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Comparison of Plasma Tumor Necrosis Factor Alpha (TNF-Alpha) Levels between Obese and Non-Obese With Graded Exercise

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Methods: The effect of single bout of moderate exercise and a single bout of strenuous exercise and one month of regular moderate exercise on plasma TNF- α level was estimated. 24 healthy non-obese subjects (15 males and 9 females) with mean age, 20.81 years and mean BMI; 21.49 ± 1.23 kg/m² were recruited. 8 obese, but otherwise healthy individuals (5 males and 3 females) with mean age 20.92 years, mean BMI; 31.78 ± 3.38 kg/m² were inducted into the study. Age range of subjects in both groups was 18-25 years. Standardized 10m Shuttle Walk Test regime was used for performing the exercise. Plasma TNF- α was measured by Sandwich ELISA technique. The reagent kit used was from DuoSet ELISA Development System (R & D Systems Europe Ltd). The readings were taken at 450nm using Organon Teknika Reader 230S.

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Comparison of Plasma Tumor Necrosis Factor Alpha (TNF- Alpha) Levels between Obese and Non-Obese With Graded Exercise

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Statistical methods: Repeated measures ANOVA was used to find the differences in TNF-α level with different grades of exercise. Post hoc tests using the Bonferroni correction was employed to compare the cytokine levels between the 2 groups.

Results: Amongst the non-obese, mean and SD values of TNF-α (in picograms per ml) for baseline (no exercise) was: 9.79 ± 1.15 , for acute moderate exercise: 13.98 ± 2.66 , for acute strenuous exercise: 48.28 ± 5.90 and after one month of regular moderate exercise: 5.89 ± 0.45 . Amongst the obese, the TNF-α level were as follows: baseline; 14.57 ± 2.36 , acute moderate exercise; 45.50 ± 15.77 , acute strenuous exercise; 95.82 ± 4.16 , and at end of one month of regular moderate exercise; 13.30 ± 2.76 . TNF-α level showed significant difference between; a) baseline and moderate exercise, b) baseline and strenuous exercise, c) moderate and strenuous exercise, d) strenuous exercise and end of one month of regular moderate exercise, e) baseline and end of one month of regular moderate exercise, f) moderate exercise and end of one month of regular moderate exercise in both obese and non obese. The TNF-α level differed significantly between obese and non obese groups in each grade of exercise. TNF-α showed overall significance between different grades of exercise in both groups ($p < 0.05$).

Conclusions: The plasma TNF-α levels were higher in the obese group compared to the non-obese group in all grades

of exercise. In both groups, plasma TNF-α increases with one bout of acute moderate exercise and increases further with one bout of acute strenuous exercise and decreases to below baseline value at end of one month of regular moderate exercise. This shows that regular moderate exercise has beneficial effects on health by way of decreasing TNF-α level.

Keywords: obese, non-obese, tumour necrosis factor alpha, exercise, inflammation.

I. INTRODUCTION

Tumour Necrosis Factor alpha (TNF-α) is a pro-inflammatory cytokine [1,2]. Higher levels of inflammatory cytokines like TNF-α and IL-6 is positively correlated with the increased prevalence and complications of life style diseases [3, 4]. Obesity is also associated with increased incidence of metabolic syndrome and other life style disorders. Unaccustomed physical activity can have harmful effects on health [5]. It increases serum IL-6 levels and the hsCRP (highly sensitive C reactive protein) to correlate with increased incidence of cardiovascular diseases [6]. Persisting physical stress increases secretion of TNF-α and IL-6 which in turn leads to premature onset of lifestyle disorders [7]. Moderate exercise performed regularly decreases severity of inflammation in rheumatoid arthritis [8],[9]. The performance of immune system improves with daily practice of moderate exercise [10]. Regular moderate exercise improves overall health in all age groups [11],[12].

Scientists have observed the plasma cytokine changes with different modes of exercises like mara - thon, military training, downhill running on a treadmill, cycling, etc., on different groups of individuals in different parts of the world [13][14][15][16]. We undertook this study in order to understand the impact of moderate and strenuous exercise on the plasma levels of TNF-α in unaccustomed obese and non-obese individuals and the benefit of exercise on accustomisation by the same individuals.

II. MATERIALS AND METHODS

24 healthy non-obese subjects (15 males and 9 females) with mean age, 20.81years and mean BMI; 21.49 ± 1.23 kg/m² were recruited. 8 obese, but otherwise healthy individuals (5 males and 3 females)

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with mean age 20.92 years, mean BMI; 31.78 ± 3.38 kg/m² were inducted into the study. Age range of subjects in both groups was 18-25 years. Subjects in both the groups were not performing any form of regular exercise. Prior consent was obtained before inducting them into the study. Clearance was obtained from the institutional ethical committee for the study. The approved number of subjects was 40.

The subjects in both groups were made to perform one bout of moderate exercise (acute moderate exercise), one bout of strenuous exercise (acute strenuous exercise) and one month of scheduled moderate exercise on a daily basis. The subjects were made to perform acute moderate exercise on the first day and acute strenuous exercise on the second day. They were made to perform scheduled regular moderate exercise from the third day onwards, for 30 days. The exercise was performed under supervision. During one month of scheduled moderate exercise, the subjects were made to perform single bout of moderate exercise daily for 30 days. The exercise was graded as moderate or strenuous based on the rise in heart rate. It was labelled as moderate when the heart rate increased by 50% from resting level and was labelled as strenuous when heart rate increased by 100% [18].

Shuttle Walk Test Protocol The exercise regime chosen was the standardized 10m Shuttle Walking test regime, described by Glenfield Hospital, Leicester, United Kingdom in collaboration with the department of Physical Education and Sports Science, Loughborough University of Technology, United Kingdom [19][20][21][22]. In this exercise protocol, the subjects walk on a 10 meter plain path at the two ends of which are placed marker cones. The subjects walk between the cones corresponding to the beeps given out by a record player. Subjects have to increase their speed of walking gradually in tandem with the shortening of intervals between the consecutive beeps as time progresses. The level of the shuttle walk regime at which the heart rate increased by 50% of the baseline was chosen as moderate exercise. The level at which the heart rate increased by 100%, i.e. doubled was considered as strenuous exercise.

A venous blood sample from cubital vein (using vacutainers) just before acute moderate exercise (baseline) was collected. Another sample was collected immediately after acute moderate exercise on the same day. After performance of acute strenuous exercise on the next day, third sample was obtained. A sample was obtained after one month of scheduled regular moderate exercise on the last day after exercise. Baseline sample just before acute strenuous exercise, and just before performance of exercise on the last day of one month regular moderate exercise was not obtained. The samples collected from each individual were aliquoted and stored at -400C till analysis.

Plasma sample was used to estimate the level of TNF- α , by using ELISA (Enzyme linked Immunosorbent Assay) method. ELISA was performed using DuoSet ELISA development system as per the manufacturer's instructions (R&D systems, USA).

Estimation of TNF- α :

Polystyrene microtiter plates (NUNC, U16 Maxisorp type, Denmark) were coated with monoclonal capture antibody (antihuman TNF- α) obtained from mouse (R&D systems, USA) and incubated at 4°C overnight. The following day, the plates were blocked and then incubated for 2 hours with plasma. This was followed by addition of corresponding biotinylated detection antibody obtained from goat (R&D systems, USA) and incubated for 2 hours. Streptavidin, horseradish peroxidase conjugate and then, 3,3',5,5'-tetramethylbenzidine substrate (Bangalore Genie, India) followed this incubation. The reaction was stopped using 2 N sulphuric acid and optical density (O.D) reading was taken at 450nm (Organon Teknika Microwell system, Reader 230s, Germany). All the experiments were conducted in duplicates. A standard curve was obtained based on the standards provided by the manufacturer. The results were expressed as concentration of cytokines (in pg/ml) read from the standard curve (concentration in range: minimum of 5 pg/ml, to maximum of 100 pg/ml).

a) Statistical Analysis

Data was entered in MS Excel and was analyzed using SPSS Version 20.0 (SPSS Inc. Chicago, USA). All the continuous variables were summarized in terms of mean and standard deviation and categorical variables as proportions. In order to test for statistical significance for differences in the mean values of TNF- α at different time points (i.e.; during various grades of exercise), in each group (obese and non-obese), repeated measures of ANOVA was employed. Further, pair wise differences were tested using Bonferroni's test. Pearson's correlation coefficient was used to find the correlation between BMI and TNF- α in both groups.

III. RESULTS

8 obese and 24 non-obese individuals took part in the study. Plasma TNF- α level was studied with different grades of exercises. Among the non obese, 15 (62.5%) were males and 9 (37.5%) were females. Among the obese, 5 (62.5%) were males and 3 (37.5%) were females. The mean BMI was 21.49 ± 1.23 kg/m² among the non-obese and 31.78 ± 3.38 kg/m² among the obese.

A repeated measures ANOVA determined that mean TNF- α levels differed statistically significantly between the various exercise levels in obese group and non-obese group ($P < 0.01$). Post hoc tests using the Bonferroni correction revealed that exercise elicited

decrease in TNF- α concentration in obese [19 ± 0.54 (Mean \pm SEM)] and non-obese group [$(42.30 \pm 0.94$ (Mean \pm SEM)] which was statistically significant ($p < 0.01$). Therefore, we can conclude that a long-term exercise elicits a statistically significant reduction in TNF- α level.

There was a significant increase in the levels of this cytokine with both acute moderate exercise ($p=0.003$ and $p=0.002$ in obese and non-obese respectively) and acute strenuous exercise ($p=0.005$ and $p=0.003$ in obese and non-obese respectively) compared to baseline value.

There was a significant rise in its levels after acute strenuous exercise when compared to moderate exercise ($p=0.043$ and $p=0.002$ in obese and non-obese respectively). The fall of TNF- α after one month of regular moderate exercise was also significant compared to baseline value ($p=0.001$ and $p=0.001$ respectively). That is, the TNF- α level decreased to below baseline level after the bout of moderate exercise on the last day of one month of regular moderate exercise regime in both groups (Table: 1, Figures: 1, 2).

Table 1 : Comparison of mean TNF alpha levels during various grades of exercise in obese and non obese group

	Group	Mean \pm SD
TNF- α at baseline	Non-obese	9.79 ± 1.15
	Obese	$14.57 \pm 2.36^*$
TNF- α after a bout of moderate exercise	Non-obese	13.98 ± 2.66
	Obese	$45.50 \pm 15.77^*$
TNF- α after a bout of strenuous exercise	Non-obese	48.28 ± 5.90
	Obese	$95.82 \pm 4.16^*$
TNF- α after 1 month regular moderate exercise	Non-obese	5.89 ± 0.45
	Obese	$13.30 \pm 2.76^*$

*TNF- α alpha in pg/dl

$n=24$ for obese and $n=8$ for non obese

* $p < 0.05$: TNF- is statistically significant between different grades of exercise and between obese and non-obese groups.

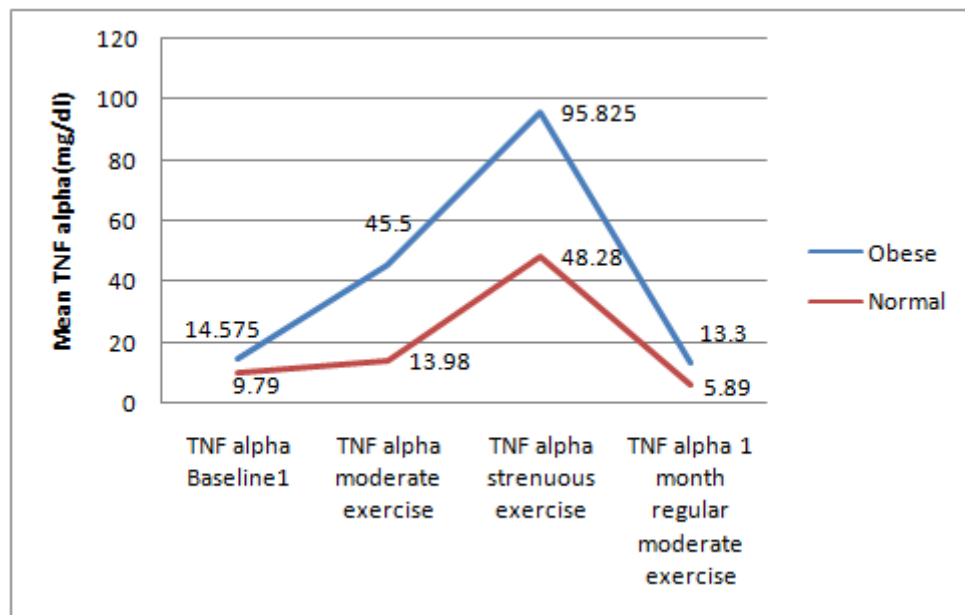


Figure 1: Changes in the TNF- α level (pg/ml) in obese and non-obese (normal) groups with different grades of exercise

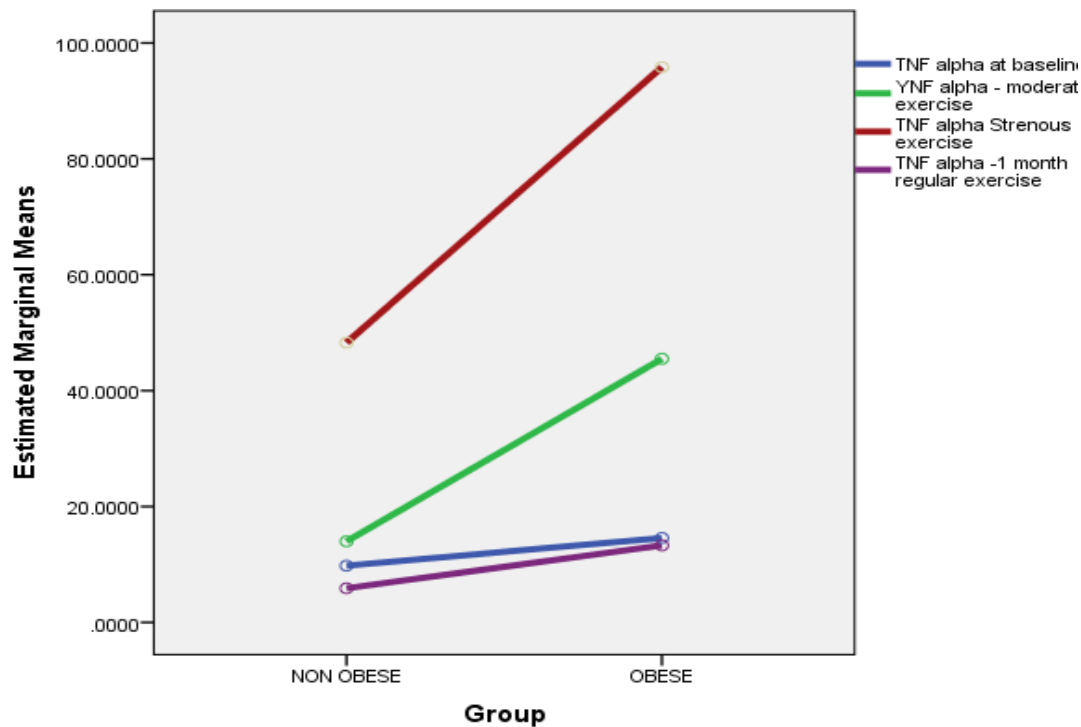


Figure 2 : Comparison of TNF- α level (pg/ml) between obese and non-obese at different grades of exercise

There was a positive correlation in both obese and non-obese groups at baseline (no exercise) but it was not statistically significant in both groups. It was found that BMI had a significant positive correlation with TNF- α in both obese and non obese groups but the correlation was high ($r=0.975$, $p<0.001$) in obese as compared to non obese group ($r=0.76$, $p<0.05$) after a bout of moderate exercise. There was a positive correlation of BMI and TNF- α alpha during strenuous exercise in obese ($r=0.59$) which was not statistically significant. There was correlation between BMI and TNF- α in non-obese group for strenuous exercise but it was not statistically significant ($r=0.16$). There was a negative correlation between TNF- α and BMI after one month of regular moderate exercise, but it was not statistically significant in both obese ($r=-0.25$) and non-obese ($r=-0.17$) groups.

IV. DISCUSSION

Obesity is a health hazard. This study was undertaken to see if there is any difference in the behavior of plasma levels of the pro-inflammatory cytokine TNF- α , when the obese subjects and non-obese subjects were made to undergo identical physical stress. Sudden and excessive physical activity is hazardous to health [23]. Physical injury and unaccustomed physical stress/exercise has similar effects on immune system. [24]. There is production of pro-inflammatory cytokines when the human body is made to undergo acute physical exercise. [25],[26]. The percentage of T cells decrease in circulation on performance of long term severe exercise. [27]. Regular

practice of moderate exercise is inversely correlated with levels of pro-inflammatory cytokines in coronary heart disease patients retarding the process of atherosclerosis [28]. Therefore, higher levels of pro-inflammatory cytokines like tumor necrosis factor alpha are harmful to health [29].

TNF- α has pro-inflammatory properties. In this study, in both the obese and the non-obese groups, TNF- α levels increased after a bout of moderate exercise and there was a further significant increase following a bout of strenuous exercise and decreased significantly compared to baseline levels when compared with one month of scheduled regular moderate exercise done on a daily basis; that is, in both the groups, TNF- α levels decreased to below baseline level after the single bout of moderate exercise on the last day of one month of scheduled moderate exercise when compared to single bout of moderate exercise without accustomisation to regular moderate exercise, in the same individuals. It can be noted that the TNF- α levels are higher in obese subjects at baseline (no exercise) level as well as at all other grades of exercise. In those subjects who perform moderate exercise on a daily basis, sudden increase in pro-inflammatory cytokine may not occur if such individuals were to perform severe bouts of unaccustomed physical activity intermittently. This may help them to tolerate sudden and unaccustomed physical stresses in life better than those who do not exercise regularly..

The immune status improves markedly with regular moderate exercise [6][7][8][10][11]. Since this study shows a fall in TNF- α level with regular moderate

exercise in both groups, a fall in TNF- α level should also be beneficial for maintaining health and immunity. TNF- α is pro-inflammatory cytokine, so its altered production leads to unnecessary inflammation and tissue damage [30]. Thus regular moderate exercise seems to modulate its release and alters its levels to the optimum levels necessary for human body to maintain good health.

Mental stress is also known to increase the level TNF- α [31]. The adaptive cytokine response may also help individuals adhering to regular moderate exercise to cope with bouts of psychological stresses encountered in daily life [32]. The levels of TNF- α may not rise drastically either [33].

Elevated levels of TNF- α interleukin-6 (IL-6) are observed in atherosclerosis, coronary artery disease and diabetes mellitus, etc [30]. Stressful bursts of physical activity in daily life, in such patients increases their levels much further and leads to exacerbation of the disease. It can be postulated that the drastic rise in TNF- α and IL-6 with bursts of physical activity or with 'acute on chronic infections' tends to become mild if such patients perform moderate exercises regularly.

Certain autoimmune disorders like systemic lupus erythematosus and rheumatoid arthritis are associated with increased plasma levels of pro inflammatory cytokines like TNF- α and IL-6, which increased inflammation [34]. Increased levels of TNF- α leads to cachexia, increased levels of C-reactive protein and other acute phase proteins, activates macrophages, increases tumour cytotoxicity, activates neutrophils and increases phagocytosis and induces secretion of other pro-inflammatory cytokines like IL-6 [36]. This study shows a positive correlation between TNF- α and BMI baseline (no exercise) though not statistically significant in both obese and non-obese groups. It was found that BMI had a significant positive correlation with TNF- α in both obese and non obese groups but the correlation was high ($r=0.975$, $p<0.001$) in obese as compared to non obese group ($r=0.76$, $p<0.05$) after a bout of moderate exercise. There was a positive correlation of BMI and TNF- α after strenuous exercise in obese ($r=0.79$, $p<0.05$) which was statistically significant. This demonstrates that the obese are more prone to secrete higher levels of pro-inflammatory cytokines like TNF- α on stressful physical activity to which they are not accustomed. There was correlation between BMI and TNF- α in non-obese group for strenuous exercise but it was not statistically significant ($r=0.16$). There was a negative correlation between TNF- α and BMI after one month of regular moderate exercise, but it was not statistically significant in both obese ($r=-0.25$) and non-obese ($r=-0.17$) groups. This may indicate that obesity predisposes to increased levels of pro-inflammatory cytokines, especially when the obese are exposed to unaccustomed physical stress. Interestingly, we found a negative correlation

between BMI and TNF- α , though not significant at end of one month of regular moderate exercise in both groups. This may be because of the increase in healthy lean body mass/muscle mass at end of one month of exercise and decrease in adiposity [37]. Till date very few studies have been undertaken simultaneously in the obese and non-obese groups of human subjects to study the effects of physical stress/exercise on plasma level of TNF- α . One of the reasons for this may be that it is difficult to convince obese subjects to perform physical exercises, especially under supervision, which are both physically and psychologically stressful for them [38]. Obesity associated inflammation is a known entity, but the mechanism controlling this pathway is still being investigated and is not clearly known [39]. Regular moderate exercise may not only benefit obese individuals but also those patients suffering from disorders related to metabolic syndrome like diabetes, inflammatory diseases and auto-immune disorders by bringing down the levels of pro-inflammatory cytokines like TNF- α . Since the behavior of plasma TNF- α level differs in obese and non-obese subjects with different grades of physical exercise, we propose that this study has potential for clinical application.

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