Prolonged health benefits by alteration in plasma Interleukin-6 and fasting blood sugar with graded exercise in individuals with varied BMI

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Abstract

Background: Exercise confers good health. Unaccustomed exercise is stressful and has harmful effects. Interleukin-6 (IL-6) is a marker of inflammation which increases with physical stress. We observed the changes in plasma Interleukin-6 and Fasting blood Sugar with different grades of exercise in healthy individuals of varied BMI.

Methods: Fifty four healthy volunteers (26 males and 28 females) in the age group 18 to 30 years not used to any form of exercise, were recruited. BMI of the subjects varied between 16.5 to 26.5 kg/m^2. Subject performed a bout of moderate exercise; a bout of strenuous exercise, followed by one month regular moderate exercise using Shuttle Walk Test. Plasma IL-6 was estimated before and after exercise by Elisa technique. Exercise protocol used was Standardized Shuttle Walk. FBS was assessed just before moderate exercise on first day and 24 hours after moderate and strenuous exercise and on last day of one month of regular moderate exercise.

Results: IL-6 level was significantly higher after a bout of moderate exercise compared to baseline (mean & SD: 32.6 $\pm$ 1.9 pg/ml and 63.1 $\pm$ 2.8 pg/ml respectively). It increased significantly after strenuous exercise (116.2 $\pm$ 4.1pg/ml). IL-6 level dipped significantly after one month of regular moderate exercise (15.9 $\pm$ 0.7pg/ml). FBS decreased 24 hours after a bout of moderate exercise (mean & SD: 92.9 $\pm$ 3.1 mg/dl and 83.2 $\pm$ 5.4 mg/dl respectively). It decreased further 24 hours after a bout of strenuous exercise (78.4 $\pm$ 5.6 mg/dl). It was lowest after a month of regular moderate exercise (73.96 $\pm$ 3.1 mg/dl). There was significant positive correlation between BMI and IL-6 for strenuous exercise for subjects above BMI of 23kg/m^2. There was significant positive correlation between FBS and BMI for subjects of BMI above 23kg/m^2.

Conclusions: Unaccustomed physical stress in form of a bout of moderate or strenuous exercise increases IL-6 level. Regular moderate exercise has a buffering effect by preventing the shoot up of IL-6 when challenged with acute physical stress. Exercise has a good "after effect" on FBS even up to 24 hours. Positive correlation between higher BMI and FBS indicates the importance of maintaining BMI within prescribed limits.

Key words: Interleukin-6, FBS, BMI, Exercise, Inflammation.

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Introduction

The way to maintain a healthy lifestyle is good physical exercise[1]. Although exercising regularly gives health benefits, unaccustomed exercise is harmful[2]. Various markers of stress like the hsCRP (highly selective C Reactive protein) increase in the blood when we undergo physical stress either in the form of exercise or infection[3]. Cytokines are a group of protein molecules released by cells of immune system into blood[4]. Cytokines are mediators of the immune system. There are two subtypes of cytokines called as the pro-inflammatory and anti-inflammatory cytokines[5]. Interleukin-6 is a pro-inflammatory cytokine, since its levels increases whenever the body has inflammation or even when it is pre-disposed to inflammation[6]. It is increased in inflammatory diseases like rheumatoid arthritis and systemic lupus Erythematosus[7]. An increase in IL-6 levels is also seen in cardiometobolic disorders like atherosclerosis and coronary artery disease since inflammation is associated with such diseases[8]. Complications of diabetes and hypertension are associated with inflammation; hence, levels of IL-6 shoot up in such conditions too[9]. Unaccustomed physical stress can pre-dispose to inflammation[10]. Therefore, IL-6 can be used as a tool to assess the ill-effects and good effects of different grades of exercise. We undertook this study to observe the behavior of plasma IL-6 to different severity of exercises and to see if there was any accustumisation or conditioning of the human body to bursts of physical stress, once the individual takes up regular moderate exercise on a daily basis. It is not clear how long the beneficial effects of exercise last after the performance of a bout of exercise. It is also not clear if there is any relation between BMI, FBS and IL-6 within the normal range. It would be

beneficial to know if lower BMI and FBS within normal limits lower the IL-6 level thus decreasing the probability of predisposition to inflammatory conditions. This study was undertaken to observe if there was any good effect of a bout of exercise on FBS and whether the effect of FBS would last up to 24 hours after a bout of moderate or strenuous exercise. We observed whether there is any correlation between IL-6 and BMI and also between FBS and BMI. This is relevant in the present day scenario all over the world where prevalence of obesity and high blood sugars contribute to higher incidence of cardiometobolic disorders[11].

Materials and Methods

Anthropometrically matched 26 male and 28 female volunteers in the age group of 18 to 30 years (Mean and SD: 22.87±3.4), who did not do any form of exercise previously, took part in the study. BMI of the subjects was between 16.5 to 26.5 kg/m^2 (mean and SD: 21.5±3.1). A previous study was used for sample size calculation[12]. The sample size was estimated to be 30, with power of 85 percent, effect size of 0.69 and 5% significance. Consent to participate in the study was obtained from all the subjects after explaining about the study. Clearance was procured from the institutional ethics committee for undertaking this study.

The Each subject performed one bout of moderate exercise, one bout of strenuous exercise the next day, followed by one month of regular moderate exercise from the third day onwards. During one month of regular moderate exercise, the subjects were made to perform one bout of moderate exercise every day for 30 days continuously under supervision. Plasma IL-6 was assessed before one bout of moderate exercise after the bout of moderate exercise, after one bout of strenuous exercise and at end of one month of regular moderate exercise. The grading of exercise into moderate and severe was done based on the WHO classification using the increase in heart rate. The subject is said to have performed moderate exercise when the heart rate has increased by 50 percent from resting state. To achieve strenuous exercise, the subject performs the exercise till the heart rate doubles (i.e. increases by 100%) from the resting state[13].

Exercise protocol

Exercise protocol used in this study is the Standardized 10m Shuttle Walk Test (SWT)[14]. The protocol for this exercise regime was established by Glenfield Hospital, Leicester, England and the Department of Sports Science, Loughborough University, England. The volunteers have to tread a 10m plain path. The two ends of the path are marked clearly. A pre-recorded CD player keeps giving out beeps at regular intervals. The subject starts walking at the beginning of the initial beep. He/she has to reach the end of the 10m stretch by the time the subsequent beep is sounded. After a few rounds a triple beep is heard indicating shortening of time between the beeps. This is the beginning of the next level. The intervals between the beeps keep on decreasing as time progresses. The subject has to increase the pace accordingly and walk faster. The level of the Shuttle Walk Test at which the heart rate increases by 50 percent is considered as moderate exercise. The level at which the heart rate doubles is labeled as strenuous exercise. Male subjects attained moderate exercise and strenuous exercise at 7th and 11th levels respectively of SWT. Female subjects crossed the 6th and 10th level of SWT to attain moderate and strenuous exercise respectively.

Venous blood from cubital fossa was obtained and aliquoted. Precaution was taken to obtain the blood with a single prick, since pain and inflammation due to repeated phlebotomy can lead to increased local inflammation and hence lead to increase in IL-6. IL-6 was estimated by the Sandwich Elisa method by using plasma sample of the subjects[15]. Duoset Elisa Development System (R&D Systems USA) was used for estimating IL-6. Monoclonal capture antibody (antihuman IL-6) of mouse was coated on the polystyrene microtitre plates. This was incubated at 4^{0}C for 10 hours through the night. Plasma was added after blocking and then incubated for 2 hours. Biotinylated detection antibody was added and incubated for another 2 hours. Streptavidin horseradish Peroxidase and tetramethylbenzidine (substrate) was added. 2N sulphuric acid was used to stop the reaction. Organon Teknika Elisa reader of Microwell system, Germany was used to take optical density reading at 450nm. All plasma samples were run in duplicates. Based on the standards supplied by the manufacturing company, a standard curve was obtained. Using this standard curve, concentration of IL-6 (in pg/ml) was obtained.

FBS was assessed before the first bout of moderate exercise, before strenuous exercise on next day, before moderate exercise on third day and before the bout of moderate exercise on the last day of one month of regular moderate exercise. FBS was estimated by the glucose oxidase-peroxidase method using a standard kit[16].

Statistical Methods

Statistics were run using SPSS software version 20 (SPSS Inc. Chicago, USA). Repeated measures ANOVA was employed to analyze the differences in plasma IL-6 and FBS between different grades of exercise. Pearson's correlation coefficient was used to find the correlation between BMI, IL-6 and FBS. P value less than 0.05 was considered as statistically significant.

Results

Mean and SD of IL-6 before one bout of moderate exercise on first day was 32.6 ± 0.9 pg/ml. IL-6

increased to 63.1±0.8 pg/ml immediately after one bout of moderate exercise on the same day, which was statistically significant. It further increased to 116.2±1.1pg/ml immediately after a bout of strenuous exercise the following day. This difference in the IL-6 levels between moderate and strenuous exercise was also statistically significant. IL-6 level was lowest after one bout of moderate exercise on last day of one month of regular moderate exercise with a value of 15.9 ± 0.7pg/ml. This was statistically significant compared to baseline (no exercise) value, indicating the good effect of regular moderate exercise in decreasing the level of the inflammatory cytokine IL-6. There was overall significance seen in the IL-6 values between the different grades of exercises (Fig. 1). However, no statistical significance was observed in the IL-6 levels between the two genders.

The Mean and SD of FBS before one bout of moderate exercise one first day was 92.9 ± 3.1 mg/dl (FBS 1). FBS next day morning (before one bout of strenuous exercise) was 83.2 ± 5.4 mg/dl. It decreased further to 78.4 ± 5.6 mg/dl the following morning (i.e. one day after a bout of strenuous exercise. FBS was the least on last day of one month of regular moderate exercise; 73.96 ± 3.1 mg/dl demonstrating that regular moderate exercise is good for health. It is interesting to note that the effect of single bout of moderate exercise and single bout of strenuous exercise have their effects on FBS till next day morning by decreasing the FBS levels. The FBS is least, yet within normal range after one month of regular moderate exercise (Fig. 2). Over all, the males had a higher FBS compared to the females though it was not statistically significant. FBS for baseline (no exercise) was 94.7±2.5 and 91.3±2.8 for males and females respectively. FBS after 24 hours after a bout of moderate exercise for males and females was 86.2±4.9 and 80.4±4.3 respectively. Twenty four hours after a bout of strenuous exercise, the FBS for males and females was 81.3±5.0 and 75.6±4.7 respectively. The FBS after one month of regular moderate exercise was 75.7±2.5 and 72.4±2.3 for males and females respectively (Table 1). Though there was no statistical significance in the FBS values between males and females for different grades of exercise, the mean difference between the values increased for a bout of moderate (5.8±0.6) and a bout of strenuous exercise (5.7±0.3), compared to the end of one month of regular moderate exercise (3.3±0.2). IL-6 levels increased after one bout of moderate exercise and shot up further after one bout of strenuous exercise the next day. IL-6 dipped after one month of regular moderate exercise demonstrating that regular exercise is beneficial by maintaining low levels of such pro-inflammatory cytokines.

There was significant positive correlation between BMI and IL-6 for strenuous exercise for subjects above BMI of 23kg/m^2. There was significant positive correlation between FBS and BMI for subjects of BMI above 23kg/m^2 (Table 2).

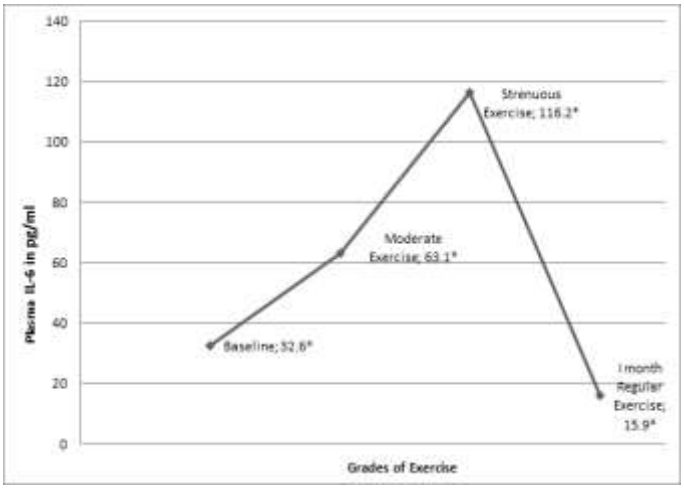

Fig. 1: Plasma Interleukin-6 (pg/ml) with different grades of exercise

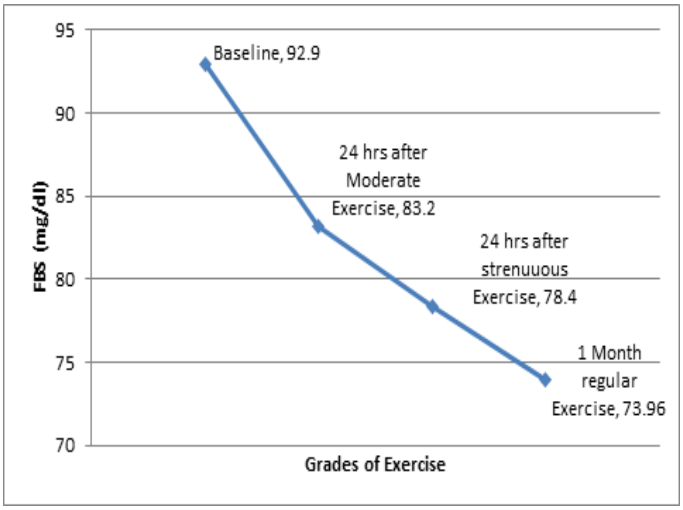

Fig. 2: Fasting Blood Sugar (mg/dl) with different grades of exercise

Table 1: Comparison of FBS between males and females with different grades of exercise (mg/dl)

	FBS Baseline	FBS 24 hours after a bout of moderate exercise	FBS 24 hours after a bout of strenuous exercise	FBS at end of 1 month moderate exercise
Males (n=26)	94.7±2.5	86.2±4.9	81.3±5.0	75.7±2.5
Females (n=28)	91.3±2.8	80.4±4.3	75.6±4.7	72.4±2.3

Table 2: Correlation between BMI, FBS (mg/dl) and IL-6 (pg/ml)

		FBS Baseline	FBS 24 hours after a bout of moderate exercise	FBS 24 hours after a bout of strenuous exercise	FBS at end of 1 month moderate exercise	IL-6 after a bout of strenuous exercise
BMI (>23kg/m^2)	Pearson's correlation	0.996	0.997	0.981	0.996	0.932
	Significance	0.003*	0.011*	0.004*	0.003*	0.015*
	n	54	54	54	54	54

*p < 0.05

Discussion

Maintenance of good health has become the primary concern of mankind today, since ill health leads to loss of precious man-hours, money, peace of mind, etc. There are different modalities to keep healthy. Physical activity is one of them. Exercise is undoubtedly good for health. Yet, it has proven to be harmful if an individual who is not accustomed to physical activity suddenly takes its up with the intention of improving one's health. Unaccustomed and/or severe forms of exercise lead to suppression of the immune system. This leads to increased susceptibility to various diseases[17].

There is a need to observe the effect of different grades of exercise on the human body at the molecular level in our immune system. Cytokines are protein molecules which act as mediators of the immune system. They are produced by the cells of the immune system to maintain a good immune status. For example, Interleukin-6 (IL-6) is a pro-inflammatory cytokine. Optimum levels of this cytokine are needed for the process of inflammation. Inflammation is necessary for normal body processes like wound repair. But excessive inflammation is harmful. Increased inflammation is a pre-disposition for inflammatory disorders and complications of cardiometobolic disorders like diabetes mellitus, hypertension, metoabolic syndrome, etc. In fact, the levels of IL-6 and another pro-inflammatory cytokine, Tumor Necrosis Factor alpha (TNF-α) are increased in inflammatory disorders like rheumatoid arthritis, Systemic Lupus Erythematosus, etc. IL-6 and TNF-α are increased in patients suffering from complications of cardiometabolic disorders. So the cytokines mediating inflammation act like double edged sword[18].

In this study, we observed the effect of one bout of moderate exercise, one bout of strenuous exercise and one month of regular moderate exercise on levels of IL-6 and Fasting Blood Glucose (FBS). With the advent of glucometers which are user friendly, FBS is one of the

most commonly used tools today by the lay public to know if they are maintaining a good health status[19]. FBS, like the oral glucose tolerance test, has been used as a predictor for onset of diabetes in normal individuals who are not yet diabetic[20]. So we included it in this study keeping in mind the needs of present generation who are health conscious and like to prevent and postpone onset of hereditary disorders like diabetes mellitus.

IL-6 levels increased after one bout of moderate exercise and shot up further after a bout of strenuous exercise compared to baseline levels. The IL-6 level immediately after one bout of moderate exercise on last day of one month regular exercise was significantly lower than baseline. All the subjects were not accustomed to any form of exercise before being recruited to this study. This demonstrates that unaccustomed exercise acts as a physical stress by increasing the IL-6 levels. Similar immune response occurs when the body is exposed to injury[21]. A single bout of moderate exercise or a single bout of strenuous exercise is deleterious to health. IL-6 level was least at the end of one month of regular moderate exercise. On the last day of one month of regular exercise, the blood sample was collected immediately after a bout of moderate exercise. Though the subjects underwent the physical stress of one bout of moderate exercise, the IL-6 was at its lowest. This indicates that regular moderate exercise conditions the human body to nullify the effect of physical stresses when we take up regular exercise on a daily basis. It also indicates that performing "binge" exercises on and off is more harmful than giving any benefits, since it may predispose the body to unwanted inflammation by increasing levels of inflammatory cytokines like IL-6. There is a significant positive correlation between IL-6 levels after a bout of strenuous exercise for individuals with BMI greater than 23kg/m^2. This shows that higher BMI is a predisposition to higher levels of inflammatory cytokines and thus inflammation, when he/she is exposed to unaccustomed physical stress.

We observed that the FBS level was lower compared to previous level even after 24 hours after a bout of exercise. The FBS level was lower than baseline when the blood sample was taken 24 hours after one bout of moderate exercise; it further lowered 24 hours after one bout of strenuous exercise, though it was well within normal limits. This shows that the good effect of exercise lasts for about a day after the bout of exercise is performed, by beneficially altering the FBS level. It was lowest on last day of one month of regular moderate exercise. So a cumulative decreasing trend was observed in the FBS levels from moderate too strenuous to regular moderate exercise. There is a trend for the males to have a higher FBS value consistently compared to the females. Males have a higher predisposition to getting diabetes than the females[22]. On the other hand, the males also had a higher BMI compared to females. So both male gender and higher BMI may contribute to higher FBS making males more vulnerable to get diabetes than the females. The mean difference of FBS between males and females was higher after 24 hours each after a bout of moderate exercise, and a bout of strenuous exercise. This difference tapered at the end of one month of regular moderate exercise. This indicates that moderate exercise performed on a daily basis decreases the probability of FBS being higher in males and decrease the predisposition to diabetes mellitus. A significant positive correlation exists between BMI greater than 23kg/m^2 and FBS at all levels of exercise. This indicates that persons with higher BMI may be more prone to getting diabetes mellitus.

Therefore in our study, we observe that a bout of moderate exercise or better still, a bout of strenuous exercise has benefits of decreasing FBS levels within the normal range. But performing moderate exercise on daily basis has the greatest benefit as it keeps both IL-6 and FBS to a minimum.

Conclusions

1. Moderate exercise performed on a daily basis helps to maintain both IL-6 and FBS at optimum levels.
2. Performing physical activity "on and off" in "binges" exercise is harmful as it increases the inflammatory cytokine levels.
3. The beneficial effect of exercise in decreasing the FBS within normal limits lasts for up to 24 hours after the exercise is performed.
4. Individuals of higher BMI have a greater predisposition to inflammation when they are exposed to physical stress.

Limitations of the study

1. IL-6 level was not estimated before the bout of strenuous exercise.
2. No significance was seen in FBS levels with different grades of exercise. This may be achieved if we increase the sample size as we take the study further.

Authors' contributions

Dr. Ambarish Vijayaraghava was instrumental in the study design, implementation, execution of the project and manuscript writing. Dr. Kirthana Kunikullaya was involved in implementation and supervision of project and manuscript writing along with data collection along with the first author. Ms. Radhika Kunnavil was involved in sample size calculation and the statistical analysis.

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Conflict of interest: None

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