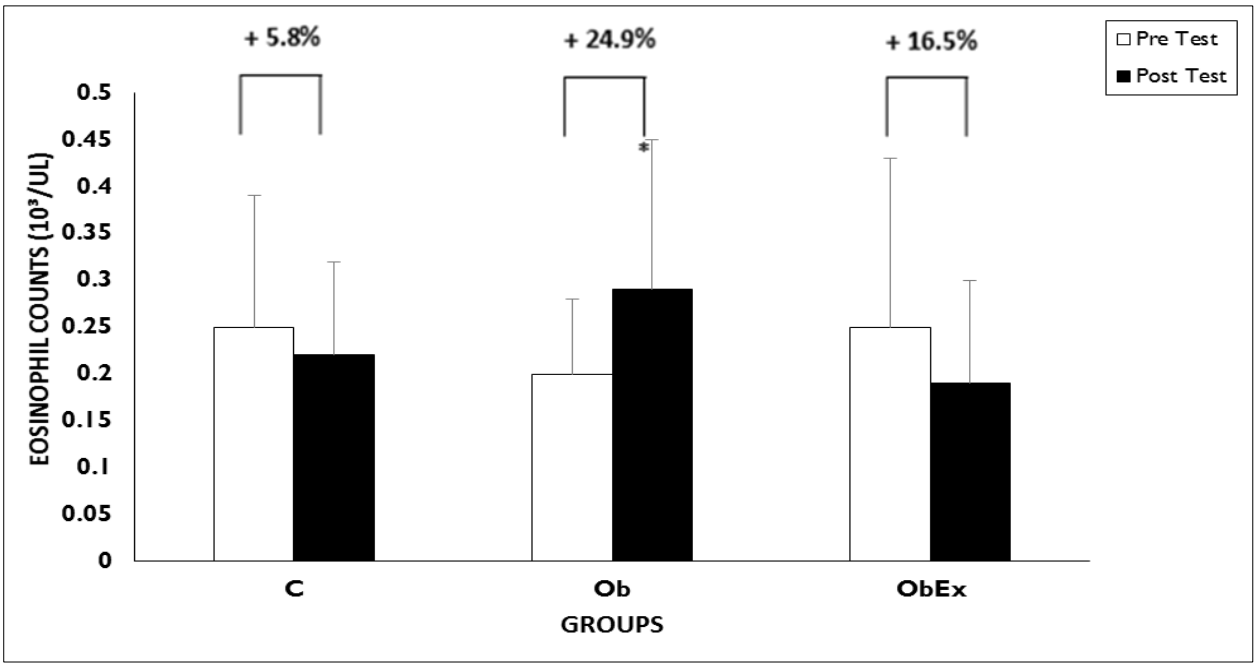
Abbreviations: C, control group; OB, oat bran supplementation alone group; ObEx, combined oat bran supplementation and exercise group

Figure 1: Means eosinophil count at pre- and post tests.



*, p<0.05 significantly different from pre test
Abbreviations: C, control group; OB, oat bran supplementation alone group; ObEx, combined oat bran supplementation and exercise group.

Figure 2: Means neutrophil count at pre- and post tests.



*, p<0.05 significantly different from pre test
Abbreviations: C, control group; OB, oat bran supplementation alone group; ObEx, combined oat bran supplementation and exercise group.

Basophil, total lymphocyte, T lymphocyte ($CD3^+$) and T helper ($CD4^+$) counts

Means basophil, total lymphocyte, T lymphocyte ($CD3^+$) and T helper ($CD4^+$) count of all groups are presented in Table 3. At pre- and post tests, there were no significant differences in all these parameters among all the experimental groups. After 6 weeks of the experimental period, there were also no significant differences in these parameters between pre- and post tests in C, Ob and ObEx groups.

T cytotoxic ($CD8^+$) and natural killer cell ($CD16^+$) counts

Means T cytotoxic ($CD8^+$) and natural killer cell ($CD16^+$) absolute count of all groups are presented in Table 4. At pre test, T cytotoxic ($CD8^+$) absolute counts were significantly higher in ObEx group compared to Ob group ($p=0.049$). At post test, there were no significant differences in T cytotoxic ($CD8^+$) absolute counts among all the experimental groups. After 6 weeks of the experimental period, T cytotoxic ($CD8^+$) absolute counts in ObEx group decreased significantly ($p=0.027$) compared to pre test value. The percentage decrease of T cytotoxic ($CD8^+$) absolute counts in ObEx group was the highest (-11.1%) among the groups.

Table 3: Means basophil, total lymphocyte, T lymphocyte ($CD3^+$) and T helper ($CD4^+$) count at pre- and post tests

Groups	Basophils counts ($10^3/\mu L$) (Mean±SD)			Percent difference compared to pre test (%)	Total lymphocyte counts ($10^3/\mu L$) (Mean±SD)			Percent difference compared to pre test (%)
	Pre Test	Post Test	Mean difference between pre- and post tests		Pre Test	Post Test	Mean difference between pre- and post tests	
C	0.03 ± 0.02	0.03 ± 0.02	0.00 ± 0.01	0.0	2.33 ± 0.50	2.47 ± 0.49	0.13 ± 0.40	+6.0
Ob	0.03 ± 0.01	0.02 ± 0.01	0.00 ± 0.01	-33.3	2.42 ± 0.50	2.30 ± 0.61	-0.12 ± 0.40	-5.0
ObEx	0.03 ± 0.01	0.03 ± 0.01	0.00 ± 0.01	0.0	2.79 ± 0.63	2.62 ± 0.74	-0.17 ± 0.33	-6.1

Groups	Total T lymphocyte ($CD3^+$) absolute counts ($cells/mm^3$) (Mean±SD)			Percent difference compared to pre test (%)	T helper ($CD4^+$) absolute counts ($cells/mm^3$) (Mean±SD)			Percent difference compared to pre test (%)
	Pre Test	Post Test	Mean difference between pre- and post tests		Pre Test	Post Test	Mean difference between pre- and post tests	
C	1554.49 ± 400.46	1641.79 ± 373.65	87.30 ± 253.87	+5.6	860.09 ± 201.71	873.70 ± 159.49	13.62 ± 161.29	+1.6
Ob	1552.53 ± 409.35	1481.94 ± 458.24	-70.59 ± 271.52	-4.5	974.13 ± 258.63	912.32 ± 310.99	-61.81 ± 180.20	-6.3
ObEx	1615.29 ± 379.71	1464.59 ± 337.09	-150.70 ± 114.94	-9.3	875.07 ± 163.26	875.70 ± 181.08	0.62 ± 96.92	+0.1

*, $p<0.05$ significantly different from pre test

Abbreviations: C, control group; OB, oat bran supplementation alone group; ObEx, combined oat bran supplementation and exercise group.

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Table 4: Means T cytotoxic ($CD8^+$) and natural killer cell ($CD16^+$) absolute count at pre- and post tests

Groups	T cytotoxic ($CD8^+$) absolute counts ($cells/mm^3$) (Mean±SD)			Percent difference compared to pre test (%)	Natural killer cell ($CD16^+$) absolute counts ($cells/mm^3$) (Mean±SD)			Percent difference compared to pre test (%)
	Pre Test	Post Test	Mean difference between pre- and post tests		Pre Test	Post Test	Mean difference between pre- and post test	
C	525.34 ± 183.70	572.38 ± 184.15	47.04 ± 94.53	+9.0	426.37 ± 152.27	422.92 ± 114.19	3.45 ± 145.50	-0.81
Ob	447.88 ± 124.04	434.97 ± 153.88	-12.90 ± 92.81	-2.9	458.74 ± 163.62	438.55 ± 131.12	20.19 ± 129.54	-4.40
ObEx	623.70 ± 245.58$^{\#}$	554.74 ± 220.56*	-68.95 ± 93.77	-11.1	651.45 ± 243.04$^{+,\#}$	559.68 ± 205.82$^{*,+}$	91.76 ± 138.92	-14.09

*, $p<0.05$ significantly different from pre test
$^{+}$, $p<0.05$ significantly different from respective control (C) group
$^{\#}$, $p<0.05$ significantly different from respective Ob group
Abbreviations: C, control group; OB, oat bran supplementation alone group; ObEx, combined oat bran supplementation and exercise group.

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At pre test, natural killer cell ($CD16^+$) absolute counts were significantly higher in ObEx group compared to Ob group ($p=0.024$) and C group ($p=0.010$) respectively. At post test, natural killer cell ($CD16^+$) absolute counts were significantly higher in ObEx group compared to C group ($p=0.048$). After 6 weeks of the experimental period, natural killer cell ($CD16^+$) absolute counts in ObEx group decreased significantly ($p=0.035$) compared to pre test value. The percentage decrease of natural killer cell ($CD16^+$) absolute counts in ObEx group was the highest (-14.09%) among the groups.

DISCUSSION

One of the notable findings in immune function parameters of the present study was that eosinophil and neutrophil were significantly increased in Ob group compared to pre test. In eosinophil, the percentage increase was the highest in Ob group among the groups. Meanwhile, for the neutrophil, Ob group also exhibited the highest percentage increase among the groups. These findings imply that oat bran alone may elicit greater beneficial effects than combined ObEx and sedentary without oat bran consumption in increasing eosinophil and neutrophil counts. The increasing of neutrophil and eosinophil counts in the present study are consistent with two previous studies which reported the beneficial effects of oat bran β-glucan on immune functions (Volman et al., 2008; Yun et al., 2003). Conversely, inconsistent with the present study, Davis et al. (2004) found that oat β-glucan did not elicit significant effects on the immune system either alone or combined with exercise. The present study also observed that there were no significant changes in white blood cells, monocytes, basophils, total lymphocytes, T lymphocytes ($CD3^+$), T helpers ($CD4^+$), T cytotoxics ($CD8^+$) and natural killer cells ($CD16^+$) in the oat bran alone group. These findings reflect that β-glucan contained in the oat bran can elicit beneficial effects in elevating neutrophil and eosinophil counts but not another measured blood parameters of immune function in 40 to 50 years hypercholesterolemic old women of this study.

Neutrophils and eosinophils are granulocyte that plays prominent role in immune functions of individuals (Chaplin, 2010). According to Hong et al. (2004), β-glucan can enhance stimulation of granulocytes such as neutrophils and eosinophils to kill tumor cells in mice. Liang et al. (1998) also reported that β-glucan exhibits high absolute neutrophil counts and can enhance neutrophil oxidative burst responses in rats. The present study findings have supported the above statements. The present observation of eosinophils and neutrophils were significantly increased in Ob group after 6 weeks of supplementation period showed that oat bran which contained β-glucan can increase eosinophil and neutrophil counts, implying that β-glucan contained in oat bran in the present study has played its role in increasing immune function of 40 to 50 years hypercholesterolemic old women.

It was hypothesised that when exercise and oat bran consumption combined together in the present study, this combination may enhance immune function of the participants greater than oat bran consumption alone and sedentary without oat bran consumption. However, this study found that there were decreases in T cytotoxics ($CD8^+$) and natural killer cells ($CD16^+$) in combined oat bran consumption and brisk walking exercise group (ObEx). This was based on the evidence that T cytotoxics ($CD8^+$) and natural killer cells ($CD16^+$) in ObEx group significantly decreased in post test compared to pre test. In both T cytotoxic ($CD8^+$) and natural killer cells ($CD16^+$), ObEx also showed the highest percentage decreases among the groups. These findings did not support our hypothesis. The present findings was inconsistent with two previous studies which reported the combined effect of exercise with nutritional supplementation. For instances, Ooi et al. (2015) reported that no significant changes were observed in T cytotoxic ($CD8^+$) in adult male participants performing circuit training exercise combined with Eurycoma longifolia jack supplementation. Another previous study by Mohamed and Ooi (2013) showed that combination of honey supplementation and circuit training exercise increase natural killer cells ($CD16^+$) in young male participants. This discrepancy could be attributed to differences in study design such as gender and age of the participants, nutritional supplementation, and type of exercise prescribed. As example, the participants recruited by Ooi et al. (2015) were 35-55 years old men who consumed Eurycoma longifolia jack supplementation and performed circuit training programme. Meanwhile, participants recruited by Mohamed and Ooi (2013) were 19-29 years old males who consumed honey supplementation and performed circuit training programme. However, in the present study, 40-50 years old hypercholesterolemic women were recruited, oat bran drink was given as supplementation and brisk walking exercise was prescribed.

In this study, we observed that there were significant changes in eosinophils and neutrophils with oat bran supplementation alone, however we also observed that there were no significant changes in white blood cells, total lymphocytes, eosinophils, neutrophils, monocytes, basophils, T lymphocytes ($CD3^+$) and T helpers ($CD4^+$) in combined ObEx group in post test compared to pre test. Regarding effect of moderate exercise on the immune system, Nieman (2019) mentioned that each exercise bout enhances the antipathogen activity of tissue macrophages in parallel with an increased recirculation of immunoglobulins, anti-inflammatory cytokines, neutrophils, NK cells, cytotoxic T cells, and immature B cells. Furthermore, a previous study on animal by Davis et al. (2004) also supported that moderate exercise can enhance immune function and lower the susceptibility to infection. Unexpectedly, the present findings do not show the changes in immune function parameters such as white blood cells, total lymphocytes, eosinophils, neutrophils, monocytes, basophils, T lymphocytes ($CD3^+$) and T helpers ($CD4^+$) in ObEx group after 6 weeks of experimental period.

The reduction of T cytotoxics ($CD8^+$) and natural killer cells ($CD16^+$) in ObEx group may reflect that both duration and the intensity of exercise prescribed in the present study are needed to be reconsidered. The questions raised are whether the participants in the combined exercise and oat bran consumption group perceived the prescribed exercise in the current study as a type of stress, even though the mean participants' heart rates recorded during brisk walking exercise was 118±8.46 beats.min^{-1}, which was equivalent to 55-70% of the participants' maximum heart rate, reflecting that the intensity of the prescribed exercise in the present study was considered moderate. The reductions in T cytotoxic ($CD8^+$) and natural killer cell ($CD16^+$) counts in combined brisk walking exercise and oat bran consumption group may imply that the prescribed exercise of this study may have acted as a stress to reduce the T cytotoxic ($CD8^+$) and natural killer cell ($CD16^+$) counts in 40 to 50 years old hypercholesterolemic women. This speculation is based on previous studies that investigated the effects of exercise on immune function which reported that after intense long duration and high intensity exercise, immune function can be impaired (Pedersen & Toft, 2000; Gleeson, 2007), whereas moderate, regular exercise is associated with improved immune functions (Nehlsen-Cannarella et al., 1991; Nieman, 2019). In addition, the status of hypercholesterolemia in the participants may impaired aerobic capacity and cause participants to perceive the prescribed exercise as a type of stress. This speculation is based on previous studies that reported hypercholesterolemia can impaired exercise capacity. Exercise capacity such as oxygen uptake, anaerobic threshold, overall work performance and running distance, all declined in proportion to the degree of hypercholesterolemia (Maxwell et al., 2009). Niebauer et al. (1999) also reported that hypercholesterolemia impaired aerobic capacity in mice.

The present study findings reflect the chronic effects of 6 weeks combined oat bran consumption and brisk walking exercise on the measured parameters, but not the acute effects such as single session of nutritional supplementation and exercise on immune function parameters. In a previous study by Nieman et al. (1993) it was reported that 12 weeks of moderate intensity exercise with 60% of heart rate reserve failed to significantly increase NK cell activity or T cell function in sedentary elderly women. It is speculated that the duration of 6 weeks of brisk walking exercise may not be long enough to elicit beneficial effects on measured immune functions in 40 to 50 years old hypercholesterolemic women in this present study, as the participants may need to adapt to the stress induced by brisk walking exercise physiologically, and this adaptation requires time.

Time variation of blood sampling also could possibly have affected the present findings. Based on Natale et al. (2003), their study showed that total leukocyte, neutrophil and monocyte counts remained high for 3 hours after exercise. Furthermore, lymphocyte and its subpopulation had returned to baseline by 3 hours after exercise. The present study findings reflect the immune status of the participants where blood taking was carried out 14 hours after exercise in ObEx group. Therefore, the sampling time in the present study might have failed to detect any significant changes in white blood cell, monocyte, basophil, eosinophil, neutrophil, total lymphocyte, T lymphocyte ($CD3^+$) and T helper ($CD4^+$) in the ObEx group at that time point. Based on that, it is speculated that different sampling period either shorter or longer duration, such as during exercise, 1 to 3 hours post exercise, 24 hours or even few days post exercise, may have produced different results from the present findings.

Lymphocytes are cells that are responsible for antibody reproduction, a direct cell-mediated killing of virus-infected and tumor cells, and regulation of the immune response (Larosa & Orange, 2008). Thus, lymphocytes play an important role in specific immunity. In the present study, there was a significant decrease in T cytotoxic ($CD8^+$) in the ObEx group in post test compared to pre test. The present finding is different from Liew et al. (2013) which reported that there was no significant change in T cytotoxic ($CD8^+$) in 6 weeks of combined chocolate malt drink supplementation and circuit training exercise group. There was also no significant changes in T cytotoxic ($CD8^+$), total lymphocyte and T lymphocyte ($CD3^+$) in combined Eurycoma longifolia jack supplementation and circuit training exercise group in another previous study by Ooi et al. (2015). The discrepancy between previous studies and the present study may be due to differences in gender and age of the subjects, types of nutritional supplementation and exercise prescribed. Participants recruited by Liew et al. (2013) were 19-25 years old males who consumed chocolate malt drink and performed circuit training programme. Meanwhile, participants recruited by Ooi et al. (2015) were 35-55 years old men who consumed Eurycoma longifolia jack supplementation and performed circuit training programme. However, in the present study, 40-50 years old hypercholesterolemic women were recruited, oat bran drink was given as supplementation and brisk walking exercise was prescribed.

Natural killer cells ($CD16^+$) play a critical role in the innate immune response against infections and tumors, which are seen with increasing incidence during ageing (Bruunsgaard & Pedersen, 2000). According to Ogata et al. (2001), low natural killer cell activity is associated with the development of infections and death due to infection in immunologically normal elderly subjects. In the present study, it was found that natural killer cells ($CD16^+$) significantly decreased in ObEx group compared to pre test. In contrast with Mohamed and Ooi (2013), the combination of circuit training programme with honey supplementation has been reported could significantly increase natural killer cells in young males participants. The discrepancy between the present study and Mohamed and Ooi (2013) could be attributed to differences in study design such as gender and age of the participants, nutritional supplementation and also the type of exercise prescribed.

In general, the discrepancy of the finding of the measured parameters between the present study and previous studies may be due to differences in the type of exercise and duration of exercise prescribed, the age range of the participants and particularly the time of blood withdrawal after exercise. It is suggested that future studies with different exercise intensity, longer intervention period and repeated blood withdrawing after exercise are needed. The presence of exercise alone group is also needed to be included as one of the study group in future studies to determine the effect of exercise alone on the immune system.

CONCLUSION

In conclusion, oat bran consumption alone may have potential to be proposed for formulating guidelines in nutritional promotion programmes for increasing immune function in 40 to 50 years old hypercholesterolemic women. Future studies with larger sample size are warranted to investigate the beneficial effects of combined oat bran with walking exercise on blood immune function parameters in aging hypercholesterolemic women.

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CONFLICT OF INTREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

MAHA conducted the study and wrote the manuscript, FKOoi designed, conducted the study and edied the manuscript, FNFN and NAGL conducted the study. All authors participated in the final approval of the manuscript.

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