RESEARCH ARTICLE

Behavior of plasma interferon-gamma with graded exercise in individuals with varied body mass index and age: Risk stratification of predisposition to inflammation

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ABSTRACT

Background: Increasing body mass index (BMI) and age predispose one to cardiometabolic disorders and their complications. Cardiometabolic disorders and their complications are associated with excessive inflammation leading to further exacerbation. Regular moderate exercise modulates inflammatory processes. Interferon-gamma (IFN-γ) is an anti-inflammatory cytokine which protects against excessive inflammation. We assessed the plasma levels of IFN-γ after different grades of exercise in normal healthy individuals with variable BMI and age. **Aims and Objective:** To study the behavior of plasma IFN-γ with graded exercise in individuals with varied BMI and age. **Materials and Methods:** A total of 40 healthy volunteers (21 males and 19 females) between the ages of 18 and 35 years were enlisted into the study. Each volunteer performed a bout of moderate exercise, a bout of severe exercise, and 1 month regular moderate exercise under supervision. Mean and standard deviation (SD) of BMI was 22.26 ± 3.3 kg/m^2. BMI ranged between 19 kg/m^2 and 26 kg/m^2. Blood samples from cubital vein were collected before a bout of moderate exercise (baseline), after moderate exercise, after a bout of severe exercise, and at the end of 1 month of regular moderate exercise. Plasma IFN-γ was measured by the enzyme-linked immunosorbent assay technique. **Results:** Mean and SD of IFN-γ at baseline (no exercise) was 10.54 ± 2.21 pg/ml. After a bout of moderate exercise, its levels increased to 21.21 ± 1.34 pg/ml. Mean and SD of IFN-γ after a bout of strenuous exercise was 8.32 ± 1.45 pg/ml. The IFN-γ levels increased to 38.98 ± 2.33 pg/ml at the end of 1 month of regular moderate exercise. Its levels were higher in healthy individuals with lower BMI and lower age. But on the performance of 1 month of regular moderate exercise, the IFN-γ levels of higher age group and higher BMI came closer to that of the lower age group with lower BMI. This indicates that regular physical activity confers protection against excessive inflammation in spite of higher BMI and age. **Conclusion:** Although an increase in physical stress, BMI and age predispose one to inflammation, regular physical activity of moderate nature protects against inflammation by increasing levels of anti-inflammatory cytokine, IFN-γ.

KEY WORDS: Exercise; Inflammation; Age; Body Mass Index; Interferon-gamma

Access this article online	
Website: www.njppp.com	**Quick Response code**
DOI: 10.5455/njppp.2017.7.1030213112016	

INTRODUCTION

Inflammation can be both beneficial and harmful.[1] It is needed for wound healing and body repair.[2] On the other hand, exacerbation of inflammation can lead to a predisposition to inflammatory disorders.[3] Unwanted inflammation can also result from cardiometabolic disorders.[4] Therefore, the

human body maintains a delicate balance of inflammatory status through homeostatic mechanisms.[5] An increase in body mass index (BMI) and age predispose one to inflammation or complications of disorders like diabetes that lead to inflammation.[6] On the contrary, individuals with greater BMI and higher age are sometimes more healthy than leaner individuals of younger age.[7] We postulated that one of the major reasons for this physiological aberration could be physical activity performed on a daily basis with or without one's knowledge.

The mediators of the immune system which modulate the inflammatory processes are protein molecules called as cytokines. Cytokines are released by the cells of immune system.[8] They are classified into two broad categories as pro-inflammatory and anti-inflammatory cytokines. The delicate balance of these two opposing cytokine groups maintains the healthy status of individual by mediating inflammatory processes to the extent required. For example, interferon-gamma (IFN-γ) is an anti-inflammatory cytokine, whereas tumor necrosis factor alpha (TNF-α) is a pro-inflammatory cytokine.[9] An excess of pro-inflammatory cytokines in circulation can be a predisposition to unwanted inflammation. On the other hand, an increase in anti-inflammatory cytokines can be beneficial by preventing predisposition to inflammation.[10] Hence, apart from inflammatory markers like highly sensitive c-reactive protein, these cytokines are used as biomarkers of inflammation.[11] There are quite a few studies relating to pro-inflammatory cytokines such as TNF-α and interleukin-6 (IL-6) while the research into anti-inflammatory cytokines like IFN-γ is relatively less.[12] We felt it was imperative to observe if physical activity beneficially altered levels of IFN-γ in such a way that it had a protective effect in individuals with increased BMI and age.

MATERIALS AND METHODS

A total of 40 healthy volunteers (21 males and 19 females) not accustomed to any form of exercise, between the ages of 18 and 35 years were enlisted into the study. Each volunteer performed a bout of moderate exercise, a bout of severe exercise, and 1 month regular moderate exercise under supervision. Mean and standard deviation (SD) of BMI was 22.26 ± 3.3 kg/m^2. BMI ranged between 19 and 26 kg/m^2. Calculation of the sample size was done using another study based on IFN-γ estimation.[13] The sample size calculation came to 16, with an effect size of 0.69, 90% power and 5% significance. The study was explained to all the subjects and signed consent forms were obtained from them after clarifying any doubts they had about the study. The study was started after obtaining permission from the Institutional Ethical Committee.

Blood samples from cubital vein were collected into vacutainer before a bout of moderate exercise and immediately after the moderate exercise on the 1st day. The second sample was collected after a bout of severe exercise on the 2nd day after 24 h. The third sample was collected at the end of 1 month of regular moderate exercise, soon after the bout of moderate exercise on the last day. All the grades of exercises were performed between 7 and 8 AM to nullify effect of circadian rhythm on IFN-γ levels if any.[14] The different grades of exercises were monitored. Heart rate was used for grading of exercises. When the heart rate increased by 50% from resting level, it was considered as moderate exercise. The level for strenuous exercise was when the heart rate doubled compared to the resting value, i.e., it increased by 100% from baseline.

Exercise Method

The exercise method chosen for this study was the 10 m shuttle walk test (10 M SWT) protocol.[15,16] The subjects walked on a plain surface between two markers placed 10 m apart. They would start walking when sound signal was given out by a compact disc player. The subject is supposed to reach the end of the 10 m path by the time the successive sound signal emanates. In the first level, the intervals between the sound signals are more. This means the pace of walking will be slow. With the progression of time, the time gap between the sound signals keep decreasing so that the subject will have to increase the pace of walking. The subject is deemed to have entered the successive level of SWT when the time gap decreases. For example, the time gap to cover the distance of 10 m in the first level will be 20 s. In the first level, the subject walks to and fro between the markers for 4 times. In the second level, the time gap decreases to 15 s and so on. The subjects reached the grade of moderate exercise at the 7th level and strenuous or severe exercise at 10th level based on the heart rate.

IFN-γ Assay

The level of IFN-γ was measured by the sandwich enzyme-linked immunosorbent assay technique (ELISA) using Duoset ELISA kit of R&D Systems, USA. Polystyrene microtiter plates were coated with a film of anti-human IFN-γ - monoclonal capture antibody and was incubated (4°C overnight). 2 h of incubation followed after blocking this and adding plasma. Further 2 h of incubation was done after adding biotinylated detection antibody. Next, streptavidin-horseradish peroxidase and tetramethylbenzidine substrate were administered. At the end, the whole reaction was stopped by administering 2 N sulfuric acid. The optical density reading was taken at 450 nm using an ELISA reader. Sample testing was performed in duplicates as per instructions in the kit insert. Finally, the standard curve was drawn using the standards provided by the reagent manufacturing company. With the help of the standard curve, plasma IFN-γ (pg/ml) was derived.[17]

Statistical Methods

Version 20 of SPSS software (Chicago, USA) was used for analysis. To estimate the differences of plasma IFN-γ with different levels of exercise, repeated measures analysis of variance was used. For finding, the correlation between IFN-γ, age and BMI, Pearson's correlation coefficient was used. It was considered to be statistically significant when $P < 0.05$.

RESULTS

About 20 healthy human subjects (equal number of males and females) between the ages of 18 and 35 years (mean and SD: 25.52 ± 2.41) were recruited into the study. All subjects performed a single bout of moderate exercise, a single bout of severe exercise (next day), and 1 month regular moderate exercise. Mean and SD of IFN-γ at baseline (no exercise) was 10.54 ± 2.21 pg/ml. After a bout of moderate exercise, its levels increased to 21.21 ± 1.34 pg/ml. A significant rise in the cytokine level was observed between the baseline level and after a single bout of moderate exercise ($P = 0.021$). Mean and SD of IFN-γ after a bout of strenuous exercise dropped to a value below baseline level and was 8.32 ± 1.45 pg/ml. There was a statistically significant drop between moderate exercise level and severe exercise level ($P = 0.032$). The IFN-γ levels increased to 38.98 ± 2.33 pg/ml at the end of 1 month of regular moderate exercise. Statistically significant increase was seen between 1 month regular exercise level and baseline ($P = 0.003$). The rise in the cytokine level was significant after 1 month exercise when compared to single bout of moderate exercise also ($P = 0.015$). Compared to one bout of severe exercise, the cytokine level after 1 month regular moderate exercise was highly significant ($P = 0.001$) (Figure 1). Its levels were higher in healthy individuals with lower BMI and lower age. But on performance of 1 month of regular moderate exercise, the IFN-γ levels of higher age group and higher BMI came closer to that of the lower age group with lower BMI (Tables 1 and 2). This indicates that regular physical activity confers protection against excessive inflammation in spite of higher BMI and age.
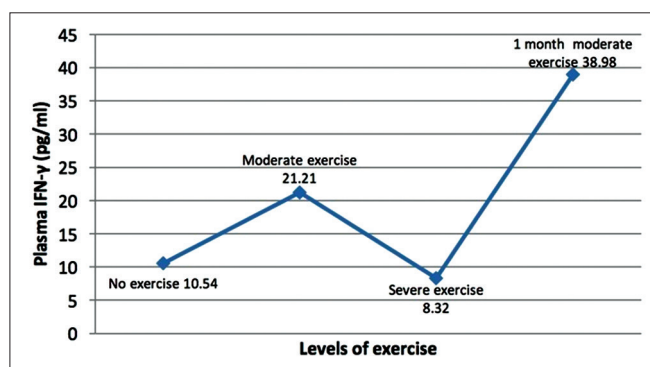

Figure 1: Plasma interferon-gamma with various levels of exercise

DISCUSSION

The health benefits of exercise are beyond doubt. Studies have shown the beneficial effects by taking into account different modes of exercise and a variety of parameters to assess the positive health outcomes. Regular exercise protects us in many ways.[18] On the other hand, excessive and unaccustomed physical exercise can be harmful to health.[19]

Inflammation is necessary for wound healing and body repair.[2] At the same time, unabated inflammation is the underlying pathophysiology of debilitating autoimmune diseases like rheumatoid arthritis.[3] Hence, inflammation is considered to be a double-edged sword.[20] In healthy individuals, there is a fine balance between agents propagating inflammation and those suppressing it. That is, the pro- and anti-inflammatory cytokines strike a fine balance through homeostatic mechanisms in our immune system to let us live in peace.[5] There is an increased predisposition to inflammation in metabolic syndrome and other cardiometabolic disorders many of which are associated with the modern sedentary life. In such individuals, pro-inflammatory cytokines such as TNF-α and IL-6 are secreted more by the cells of the immune system.[21] Therefore, we proposed that healthy individuals performing physical activity in the form of exercises should have more anti-inflammatory cytokines in circulation since unwanted inflammatory processes are kept in check because of the healthy lifestyle.

In this study, we observed the levels of IFN-γ, to assess the effect of different grades of physical activity on the immune system. IFN-γ is an anti-inflammatory cytokine. We checked if age and BMI was associated with the levels of circulating IFN-γ during the different grades of physical activity.

We observed that a single bout of moderate exercise was good enough to show its beneficial effects by significantly increasing the levels of plasma IFN-γ. This proves that a bout of moderate exercise is beneficial even if it is done intermittently in untrained individuals. It is not yet clear how long the beneficial effects of moderate exercise performed in intermittent bouts last. More often than not, it is difficult to stick to a regular exercise regime in our daily lives. On the contrary, a single bout of severe exercise reduced the plasma IFN-γ levels drastically, making one more prone to inflammation. This probably proves that in an untrained individual, it is ill advised to take up "one off" over-exercising or working out at one's whim.

Surprisingly, the levels of plasma IFN-γ were significantly the highest compared to all previous levels on the last day of 1 month of regular moderate exercise. This blood sample was collected immediately after the bout of moderate exercise on the last day of 1 month of the daily performance of regular moderate exercise. Therefore, consistency in physical activity, particularly, following a regime in which

Table 1: Correlation between BMI (kg/m²) and IFN-γ (pg/ml)				
BMI (kg/m²)	IFN-γ baseline	IFN-γ moderate exercise	IFN-γ severe exercise	IFN-γ 1 month moderate exercise
<23				
Pearson's correlation	−0.632	−0.776	−0.275	−0.936
Significance	0.022*	0.028*	0.137	0.001*
>23				
Pearson's correlation	−0.123	−0.314	−0.021	−0.934
Significance	0.145	0.010	0.211	0.001*

*$P<0.05$. BMI: Body mass index, IFN-γ: Interferon-gamma

Table 2: Correlation between age and IFN-γ after 1 month moderate exercise in subjects with age<26 years and age>26 years				
Age (years)	IFN-γ baseline	IFN-γ moderate exercise	IFN-γ severe exercise	IFN-γ 1 month moderate exercise
<26				
Pearson's correlation	−0.732	-0.810	−0.301	−0.835
Significance	0.033*	0.038*	0.092	0.001*
>26				
Pearson's correlation	−0.157	−0.341	−0.172	−0.798
Significance	0.235	0.012	0.122	0.021*

*$P<0.05$. BMI: Body mass index, IFN-γ: Interferon-gamma

one performs one bout of moderate exercise every day has more health benefits by boosting the levels of plasma IFN-γ. Clearly, regular practice of moderate exercise is more beneficial than intermittent performance of moderate exercise.

A BMI more than normal is associated with various cardiometabolic disorders. Resting levels of pro-inflammatory cytokines are high and that of anti-inflammatory cytokines low in those with abnormal BMI. For the Asian population, BMI <23 kg/m² is considered to be ideal.[22] Hence, we checked if there was any difference in association between plasma IFN-γ levels with different grades of exercise between subjects with BMI below 23 kg/m² and those above BMI of 23 kg/m². We observed that there was a significant negative correlation between BMI and IFN-γ in individuals with BMI <23 kg/m². The correlation between BMI and IFN-γ for one bout of strenuous exercise was not significant in this group. This demonstrates that lower the BMI, higher is the plasma level of anti-inflammatory cytokine IFN-γ. In those subjects with BMI >23 kg/m², there was no significant correlation between BMI and IFN-γ levels in all grades of exercise, except at the end of 1 month of regular moderate exercise. Hence, the benefits of exercise in both groups of subjects (of BMI above and below 23 kg/m²) is the same provided moderate exercise is performed daily for prolonged periods. Therefore, the benefits of physical activity are reaped better by maintaining BMI below 23 kg/m². Even if BMI is above 23 kg/m², the exercise benefit is same as for those subjects below BMI of 23 kg/m², provided a bout of moderate exercise is performed daily for a prolonged duration of one month.

It is a known fact that incidence of maladies is directly proportional to advancement of age.[23] Hence, we observed the behavior of plasma IFN-γ with different grades of exercise in the following two groups; one with age <26 years and other with age >26 years. There was a significant negative correlation between ages <26 years and plasma IFN-γ in all grades of exercise, except after a bout of strenuous exercise. Lesser the age, higher was the plasma IFN-γ. There was no significant correlation between age >26 years and plasma IFN-γ in all levels of exercise, except after 1 month of regular moderate exercise. This means that even though older subjects (>26 years) are more prone for predisposition to inflammatory conditions, this untoward pre-disposition to inflammation decreases and compares with those of age <26 years age, on the performance of 1 month of regular moderate exercise. Hence the adage, "stay young and fit by exercising regularly," holds good even at molecular level in our immune system.

Limitations of Study

Plasma sample for assaying IFN-γ was not collected before the single bout of severe exercise. We could have had a clearer picture if we had more number of subjects with greater range of BMI and age among the subjects.

Future Direction

Blood samples to assess plasma IFN-γ could be collected at regular intervals after 1 month of regular moderate exercise to observe how long the beneficial effects of prolonged moderate exercise last.

CONCLUSIONS

Moderate exercise even if performed intermittently accrues health benefits by increasing plasma levels of anti-inflammatory cytokine IFN-γ. Severe exercise decreases plasma IFN-γ levels and is hence not beneficial. Regular moderate exercise performed on a daily basis has the best health benefits compared to all other grades of exercise. Plasma IFN-γ levels decreased significantly in all age groups and in those with BMI above and below 23 kg/m^2, indicating regular performance of moderate exercise benefits even individuals with advanced age and higher BMI similar to those with lower BMI and lesser age.

ACKNOWLEDGMENTS

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