Original Article
The antiviral immune defense may be adversely influenced by weight loss through a calorie restriction program in obese women

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Abstract: Background: Obesity and weight loss are reported to be associated with immune function. This study aimed to investigate the changes in counts of lymphocytes involved in microbial defense during weight loss in obese women. Methods: This clinical trial involved 29 women with a body mass index (BMI) ≥ 30 kg/m^2. The intervention group was prescribed a low-calorie diet (600 kcal lower than caloric requirement per day) plus Orlistat (120 mg three times daily). The control group received *ad libitum* diet. Anthropometric indices, obesity-related traits, and blood pressure were assessed every three weeks. Metabolic indices and plasma count of lymphocyte subpopulations (CD3, CD4, CD8, CD19, and CD16/56, as well as the ratio of CD4:CD8) were measured at baseline and after the intervention (after 10% weight loss). Results: After the weight loss, natural killer cells (CD16/56) decreased in the intervention group (P=0.014) even after adapting for all confounders. No significant changes were observed in other immune markers compared to the control group. Conclusions: Caloric restriction-induced weight loss might independently weaken the antiviral immune defense. Further clinical trials are warranted to better clarify the association between weight loss, calorie restriction, and immunity.

Keywords: Lymphocytes, weight loss, viral infection, immunity

Introduction

Obesity is a growing concern across different age groups worldwide and can impair the immune response. Adiposity was defined as a risk factor for increased viral disease severity and mortality in individuals infected by H1N1 [1]. It seems that weight loss should be the most effective approach to improve the immune dysfunction caused by obesity [2]. However, it is claimed that reduced energy and macronutrient intake has been associated with impairments in several aspects of the cellular, systemic, and mucosal immune defenses [3]. Additionally, based on the experience of clinicians, a complaint of obese people undergoing a weight loss program is increased incidence of flu virus infection and a more extended period of infection. These conflicts might be due to the type of the weight loss plan, since the role of various weight loss programs in the recovery of immune dysfunction has not yet been established [4]. Many factors may influence obesity and health [5].

There are few studies on the immune function of obese individuals and the effect of weight loss on impaired immune function. Moulin et al. stated that an approximately 26% weight loss induced by bariatric surgery could enhance the activity of natural killer cells (NKCs), which are involved in viral defense [6]. Jahn et al. also claimed that a three-month weight-loss program consisting of diet and physical activity could reactivate NKC functionality [7]. In contrast, Neiman et al. showed that weight loss through a combination of diet and physical exercise, even at a moderate rate, impaired specific

immune mechanisms [8, 9]. On the other hand, some studies found that weight loss through regular physical activity increases NKC activity [10]. In contrast, weight reduction through high-intensity training might weaken immune function in athletes [11, 12].

Given these conflicting results, further investigations must clarify the exact impact of weight loss through multiple weight management programs on the immune system considering various confounders. Therefore, we aimed to investigate changes in immune system markers (CD3, CD4, CD8, CD19, and CD16/56) in microbial defense in obese women under a weight loss program consisting of calorie restriction plus lipase inhibitor and as well considering important confounding indices that influence immune response.

Materials and methods

This study was a clinical trial, approved by the Research Ethics Committee in Mashhad University of Medical Sciences (registration number: MUMS900413) and registered in the clinicaltrial.gov with the code of NCT03336086, as well as in the Iranian Registry of Clinical Trials (Registration number: IRCT2014052617872N).

Participants

We used a convenient sampling method. The study protocol was explained to all individuals referred to the weight management clinic in Ghaem Hospital, Mashhad, Iran. Fifteen women with obesity were allocated to the intervention group. Participants for the control group were invited through advertisement in the hospital and they were matched for age and body fat.

Since sex differences can influence immune responses due to sex hormone differences, the effect of gender (confounding variable) on the immune system was controlled by enrolling only women in the study [13].

Inclusion criteria: (1) female gender; (2) age of 20-45 years old; (3) (BMI) ≥ 30 kg/m^2; (4) being in the normal range for HbA1C, zinc, iron, and thyroid tests (the blood tests were assessed for all the participants), and (5) having no weight loss attempt in the last two months.

Exclusion criteria: Women with diabetes mellitus, thyroid dysfunction, pregnancy, lactation, iron and zinc deficiency as well as those consuming multivitamin or mineral supplements were excluded from the study. Informed written consent was obtained from all the participants.

Design

The eligible individuals were asked to return to the weight management clinic during days 7 to 10 of their menstrual cycle and after 10-hour overnight fasting. A 10-ml venous blood sample was collected. Biochemical variables including fasting blood sugar, HbA1c, lipid profile, thyroid test, cortisol level (at 8 AM), serum iron and zinc, and complete blood count (CBC), were measured. Lymphocyte subset counts (CD3, CD4, CD8, CD19, and CD16/56) were immediately analyzed after blood sample collection through flow cytometry. Anthropometric indices including body weight (Seca, Germany), height (Seca, Germany), and BMI (equation) were measured. Bio-electrical analysis of body composition including body fat mass, trunk fat, and fat-free mass were measured using bio-electrical impedance analyzer device (Tanita 370, Japan) in both groups.

For the intervention group, a target of at least 10% weight loss of the initial body weight was determined. Participants in this group were prescribed a balanced low-calorie diet of 2512 kJ/600 kcal lower than caloric requirement, plus Orlistat (120 mg three times a day) (Venustat, Aburaihan Co., Iran). Diet macronutrient composition consisted of carbohydrates (60%), fat (20%), and protein (20%). The control group were prescribed *ad libitum* diet. Both groups were recommended to do physical activity (one-hour walking daily at VO$_2$max 60%). The follow-up visits were done every three-to-four weeks until the desired weight loss was reached. In the follow-up session, the anthropometric indices and blood pressure were measured. To verify the participants' compliance with the study protocol, they were contacted by the researcher by phone regularly. In addition, at each follow-up session, adherence to the prescribed diet was assessed through a 24-hour diet (minimum of 60% adherence as a goal).

Compliance with the physical activity was evaluated through self-record. At the end of the study, blood samples were collected to assess immune markers as well as metabolic indices.

Immune assays

Blood samples (2 ml) were collected in tubes containing EDTA. Initially, CBC was performed on each sample, and samples were prepared for flow cytometry. In this process, 5 µl of fluorescent antibodies were added to 100 µl of blood samples. Antibodies for CD3/CD4/CD8 (FITC/R-PE/CyQ conjugates), CD3/CD19/CD45 (FITC/R-PE/CyQ conjugates), and CD3/CD16/56 (FITC/R-PE conjugates) were utilized (IQ Products, Netherlands). We also used one tube of blood without antibody as control. After incubation at room temperature in darkness (10 minutes), 500 µl of lysis buffer (IQ Products, Netherlands) was added to each tube and mixed.

Contents of each tube were diluted with 2 ml of phosphate-buffered saline (PBS) and further incubated. Afterward, the tubes were washed with PBS twice by centrifugation at 1200 rpm. Following that, the cell pellet was re-suspended in 250 µl PBS. Finally, samples were tested for the proportion of stained cells using a flow cytometer (FACS Calibur, BD, USA). For each sample, 10,000 events were analyzed and absolute lymphocyte subset counts were calculated based on CBC. In addition, we computed CD4/CD8 ratio by the equation.

Statistical analysis

Descriptive characteristics of the patients were presented with statistical indices; mean (standard deviation) and frequency (relative frequency). In order to assess statistical tests, first, the normality of data distribution was evaluated using Kolmogrov-Smirnov test. In order to assess a statistically significant association among qualitative variables and in case of imitation in expected frequency, Chi-square test and Fischer's exact test were used, respectively.

Independent t-test was used to examine the baseline characteristics of demographic variables, anthropometrics, and blood indices, in the intervention and control groups, and if not normal, Mann-Whitney test was used.

Paired-sample t-test was used to compare means before and after the intervention to evaluate the effects of anthropometric and metabolic variables, and in case of non-normal-

ity, the Wilcoxon test was used, and also Independent-sample t-test was used to compare equivalence of the two means among qualitative variables (baseline characteristics of demographic variables, anthropometrics, blood index), and in case of non-normality, the Wilcoxon test was used.

To evaluate the effects of anthropometric and metabolic variables before and after the intervention, and in case of non-normality, the Wilcoxon test was used.

Finally, in order to assess the effects of confounders along with adapting for baseline values and the duration of the study, the ANCOVA model was used. In the interpretations, mean difference and confidence interval of 95% were presented as the effect size. All the analyses were done using SPSS software version 22 and significance level was considered as P<0.05.

Results

A total of 29 women with obesity were included in the final analysis (15 participants in the intervention group and 14 participants in the control group). One participant was excluded based on the exclusion criteria. The mean age of the participants in the intervention and control groups was 35.71±5.09 and 32.0±4.47 years old, respectively (P=0.296). All baseline data in **Table 1** were normally distributed. The duration of the study was about 3-4 months. The groups were not significantly different in mean body weight, FM and FM percentage, FFM and FFM percentage, SBP, as well as serum level of iron, FBS, HDL-c, LDL-c, and cortisol. In addition, there were no significant differences among immune markers of CD4, CD8, CD19, and also the ratio of CD4/CD8 between two groups.

However, significant differences were observed for several variables including BMI, WC, HC, WHR, WHtR, TF and TF percentage, DBP, as well as serum level of zinc, TG, TC, CD3, and CD16/56 between two groups at baseline. None of the participants had iron or zinc deficiency (**Table 1**). The variables with significant differences at baseline between the two groups were controlled in the further analysis to prevent confounding effects.

Dietary adherence and caloric intake percentage was 82% at the first visit, 74% at the sec-

Table 1. Baseline characteristics of the intervention and control groups

Variable	Intervention Group Mean ± SD	Control Group Mean ± SD	P-value
Age (years)	34.93 5.76	32.00±4.47	0.139
BMI (kg/m^2)	35.16±4.42	30.81±0.95	0.002*
Ht (cm)	159.93±4.73	167.36±5.42	0.001*
Wt (kg)	89.88±12.62	86.03±5.61	0.296
WC (cm)	108.53±11.89	92.07±3.07	0.001*
HC (cm)	112.13±10.96	101.59±2.95	0.002*
WHR	0.97±0.06	0.91±0.04	0.002*
WHtR	0.68±0.08	0.55±0.03	<0.001*
FM (kg)	37.10±8.57	35.6±2.89	0.537
FM (%)	40.85±4.15	41.29±2.19	0.727
TF (kg)	17.32±3.39	13.67±1.67	0.001*
TF (%)	47.22±4.86	38.57±5.21	<0.001*
FFM (kg)	52.47±5.04	50.73±5.59	0.387
FFM (%)	58.74±3.39	58.78±4.73	0.974
DBP (mmHg)	84.33±10.32	76.42±8.41	0.033*
SBP (mmHg)	123.0±10.32	122.50±7.0	0.893
Zinc (µg/dl)	66.87±7.16	98.14±15.56	<0.001*
Iron (µg/dl)	76.93±14.40	94.21±31.01	0.062
FBS (mg/dl)	96.87±3.70	94.14±6.46	0.171
HDL-c (mg/dl)	36.40±5.91	38.07±4.92	0.417
LDL-c (mg/dl)	100.07±19.30	89.93±11.65	0.101
TG (mg/dl)	174.27±20.87	154.76±11.17	0.004*
TC (mg/dl)	204.33±19.91	184.79±12.05	0.004*
Cortisol (µg/dl)	16.40±4.26	13.43±4.78	0.088
CD3 (cell/µl)	1534.80±315.79	1188.86±466.73	0.026*
CD4 (cell/µl)	882.73±239.38	748.75±351.82	0.238
CD8 (cell/µl)	570.86±186.98	440.10±155.621	0.051
CD19 (cell/µl)	307.46±273.43	238.39±53.93	0.353
CD16/56 (cell/µl)	214.06±128.43	310.25±85.01	0.026*
CD4CD8R	1.67±0.64	1.73±0.70	0.828

*Significant difference (*P*-value <0.05). Independent t-test was used. BMI: Body Mass Index, Ht: Height, Wt: Weight, WC: Waist Circumference, HC: Hip Circumference, WHR: Waist to Hip ratio, WHtR: Waist to Height ratio, DBP: Diastolic Blood Pressure, SBP: Systolic Blood Pressure, FM: Fat Mass, TF: Trunk Fat, FFM: Fat-Free Mass, FBS: Fasting blood sugar, HDL-c: HDL-cholesterol, LDL-c: LDL-cholesterol, TG: Triglyceride, TC: Total cholesterol.

ond visit, 69% at the third visit, and 62% at the end of the study. Medication adherence percentage was 91% at the first visit, 87% at the second visit, 87% at the third visit, and 83% at the end of the study. Proportion adherence percentage to the prescribed physical activity was 79% at the first visit, 74% at the second visit, 65% at the third visit, and 61% at the end of the study.

No significant adverse effects were reported during the study. All the participants in this group had about 11% weight reduction during approximately four months.

Table 2 shows that changes in BMI, Wt, WC, WHtR, FM and FM percentage, TF and TF percentage, FFM and FFM percentage in the intervention group were significant compared to the control group. **Table 3** shows that DBP, and the serum levels of FBS, HDL-c, LDL-c, TG, TC, and cortisol were significantly changed in the intervention group compared to the control group. In order to investigate the changes of immune markers during the intervention, we involved all changes of baseline variables as confounders and examined the exact effect of each to prevent bias in the final interpretation.

The absolute CD3, CD8, and CD16/56 lymphocyte subset counts were decreased, while CD4 and CD19 subsets count, as well as the CD4/CD8 ratio, were increased in the intervention group compared to the control group. Most of these changes were clinically significant, but we observed statistically significant changes only for CD16/56 after adjusting for confounders. Precisely, the changes in CD3 were considerable and clinically significant, but did not achieve a statistically significant *P*-value after adjusting for confounders (**Table 4**). In addition, we included all the anthropometric and metabolic variables in the study as confounders in the final analysis, and no changes occurred to the final results presented in **Table 4**.

Discussion and conclusions

This study found that weight loss induced by caloric restriction could decrease the absolute

Table 2. Anthropometric variables before and after the study in the control and intervention groups

Variable	Group	Mean ± SD		MD (95% CI)	P value[a]
		Before	After		
BMI (kg/m^2)	Intervention	35.15±4.42	31.30±4.44	-3.85 (-5.53 to -2.16)	<0.001[*]
	Control	30.81±0.96	30.76±1.44	-0.05 (-0.60 to 0.48)	0.818
	P-value			<0.001[b,*]	
Wt (kg)	Intervention	89.88±12.62	78.57±11.80	-11.31 (-14.2 to -8.37)	<0.001[*]
	Control	86.28±5.69	85.97±5.63	-0.31 (-1.83 to 1.21)	0.668
				<0.001[*]	
WC (cm)	Intervention	108.76±11.85	101.58±11.56	-7.18 (-8.82 to -5.53)	<0.001[*]
	Control	92.33±3.09	92.09±2.96	-0.24 (-0.81 to 0.32)	0.372
				<0.001[*]	
HC (cm)	Intervention	112.12±10.96	112.18±21.08	0.05 (-9.97 to 10.08)	0.991
	Control	101.59±2.95	101.65±2.98	0.05 (-0.14 to 0.26)	0.557
				0.999	
WHR	Intervention	0.97±0.05	0.91±0.10	-0.06 (-0.10 to 0.00)	0.065
	Control	0.90±0.03	0.90±0.03	-0.00 (-0.00 to 0.00)	0.286
				0.080	
WHtR	Intervention	0.68±0.07	0.63±0.07	-0.05 (-0.06 to -0.03)	<0.001[*]
	Control	0.55±0.02	0.55±0.02	-0.00 (-0.00 to 0.00)	0.366
				<0.001[*]	
FM (kg)	Intervention	37.10±8.57	29.70±7.24	-7.39 (-9.73 to -5.05)	<0.001[*]
	Control	35.62±2.89	35.49±2.91	-0.12 (-0.35 to 0.09)	0.234
				<0.001[*]	
FM (%)	Intervention	40.85±4.14	37.48±4.80	-3.37 (-5.50 to -1.24)	0.004[*]
	Control	41.29±2.18	41.27±1.94	-0.01 (-0.69 to 0.66)	0.962
				0.004[*]	
TF (kg)	Intervention	17.32±3.39	12.76±3.40	-4.55 (-6.04 to -3.06)	<0.001[*]
	Control	13.67±1.67	13.59±1.53	-0.08 (-0.28 to 0.10)	0.359
				<0.001[*]	
TF (%)	Intervention	47.21±4.85	42.88±5.93	-4.33 (-8.11 to -0.55)	0.027[*]
	Control	38.56±5.21	38.47±4.94	-0.08 (-0.46 to 0.29)	0.631
				0.031[*]	
FFM (kg)	Intervention	52.47±5.04	48.86±5.96	-3.60 (-5.52 to -1.69)	0.001[*]
				0.009[*]	
	Control	50.73±5.59	50.48±3.68	-0.25 (-1.91 to 1.40)	0.746
FFM (%)	Intervention	58.73±3.39	62.51±4.80	3.78 (1.70 to 5.86)	0.002[*]
	Control	58.78±4.72	58.72±1.94	-0.06 (-2.20 to 2.07)	0.950
				0.010[*]	

[*]Significant difference (P-value <0.05). Paired t-test was used. [a]This P-value shows the significance level of changes in the markers before and after the study protocol. [b]This is the P-value for changes among the two study groups. BMI: Body Mass Index, Ht: Height, Wt: Weight, WC: Waist Circumference, HC: Hip Circumference, WHR: Waist to Hip ratio, WHtR: Waist to Height ratio, FM: Fat Mass, TF: Trunk Fat, FFM: Fat-Free Mass.

count of the NKCs CD16/56 subset, which is important in viral defense and involved in belongs to humoral immunity. As part of the innate immune system, NKCs play an important role in the host defense mechanism against

microbial infections [14]. Evidence suggests that NKCs largely contribute to the control of several viral infections, such as influenza [15]. Orange claims that NKC deficiency increases susceptibility to herpes virus infection [16].

Table 3. Metabolic variables before and after the study in the control and intervention groups

Variable	Group	Mean ± SD		MD (95% CI)	P value[a]
		Before	After		
DBP (mmHg)	Intervention	84.33±10.32	77.66±9.97	-6.66 (-10.24 to -3.09)	0.001[*]
	Control	76.42±8.41	77.14±4.68	0.71 (-5.04 to 6.47)	0.793
				0.024[b,*]	
SBP (mmHg)	Intervention	123.0±12.21	117.33±8.83	-5.66 (-8.40 to -2.92)	0.001[*]
	Control	122.50±7.00	118.57±3.63	-3.92 (-6.74 to -1.11)	0.010[*]
				0.350	
Zinc (µg/dl)	Intervention	66.86±7.16	71.93±12.73	5.06 (-2.65 to 12.78)	0.181
	Control	98.14±15.56	98.42±14.91	0.28 (-0.65 to 1.23)	0.525
				0.208	
Iron (µg/dl)	Intervention	76.93±14.39	72.13±21.86	-4.80 (-14.69 to 5.09)	0.316
	Control	94.21±31.00	96.64±33.52	2.42 (-4.39 to 9.25)	0.456
				0.213	
FBS (mg/dl)	Intervention	96.86±3.70	87.66±6.33	-9.20 (-11.56 to -6.83)	<0.001[*]
	Control	94.14±6.45	90.42±7.76	-3.71 (-6.52 to -0.89)	0.014[*]
				0.003[*]	
HDL-c (mg/dl)	Intervention	36.40±5.91	42.66±6.10	6.26 (4.17 to 8.35)	<0.001[*]
	Control	38.07±4.92	39.71±6.78	1.64 (-0.59 to 3.88)	0.137
				0.003[*]	
LDL-c (mg/dl)	Intervention	100.0±19.30	88.26±16.81	-11.80 (-16.4 to -7.10)	<0.001[*]
	Control	89.92±11.64	86.42±11.20	-3.50 (-8.69 to 1.69)	0.169
				0.016[*]	
TG (mg/dl)	Intervention	174.26±20.86	159.60±18.69	-14.66 (-20.2 to -9.05)	<0.001[*]
	Control	154.78±11.17	150.64±9.83	-4.14 (-8.12 to -0.16)	0.043[*]
				0.003[*]	
TC (mg/dl)	Intervention	204.33±19.91	183.80±17.79	-20.53 (-24.3 to -16.6)	<0.001[*]
	Control	184.78±12.04	176.50±17.73	-8.28 (-14.5 to -1.97)	0.014[*]
				0.001	
Cortisol (µg/dl)	Intervention	16.40±4.25	14.40±4.20	-2.00 (-2.78 to -1.21)	<0.001[*]
	Control	13.42±4.78	12.78±4.42	-0.64 (-1.44 to 0.16)	0.108
				0.015[*]	

[*]Significant difference (P-value <0.05). [a]This P-value shows the significance level of changes in the markers before and after the study protocol. Paired samples T-test was used. [b]This is the P-value for changes among the two study groups. Independent samples T-test was used. DBP: Diastolic Blood Pressure, SBP: Systolic Blood Pressure, FBS: Fasting blood sugar, HDL-c: HDL-cholesterol, LDL-c: LDL-cholesterol, TG: Triglyceride, TC: Total cholesterol.

Changes in cellular immunity markers, such as the CD3 subset, are another important finding of this study, since CD3 plays a pivotal role in the prevention of viral infections [17]. As such, it is assumed that this weight loss program may interfere with the immune markers involved in microbial defense, predisposing the patient to viral infections. Additionally, another important marker of cellular immunity, CD8, was also observed to be decreased in our study; however, the reduction was not statistically significant.

In line with this study, the study by Fathy et al. conducted on morbidly obese individuals reported that CD4 and CD8 counts decreased significantly after 30% weight loss by bariatric surgery (laparoscopic greater curvature placation). In this study, a clinically significant decrease was observed in CD8 count, which plays an essential role in immune defense; however, we also observed a clinically significant decrease in CD3 and no significant change for CD4. This discrepancy could be due to different sample sizes, amount of weight loss,

Table 4. Lymphocyte subset counts before and after the study in the control and intervention groups

Variable	Group	Mean ± SD		MD (95% CI)	P-value[a]	P value[c]
		Before	After			
CD3 (cell/μl)	Intervention	1534.0±315.7	1480.2±298.3	-54.6 (-178.6 to 69.4)	0.361	0.626
	Control	1188.8±466.7	1230.6±434.1	41.81 (-220.4 to 304.0)	0.736	
					0.470[b]	
CD4 (cell/μl)	Intervention	882.7±239.3	891.1±214.7	8.40 (-86.2 to 103.0)	0.852	0.292
	Control	989.6±382.5	1042.0±344.8	52.36 (-121.2 to 225.9)	0.526	
					0.629	
CD8 (cell/μl)	Intervention	570.8±186.9	547.7±172.1	-23.1 (-125.5 to 79.3)	0.636	0.199
	Control	589.1±189.0	633.6±204.7	44.50 (-24.9 to 113.9)	0.190	
					0.257	
CD19 (cell/μl)	Intervention	307.4±273.4	318.0±184.5	10.53 (-136.5 to 157.6)	0.880	0.270
	Control	238.3±53.9	241.2±57.5	2.88 (-31.1 to 36.92)	0.857	
					0.917	
CD16/56 (cell/μl)	Intervention	214.0±128.4	197.9±98.6	-16.13 (-101 to 68.79)	0.690	0.014[*]
	Control	310.2±85.0	331.5±89.7	21.31 (-8.12 to 50.75)	0.142	
					0.393	
CD4CD8R	Intervention	1.67±0.63	1.73±0.56	0.06 (-0.29 to 0.42)	0.715	0.834
	Control	1.72±0.70	1.72±0.65	-0.00 (-0.17 to 0.16)	0.963	
					0.729	

[*]Significant difference (P-value <0.05), the effect of intervention along with considering all the variables as confounder. [a]This P-value shows the significance level of changes in the markers before and after the study protocol. Paired samples T-test was used. [b]This is the P-value for changes among the two study groups. Independent samples T-test. [c]This is the intervention effect P-value computed through confounding for the baseline values and duration. ANCOVA test was used.

patient follow-up period, or the applied weight reduction program.

Tanaka et al. [18], found that prescription of a very low-calorie diet until a significant weight reduction (~15 kg) occurred in obese individuals could reverse the immune dysfunction. The lymphocyte subpopulations of CD3, CD4, and CD8 were increased significantly, and the diet-induced weight loss could improve T-cell immunity in obese subjects. Furthermore, CD19, CD16, and CD57 were reported to be decreased, but the difference was not statistically significant.

Field et al. [19] investigated the impact of weight loss (13±1 kg in six weeks) induced by a very low-calorie diet (1.7 MJ/d or 406 kcal/d) in obese subjects. They found a significant decrease in the lymphocyte counts and other immune markers [19]. This study, in line with ours, showed that caloric restriction could cause a decline in lymphocyte numbers, and it would be a risk factor for susceptibility to infections; and this result might be due to a higher

amount of caloric restriction and shorter weight loss duration.

Tritto et al. found that aggressive methods used by combat athletes to rapidly reduce their body weight include increased training loads and restricted food intake, both leading to impaired immune function and increased frequency of infections. They observed that rapid weight loss could independently impair immune function through affecting immune markers other than monocytes, or neutrophils, for example through adaptive immune cells as well as humoral immunity, which could play a key role in an increased susceptibility to opportunistic infections [20]. Despite the similarity of their results to the present study, we can add that moderate caloric restriction might weaken the antiviral immune defense.

In another study, Neiman et al. [8] assessed the effect of a 12-week moderate caloric restriction (4.19-5.44 MJ or 1200-1300 kcal per day) on several aspects of immunity in healthy obese women. According to the find-

ings, even with a moderate weight loss rate (9.9±1.4 kg in 12 weeks), T-cells and B-cells were decreased significantly. They found that weight loss even at a moderate rate might have adverse effects on several aspects of immunity (5).

One innovation of this study was that we considered all the measured variables as confounders and we involved all of them in the analysis since the factors effective in immune response changed during the intervention. One limitation of this study was the lack of randomization, since we prescribed medication, and therefore, we decided to enroll participants undergoing medication therapy as the intervention group. Besides, our data could not be generalized, since we enrolled one gender due to different sex hormones and their effect on the immune response.

Thus, further studies including various interventions to better evaluate both the function and counts of immune cells during weight loss are warranted. Also, there is a need to know whether the effect of weight loss on the lymphocyte subsets is short-term or long-term. In conclusion, caloric restriction, even at a moderate rate, might independently interfere with several aspects of antiviral immune defense.

Disclosure of conflict of interest

None.

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