Original Research

Effect of Dietary Fat Intake and Exercise on Inflammatory Mediators of the Immune System in Sedentary Men and Women

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Key words: dietary fat, exercise, cytokines, inflammatory response

Objective: Dietary fat intake and exercise affect the immune system. This study determined the changes in inflammatory components of the immune system in response to maximal exercise with three levels of dietary fat intake: 19%, 30%, and 50% of total calories.

Methods: Five men and six women were randomly assigned to consume diets with 19% and 50% calories from fat for three weeks each, with a one-week washout. The habitual and washout diets were 30% calories from fat. At the beginning and the end of each diet, body composition and maximal exercise tests were performed. Blood samples were collected before and after exercise to determine the immunological parameters.

Results: The subject's energy intake was balanced to expenditure on the 30% and 50% diets, but was in negative balance on the 19% diet. Exercise led to significant increases in the concentrations of leukocytes, neutrophils, lymphocytes, monocytes, plasma tumour necrosis factor (TNF)-α, plasma interleukin (IL)-2, plasma soluble vascular cell adhesion molecule (sVCAM)-1, and the production of IL-1β and IL-6 by peripheral blood mononuclear (PBMN) cells stimulated with lipopolysaccharide (LPS), irrespective of diets ($p < 0.05$). The 19% fat diet resulted in increased plasma soluble intercellular adhesion molecule (sICAM)-1 after exercise. Leukotriene (LT) B4 concentration released by neutrophils stimulated with LPS was higher in the 50% fat diet, compared to the lower fat diets, and the sICAM-1 production of neutrophils stimulated with LPS was significantly increased after exercise only with 30% fat diet.

Conclusion: While a short, intense bout of exercise increased pro-inflammatory mediators of the immune system, decreasing fat intake to 19% on a caloric deficient diet caused a greater increase in plasma TNF-α, sVCAM-1 and sICAM-1 concentration than the 30% and 50% fat diets in male and female subjects. Increasing fat calories to 50% with caloric balance did not exacerbate pro-inflammatory mediators compared to a 30% fat diet.

It is well documented that exercise induces changes in many components of the immune system [1–10], including elevated levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α [3,4]. These cytokines are known to be involved in the regulation of the immune responses and inflammation. Although the production of pro-inflammatory cytokines is believed to benefit the host defense system, the overproduction of these cytokines may lead to inflammation subsequently causing muscle damage and weakness and increased risk of infection. During the inflammatory process, several factors such as chemokines and cell-adhesion molecules are elevated to facilitate the recruitment of immune cells to the site of inflammation. Certain chemokines and cell adhesion molecules are found to be increased by exercise [5–8]. In addition, the lipid mediator prostaglandin (PG) E_2 was reported to be increased after eccentric exercise and the elevated PGE_2 level was found to be related to muscle soreness [9].

Fat intake, in addition to being an important fuel during rest and exercise, is well recognized as a modulator of the immune system [10]. Reduced dietary fat intake may modulate lymphoid cell subsets, the CD4+/CD8+ ratio, the proliferative response to mitogens, and cytokine production [11–13]. The

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stimulatory effects of low fat intake on the immune system may enhance the inflammatory action during exercise stress, while an adequate fat intake may help to reduce the stress caused by exercise and thus the inflammatory responses. It has been shown that decreasing dietary fat intake to 17% of total calories increased the inflammatory responses to exercise in well-trained runners [14,15]. In contrast, increasing dietary fat intake raised the energy intake to match with high energy expenditure in the runners [16], and when the energy is kept balanced, high fat intake (41% calories) enhanced endurance performance of runners without detrimental effects on pro-inflammatory mediators of the immune system [14,15].

We propose that a low level of fat intake (and high carbohydrate) may compromise immune functions by modulating production of inflammatory cytokines, chemokines and cell-adhesion molecules, whereas a higher fat diet may overcome some of the adverse effects of exercise on the immune system in untrained individuals, as has previously been shown for athletes [14,15]. This study examined the effect of dietary fat intake on the levels of the primary inflammatory mediators of the immune system, specifically cytokines (IL-1β, IL-6, TNF-α, and IL-2), a chemokine (IL-8), adhesion molecules (soluble intercellular adhesion molecule (sICAM)-1, and soluble vascular cell adhesion molecule (sVCAM)-1), and lipid mediators (PG) E_2, and leukotriene (LT) B_4 in response to maximal exercise in sedentary male and female subjects.

MATERIALS AND METHODS

Subjects

This study was approved by the Health Sciences Institutional Review Board (HSIRB) at the State University of New York at Buffalo, Buffalo, NY, and all procedures followed the guidelines of American College of Sports Medicine (ACSM). Untrained healthy individuals (5 males and 6 females) participated in this study. The subjects gave informed written consent before the experiment started. They were asked to document medical history and maintain 7-day food intake and activity records. A physical examination was given to all participants to screen for exclusion criteria, which included high blood pressure, ECG abnormalities, diabetes or other metabolic disorders, and chronic use of medication.

Experimental Diets

The experimental diets were generated based on 7-day food records, activity records, and a list of food preferences. Two levels of dietary fat were prescribed: 19% and 50% of total calories from dietary fat with the same proportions of saturated, monounsaturated, and polyunsaturated fatty acids. Both diets were designed and prescribed to be calorically matched and to have the same levels of protein intake (20% of total caloric intake). The subjects were randomly assigned to follow the experimental diets, which each lasted for three weeks, with a one-week washout period. Prior to each diet period, the subjects were given sample menus and were individually instructed by dietitians how to use the American Dietetic Association exchange list diets and standard serving sizes [17]. Subjects selected and prepared their own food using the guideline of the sample diets. Daily food intake and activities were recorded and turned in to dietitians weekly for review. The dietitians followed up with weekly meetings with the subjects and phone calls in between to ensure compliance. Diet compositions were analyzed using the NutritionistPro Software 1.3 (First Databank, San Bruno, CA).

Experimental Procedures

Before and after the consumption of the experimental diets, subjects completed a graded exercise test to determine peak oxygen consumption (VO_{2peak}) and a blood sample was collected aseptically into a 3-ml EDTA and a 10-mL heparinized vacutainers to evaluate immunological parameters before and after the exercise test.

Peak Oxygen Consumption (VO_{2peak})

A modified treadmill test to exhaustion was used to test VO_{2peak}. In brief, each subject walked on a treadmill starting at 0% grade at 3.3 kph and 5 kph for 3 min each. The grade on the treadmill was then increased by 2% increments every 2 min until voluntary exhaustion. Gas exchange was measured by standard open circuit techniques in the last minute (American standard dry-gas meter and Perkin-Elmer-1200 Multiple Gas Analyzer, Pomona, CA) of each successive work rate and VO_2 was calculated by standard equations. Heart rate (HR) and blood pressure (BP) were taken at the end of each stage. The respiratory exchange ratio was calculated ($RER = VCO_2/VO_2$).

Blood Cell Analysis

The blood collected in the 3-mL EDTA vacutainer was used for blood cell examination. Blood count and differential analyzes were performed using a Sysmex XE 2100, Roche Diagnostics (Indianapolis, IN).

Isolation of Peripheral Blood Mononuclear (PBMN) Cells and Neutrophils

The blood in the heparinized vacutainer was sterilely separated into plasma, PBMN cells and neutrophils by standard histopaque density gradients (Sigma Diagnostics, St. Louis, MO).

Culturing PBMN Cells and Neutrophils for *in Vitro* Production of Cytokines

After isolation the cells were resuspended in 2-mL of RPMI-1640-complete medium containing 25 mM N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), 2

mM-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, 1 mM pyruvate, 50 μg/mL gentamycin, 0.13 μg/mL fungizone, and 10% serum (7.5% FCS and 2.5% human AB serum) (Gibco-BRL Life Technologies, Grand Island, NY). A trypan blue exclusion test was used to check viability of the cells before culturing. Viable cells were counted under the microscope using a hemocytometer.

PBMN cells at a final concentration of 1×10^6 cells/mL were cultured in the presence of lipopolysaccharide (LPS from E. coli; 100 ng/mL, Sigma Diagnostics, St. Louis, MO) for 18 hr. Cell suspensions were collected and centrifuged at 850 $\times$ g for 15 min at 4°C. The cell free supernatants were collected and stored at -70°C for determination of the concentration of cytokines.

Neutrophils (2.0×10^6 cells) were cultured in RPMI-1640 culturing medium in the presence of LPS (100 ng/mL) in a CO_2 incubator for 18 h. Cell suspensions were centrifuged and cell free supernatants were collected and stored at -70°C for determination of the concentration of LTB_4, sICAM-1, and IL-8.

Determination of Immunological Parameters

The concentrations of IL-1β, IL-6, TNF-α, IL-2, IL-8, sICAM-1, sVCAM-1 and PGE_2 in the plasma, the concentrations of IL-1β, IL-6, and TNF-α in supernatant from PBMN cells cultured with LPS, and the concentration of LTB_4, sICAM-1, and IL-8 in supernatant from neutrophils cultured with LPS were determined by the double sandwich ELISA technique, using kits purchased from R&D Systems (Minneapolis, MN) and following their protocol.

Statistical Analysis

The values are presented as the mean $\pm$ SEM. The SigmaStat Statistical Software 2.0 (SPSS Inc., NJ) was used for data analyses. Paired *t*-test was used to compare the two baseline data points and the difference between energy intake and expenditure for each diet. Individual parameters among the three diets were compared by ANOVA for repeated measures. When there were significant differences between experimental groups, pair wise multiple comparison procedures (Student-Newman-Keuls method) were performed. The 0.05 level of significance was used to test all hypotheses.

RESULTS

Eleven healthy untrained subjects (5 men and 6 women) followed two experimental diets: 19% and 50% calories from fat in a randomized order, and were compared to their regular diet (30%). One man did not comply with the diets, so his data were excluded. Their mean ages were 24.8 $\pm$ 1.0 yr for men and 22.3 $\pm$ 1.3 yr for women. Their mean weights were 79.4 $\pm$ 1.7 kg and 54.8 $\pm$ 2.5 kg, heights were 183.3 $\pm$ 3.5 cm and 160.8 $\pm$ 1.7 cm, BMIs were 23.7 $\pm$ 0.6 kg/m^2 and 21.1 kg/m^2, and body fats were 13.7 $\pm$ 1.4% and 25.9 $\pm$ 1.4% for men and women, respectively.

Dietary Composition

The compositions of the actually consumed diets are shown in Table 1. There was no significant difference in dietary fat intake at the 2 baselines so the two baseline data points were averaged and contained about 30% calories from fat. For both genders, the mean caloric intake did not change when they increased their fat calories from 30% to 50%. When they decreased their fat intake to 19% of calories the caloric intake decreased significantly; however, the decrease was only about 13%, compared with the 30% and the 50% fat diets. The

Table 1. Nutrient Composition of Experimental Diets

Macronutrients	Dietary fat		
	30%	19%	50%
Energy Intake (Kcal/d)	2010.8 $\pm$ 126.9^a	1748.1 $\pm$ 148.9^b	1994.9 $\pm$ 174.5^a
Protein Intake, kcal	304.7 $\pm$ 23.6^a	291.9 $\pm$ 26.4^a	347.3 $\pm$ 34.5^b
%E[1]	15.4 $\pm$ 1.1^a	16.9 $\pm$ 0.6^{ab}	18.5 $\pm$ 0.5^b
Carbohydrate Intake, kcal	1091.9 $\pm$ 88.2^a	1130.7 $\pm$ 100.7^a	623.3 $\pm$ 57.8^b
%E	53.6 $\pm$ 1.5^a	64.1 $\pm$ 1.1^b	31.0 $\pm$ 1.3^c
Fat Intake, kcal	600.1 $\pm$ 42.9^a	331.7 $\pm$ 33.9^b	1013.7 $\pm$ 101.7^c
%E	29.7 $\pm$ 0.9^a	18.8 $\pm$ 1.1^b	50.0 $\pm$ 1.7^c
Cholesterol, mg	268.3 $\pm$ 34.0^a	208.6 $\pm$ 35.3^a	494.8 $\pm$ 82.8^b
Saturated fat, g	23.1 $\pm$ 2.0^a	11.4 $\pm$ 1.4^b	37.6 $\pm$ 4.4^c
MUFA, g	17.2 $\pm$ 1.9^a	10.1 $\pm$ 1.1^a	36.8 $\pm$ 5.0^b
PUFA, g	8.6 $\pm$ 0.6^a	7.4 $\pm$ 1.0^a	20.5 $\pm$ 2.9^b
Oleic acid (18:1), g	12.0 $\pm$ 1.5^a	7.4 $\pm$ 0.6^a	29.7 $\pm$ 4.7^b
Linoleic acid (18:2), g	5.5 $\pm$ 0.5^a	5.3 $\pm$ 0.9^a	16.1 $\pm$ 2.8^b
Linolenic acid (18:3), g	0.6 $\pm$ 0.1^a	0.5 $\pm$ 0.1^a	1.4 $\pm$ 0.2^b

Values (means $\pm$ SEM) without common letter in one row are significantly different at $p < 0.05$.

[1] %E = percent of total energy intake.

MUFA = monounsaturated fatty acid, PUFA = polyunsaturated fatty acid.

Table 2. Blood Cell Parameters on the Experimental Diets before and after Exercise[1]

Blood cells (K/CMM)	Dietary Fat									Statistical Analysis *p*-Value		
	30% (Regular diet)			19%			50%					
	Pre-Ex	Post-Ex	% Δ^2	Pre-Ex	Post-Ex	% Δ	Pre-Ex	Post-Ex	% Δ	Diet	TP	Diet × TP
WBC	5.4 ± 0.1	6.9 ± 0.1	+28	5.6 ± 0.1	7.3 ± 0.1	+30	4.6 ± 0.1	5.8 ± 0.1	+27	0.022	0.001	NS
Neutrophils	2.9 ± 0.1	3.7 ± 0.1	+26	3.2 ± 0.1	4.0 ± 0.1	+25	2.2 ± 0.1	2.8 ± 0.1	+28	0.022	0.001	NS
Lymphocytes	1.8 ± 0.1	2.4 ± 0.1	+34	1.8 ± 0.1	2.5 ± 0.1	+40	1.9 ± 0.1	2.3 ± 0.1	+25	NS	0.004	NS
Monocytes	0.4 ± 0.0	0.6 ± 0.0	+30	0.4 ± 0.0	0.6 ± 0.0	+34	0.4 ± 0.0	0.5 ± 0.1	+37	0.010	0.001	NS
Basophils	0.029 ± 0.000	0.033 ± 0.000	+14	0.025 ± 0.000	0.026 ± 0.000	+4	0.022 ± 0.000	0.023 ± 0.000	+5	0.036	NS	NS
Eosinophils	0.146 ± 0.005	0.145 ± 0.005	−1	0.103 ± 0.005	0.120 ± 0.005	+17	0.125 ± 0.005	0.130 ± 0.005	+4	NS	NS	NS

[1] Values are means ± SEM; n = 10.

[2] %Δ = % change of blood cells before and after exercise.

TP = Time point (pre- and post-exercise), WBC = white blood cells, NS = no significant differences.

subjects maintained their daily activities at the same level throughout the study.

Physiological Parameters

VO_{2peak} was significantly higher in men (average 3.1 ± 0.1 L/min), compared with women (average 1.5 ± 0.1 L/min) ($p < 0.05$). The VO_{2peak} of both genders were not affected by diets. The RER was not significantly different between men and women, and the diet had no effect on RER at any workload. The estimated maximum HR was achieved and averaged 176.7 bpm in men and 184.3 bpm in women. The average maximum systolic BP was 217.3 mmHg and maximum diastolic BP was 79.8 mmHg in men. In women, the average maximum systolic BP was 164.1 mmHg and maximum diastolic BP was 76.7 mmHg. There were no diet effects on maximum HR and BP in male and female subjects.

Blood Components

There were no gender differences in any immunological parameters so the data were combined for statistical analysis. The numbers of blood cells at pre- and post-exercise are shown in Table 2. The numbers of leukocytes increased significantly with exercise ($p < 0.001$; 28%, 30%, and 27% increase on the 30%, 19%, and 50% fat diets, respectively). The increase in leukocyte count after exercise was accounted for by increases in neutrophils, lymphocytes, and monocytes and the increases in these blood cells were similar among the diets.

Irrespective of exercise, increasing fat caloric intake to 50% significantly decreased the number of WBC (−17%) and monocytes (−10%), compared to the lower fat intake ($p < 0.05$). The number of basophiles and neutrophils on the 50% fat diet were also significantly less than those on the 19% fat diet (−30%) and 30% fat diet (−27%), respectively ($p < 0.05$). There was no dietary effect on the number of lymphocytes and eosinophiles.

Plasma Immunological Parameters

The plasma levels of IL-1β, IL-6, TNF-α, IL-2, IL-8, sICAM-1, sVCAM-1 and PGE_2 are presented in Table 3. Plasma IL-1β, IL-6, IL-8, and PGE_2 were unchanged after exercise compared to the baseline. There was no significant effect of diet on these mediators at baseline or post-exercise.

Table 3. The Plasma Levels of Inflammatory Mediators on the Experimental Diets before and after Exercise[1]

Parameters	Dietary Fat									Statistical Analysis *p*-Value		
	30% (Regular diet)			19%			50%					
	Pre-Ex	Post-Ex	% Δ^2	Pre-Ex	Post-Ex	% Δ	Pre-Ex	Post-Ex	% Δ	Diet	TP	Diet × TP
IL-1β (pg/mL)	3.31 ± 0.1	3.09 ± 0.1	−7	3.06 ± 0.1	3.13 ± 0.1	+2	3.15 ± 0.1	3.21 ± 0.1	+2	NS	NS	NS
IL-6 (pg/mL)	7.09 ± 0.28	7.41 ± 0.28	+5	7.50 ± 0.28	7.47 ± 0.28	+0.4	6.98 ± 0.28	6.68 ± 0.28	−4	NS	NS	NS
TNF-α (pg/mL)	221.3 ± 2.5	221.2 ± 2.5	0	209.2 ± 2.5	221.8 ± 2.5	+6	213.8 ± 2.5	221.9 ± 2.5	+4	NS	0.046	NS
IL-2 (pg/mL)	5.36 ± 0.22	5.89 ± 0.22	+10	5.14 ± 0.22	5.53 ± 0.22	+8	5.92 ± 0.22	5.87 ± 0.22	−1	NS	0.012	NS
IL-8 (pg/mL)	18.6 ± 0.5	18.4 ± 0.5	−1	18.0 ± 0.5	18.8 ± 0.5	+4	19.7 ± 0.5	18.8 ± 0.5	−5	NS	NS	NS
sVCAM-1 (ng/mL)	439.08 ± 21.2	488.73 ± 21.2	+11	482.27 ± 21.2	562.35 ± 21.2	+17	480.69 ± 21.2	493.34 ± 21.2	+3	NS	0.012	NS
sICAM-1 (ng/mL)	134.2 ± 4.3	130.8 ± 4.3	−3	118.6 ± 4.3	133.6 ± 4.3	+13	125.6 ± 4.3	113.6 ± 4.3	−10	NS	NS	0.017
PGE_2 (pg/mL)	429.3 ± 27.9	471.3 ± 27.9	+10	448.0 ± 27.9	430.1 ± 27.9	−4	434.1 ± 27.9	492.0 ± 27.9	+13	NS	NS	NS

[1] Values are means ± SEM; n = 10.

[2] %Δ = % change of cytokine concentration before and after exercise.

TP = Time point (pre- and post-exercise), NS = no significant differences, IL = interleukin, TNF = tumor necrosis factor, sVCAM = soluble vascular cell adhesion molecule, sICAM = soluble intercellular cell adhesion molecule, PG = prostaglandin.

Exercise stress led to significant increases in plasma TNF-α, IL-2 and sVCAM-1 concentration ($p < 0.05$), irrespective of the diets. When the subjects decreased the fat calories to 19%, the levels of sICAM-1 increased significantly after exercise ($p = 0.017$). The increased sICAM-1 after exercise was not observed on the 30% and 50% fat diets.

Immunological Parameters Released by Immune Cells Stimulated by LPS

Mononuclear Cells. The production of IL-1β (Fig. 1) was significantly increased after exercise on the 19% and 30% diets. IL-6 (Fig. 2) by PBMN cells stimulated with LPS was significantly increased after exercise ($p < 0.05$). The levels of TNF-α (Fig. 3) production were unchanged in response to maximal exercise. There were no dietary effects on the production of IL-6. However, the diet affected TNF-α production as the levels of TNF-α were reduced when changing fat intake from 30% to either 19% or 50% of calories.

Neutrophils. There was no effect of exercise on LTB_4 (Fig. 4) and IL-8 (Fig. 5) concentration released by neutrophils stimulated with LPS. However, the level of LTB_4 was significantly increased on the 50% fat diet, compared to lower fat diets ($p < 0.05$) (Fig. 4). The production of sICAM-1 from neutrophils cultured with LPS was not affected by maximal exercise, but the level of this adhesion molecule after exercise was higher when the subjects were on the regular diet (30% fat calories) compared to the 19% and the 50% fat diets (Fig. 6).

DISCUSSION

The results of this study showed that maximal exercise lead to increases in leukocyte count, plasma TNF-α, IL-2 and sVCAM-1 concentration. The magnitude of the increase in TNF-α and sVCAM-1 concentration at post-exercise on the 19% fat diet was greater than that on the 30% and 50% fat diets. Of course it has to be recognized that on an isocaloric diet changing levels of fat requires changes in carbohydrates (when protein is kept constant) and this effect may influence the data. Plasma sICAM-1 was increased after exercise only with the 19% fat diet. In addition, the consumption of 50% of calories from fat did not enhance the inflammatory mediators of the immune system examined in this study.

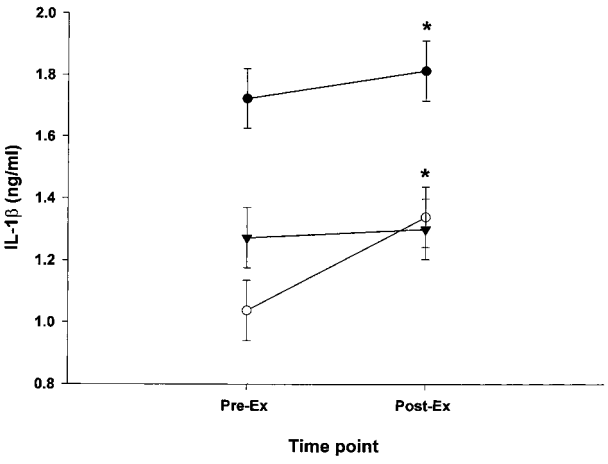

Fig. 1. *In vitro* production of IL-1β by PBMN cells stimulated with LPS. The production of IL-1β by PBMN cells stimulated with LPS at pre- and post-exercise after experimental diets. * Significant difference between pre- and post-exercise, $p < 0.05$.

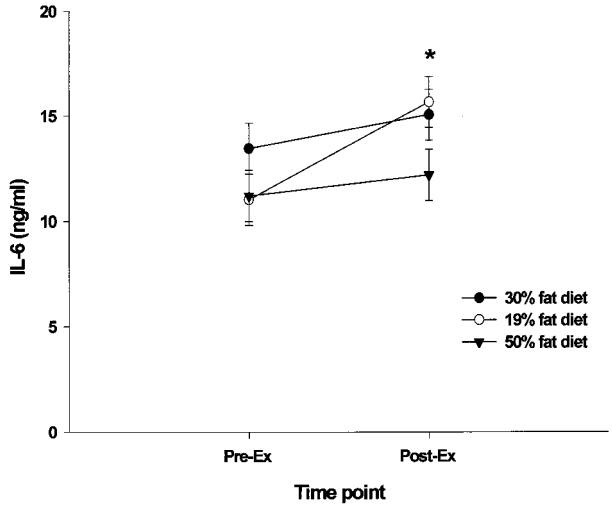

Fig. 2. *In vitro* production of IL-6 by PBMN cells stimulated with LPS. The production of IL-6 by PBMN cells stimulated with LPS at pre- and post-exercise after experimental diets. * Significant difference between pre- and post-exercise, regardless of diets, $p < 0.05$.

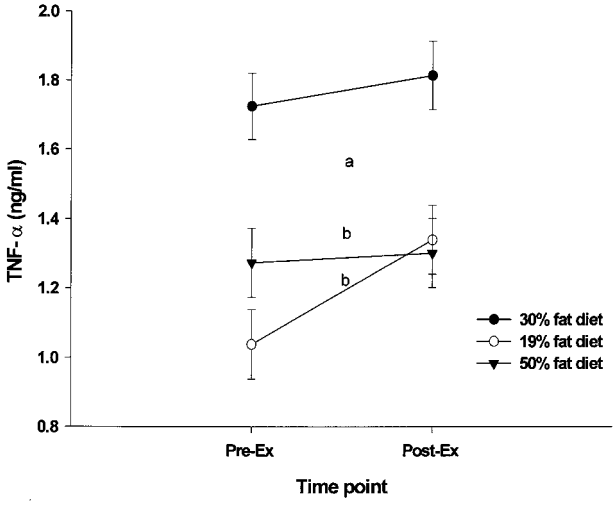

Fig. 3. *In vitro* production of TNF-α by PBMN cells stimulated with LPS. The production of TNF-α by PBMN cells stimulated with LPS at pre- and post-exercise after experimental diets. Values with different letters (a, b) are significant differences between diets, regardless of time points, $p < 0.05$.

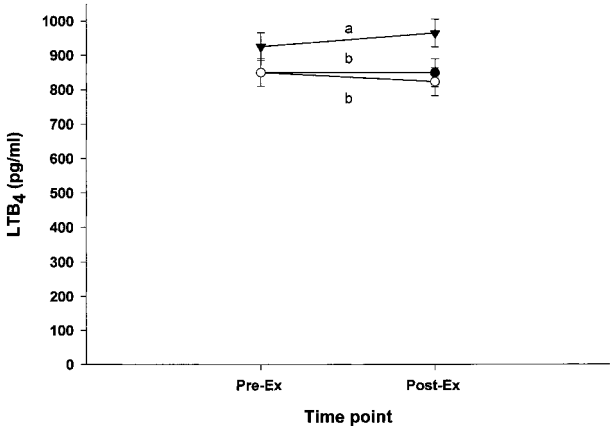

Fig. 4. *In vitro* production of LTB4 by neutrophils stimulated with LPS. The production of LTB_4 by neutrophils stimulated with LPS at pre- and post-exercise after experimental diets. Values with different letters (a, b) are significant differences between diets, regardless of time points, $p < 0.05$.

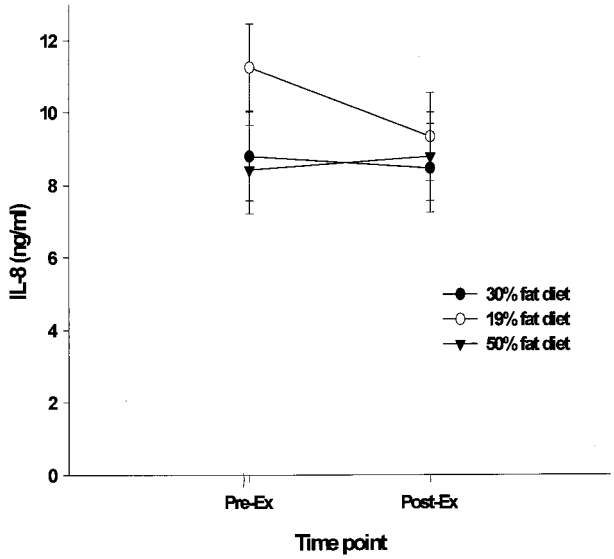

Fig. 5. *In vitro* production of IL-8 by neutrophils stimulated with LPS. The production of IL-8 by neutrophils stimulated with LPS at pre- and post-exercise after experimental diets.

Immune Cells

The immune responses to exercise stress vary depending on type, intensity, and duration of exercise [18,19]. High concentrations of leukocytes are commonly found immediately after exercise, and they may remain elevated in the blood for a few hours after exercise, before returning back to the pre-exercise levels [20,21]. Similar to previous studies [22,23], we found that maximal exercise resulted in leukocytosis. The elevated post-exercise leukocyte counts in the present study were accounted for by increased numbers of neutrophils, lymphocytes and monocytes.

It is well documented that dietary fat, both amount and type

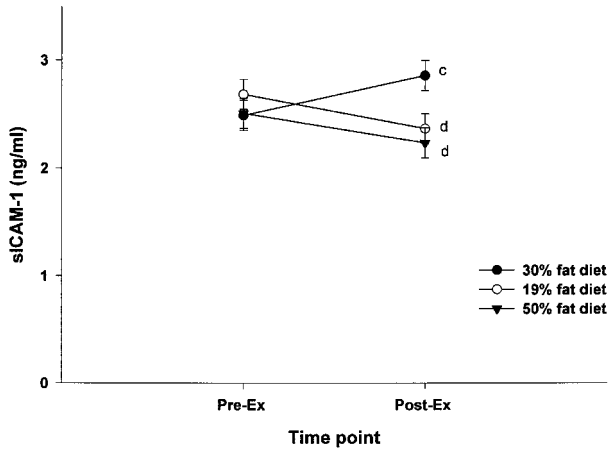

Fig. 6. *In vitro* production of sICAM-1 by neutrophils stimulated with LPS. The production of sICAM-1 by neutrophils stimulated with LPS at pre- and post-exercise after experimental diets. Values with different letters (c, d) are significant differences at post-exercise, $p < 0.05$.

of fat, modulate the responses of the immune system [1–10]. Reduction of fat intake has been shown to increase the number of circulating T- and B-lymphocytes and their proliferation [12]. We also found that the levels of dietary fat intake affected the circulating immune cell number, as on the 50% fat diet the number of leukocytes, monocytes, neutrophils and basophiles, was significantly reduced, when compared to the lower fat diets.

Plasma Immunological Parameters

IL-1β and TNF-α are pro-inflammatory cytokines that stimulate an acute phase response [24]. Although IL-6 is involved in inflammatory process, it may not be considered only a pro-inflammatory cytokine because it also has anti-inflammatory action [25]. Plasma levels of IL-1β, IL-6, and TNF-α are often found to be increased after exercise [3,4]. These cytokines may have overlapping functions and they may influence the production of each other [26,27]. In the present study, plasma IL-1β and IL-6 were unchanged in response to maximal exercise, whereas the plasma TNF-α was increased after exercise, and more on the 19% fat diet. The lack of change of plasma IL-1β and IL-6 in these sedentary male and female subjects may be due to the short duration of high intensity exercise that failed to produce an increased inflammatory response or release from skeletal muscle. Alternatively, exercise may affect local cytokine production and/or they were rapidly cleared from the circulation by the actions of cytokine inhibitors or anti-inflammatory cytokines.

Studies have shown that production of pro-inflammatory cytokines can be reduced by some dietary fatty acids, especially those containing long-chain (ω-3) polyunsaturated fatty acids [28,29,30]. In the present study, in contrast, the proportions of

saturated, monounsaturated, and polyunsaturated fatty acids in the experimental diets were similar. Thus, the effect of one specific type of fatty acid was not observed. We found that the amount of calories from fat had no effect on the levels of IL-1β, IL-6, and TNF-α pre- and post-exercise. However, the absence of effect on the low fat diet may be due to the lower caloric intake on this diet, although this was a small difference (13%).

IL-2 is produced from activated T-lymphocytes, especially CD4+ T-helper cells. It is a potent immunomodulator that plays a role in enhancing the function of T cells for host defense and maintenance of an immune response. A reduction in IL-2 level is usually reported after exercise in sedentary subjects [31]. In runners, a significant decrease in plasma IL-2 was observed with an increase in dietary fat intake in men after endurance exercise [15]. The *in vitro* IL-2 production by PBMN cells was increased after a maximal exercise with high fat intake in runners [14]. We found that plasma IL-2 in sedentary subjects was increased after exercise stress and the magnitude of increased levels tended to be less on the 50% fat diet, compared to the lower fat diets.

In addition to changes in leukocyte number and subsets and cytokine production, exercise stress also affects chemokines that are involved in a variety of immune and inflammatory responses, acting primarily as chemoattractants and activators of specific types of leukocytes [32]. Adhesion molecules expressed on leukocytes and the vascular endothelial lining play a role in leukocyte trafficking and migration, which modulate leukocyte number during and following exercise [33]. In the present study, we found that a short and maximal bout of exercise did not affect the levels of chemokine IL-8, but it affected the levels of the adhesion molecules of the immunoglobulin family. Regardless of the amount of fat intake, the maximal exercise resulted in a significant increase in plasma sVCAM-1. The elevated sICAM-1 concentration after exercise was observed only with the 19% diet suggesting that low dietary fat intake may increase the inflammatory response through a change in adhesion molecules.

PGE_2 is a potent inflammatory lipid mediator whose actions involve platelet aggregation, vasoconstriction and production of pro-inflammatory cytokines. Elevated PGE_2 was found after eccentric exercise and was associated with muscle soreness [9]. It is known that the level of PGE_2 is increased when subjects consume diets high in ω-6 fatty acids, but is decreased on diets high in ω-3 fatty acids [28]. We found no effect of exercise and dietary fat on PGE_2 levels in sedentary subjects and these results agree with previous findings in athletes [34], that high fat intake (50% of total calories) had no adverse effect on PGE_2 concentration after exercise.

Although plasma immunological parameters are often used as the end-points for the measurement of whole body immune response, the complexity of cytokine actions and other mediators is highly variable in plasma. Changes in plasma volume may confound the observations of plasma cytokines. In the present study, we did not directly measure plasma volume. It was assumed that a graded maximal exercise lasting 10 to 14 min would not result in a significant decrease in plasma volume, as has been reported for prolonged endurance exercise. The estimated change in plasma volume after exercise, derived from blood hemoglobin and hematocrit, demonstrated only a 6% decrease in plasma volume, which was similar on all diets.

Immunological Parameters *in Vitro*

The culture of immune cells *in vitro* with mitogenes allows the study of inflammatory mediators released by PBMN cells and neutrophils. Similarly to previous studies [35,36,37], we found that IL-1β, IL-6 and TNF-α were produced at high concentrations by the PBMN cells cultured with LPS. Increased productions of IL-1β [37] and IL-6 [35] by PBMN cells following exercise have been reported. In this study, the levels of IL-1β and IL-6 by the PBMN cells cultured with LPS were increased immediately after maximal exercise. The amount and type of dietary fat and carbohydrate may modulate the production of these cytokines. Some variability in the data from cultured cells may be due to the fatty acids in the sera used for culturing. As the In addition the A previous study revealed that reducing the consumption of fat calories from 36% to 27% with low fish-derived ω-3 PUFA resulted in increased levels of IL-1β, and TNF-α production by PBMN stimulated with LPS while the same low fat diet with high fish-derived ω-3 PUFA led to lowered amount of these cytokine production [13]. In the present study, changes in fat consumption of 30% total calories, either reduce to 19% of total calories or increase to 50% of total calories, lowered the TNF-α production. The type of fatty acids was unlikely to influence the cytokine production as discussed previously. We found no effect of dietary fat intake on *in vitro* secretion of IL-1β and IL-6 at pre- and post-exercise.

Neutrophils are the primary cells in host defense during inflammation. In addition to their function in phagocytolysis and killing the pathological invaders, neutrophils have the ability to produce several mediators including LTB_4 [38,39], sICAM-1 [40] and IL-8 [41]. Although exercise has been shown to affect some of the neutrophils functions [42], it did not influence the release of LTB_4, sICAM-1 and IL-8 by neutrophils stimulated with LPS in this study. We found that increased dietary fat intake (50% of calories) resulted in increased overall LTB_4 production and decreased post-exercise sICAM-1 production by neutrophils. The changes in the mediators released by neutrophils may be the result of changes in the neutrophil lipid composition by the dietary fat intake [43].

In the present study, the results of pro-inflammatory cytokine production *in vitro* disagreed with those in plasma. The increases in IL-1β and IL-6 by exercise were observed only *in vitro*, but not in plasma. The different results may suggest that circulating immune cells may not be the only factors that regulate cytokine production during exercise. This may be true as it has been shown that the number of PBMN cells did not

correlate with alterations in plasma cytokines in response to exercise [18]. A recent study reported that PBMN cells are not the only source of increased IL-6 after exercise [44]. This is no longer thought to be the case, as muscles produce IL-6 irrespective of inflammation status [45].

Interestingly, the magnitude of increase in pro-inflammatory mediators observed from plasma (TNF-α, IL-2, and sVCAM-1) and *in vitro* (IL-1β and IL-6) in response to exercise tended to be higher in the 19% fat diet, compare to the 50% fat diet. The effect of enhancing inflammatory response of the 19% fat diet was clear for sICAM-1 as the plasma concentration of sICAM-1 was significantly enhanced by exercise stress only with the 19% fat intake. The pattern of changes in sICAM-1 after exercise by the 50% diets was similar for both plasma and *in vitro.* These results suggest that a minimal amount of fat (30%) may be required to attenuate the increase in inflammatory response to exercise stress.

The energy balance between intake and expenditure in this study may be an important factor that modulates immune responses. It appeared that the subjects in the present study were able to maintain the energy balance when they were on the 30% and 50% fat diets, but not on the 19% fat diet. With caloric balance, increasing fat intake to 50% of total calories did not significantly enhance pro-inflammatory mediators in response to exercise. The finding in these sedentary subjects agree with the previous finding in athletes [14,15] that increasing fat intake (up to 50%) did not have any detrimental effects on immune status as long as there was caloric balance. Other studies showing negative effects of higher fat diets did not control caloric balance, thus subjects on higher fat diets were also taking in more calories than they were expending. In the present study, the caloric intake of the subjects decreased on the 19% fat diet leading to negative energy balance. Therefore, the stimulatory effect of the 19% fat diet on the production of pro-inflammatory mediators in response to exercise in this study may be confounded by the negative energy balance.

In conclusion, a short and maximal exercise bout resulted in an increase in the number of circulating leukocytes, neutrophils, lymphocytes and monocytes. The concentrations of plasma TNF-α, IL-2, and sVCAM-1 and the IL-1β and IL-6 production of PBMN cells stimulated with LPS were also increased in response to maximal exercise. Exercise on the 19% fat diet may have a greater tendency to induce an inflammatory response, when compared to the 50% fat diet as plasma TNF-α, sVCAM-1 increased more and sICAM-1 increased only on the 19% fat diet. The results from the present study suggest that post-exercise inflammatory response may be reduced by selecting a diet that provides sufficient caloric intake to match with the energy requirement and has a composition of at least 30% fat. In addition, if caloric balance is maintained, increasing fat intake (up to 50%) may not be harmful to immune status in healthy sedentary and athletic individuals.

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