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# Response of Anabolic Hormones and Fat Metabolism to Different Exercise Intensities

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**Abstract:** The purpose of this study was examine the effect of different exercise intensities protocol on response of anabolic hormones (growth hormone, insulin-like growth factor-1, testosterone) and fat metabolism (free fatty acids and triglycerides) immediately after exercise and after 1 h recovery period. A nine male active from varying degrees in different sports participate in this study, (age 18.56 ±0.23years, body height 182.22±0.39 cm, body mass index 25.05 ±0.29 and body fat 11.63 ±0.48%). Subjects underwent a different intensity exercise protocol on treadmill for 24 min. During treadmill test, speed started 7 Km/h for 3 min this was followed by Seven additional consecutive runs of 3-min duration at treadmill speed of 4, 8, 7, 10, 12, 10, 12 Km/h. Oxygen uptake during exercise protocol on treadmill was measure by a gas analyzer (GmbH, Germany). Blood samples were collected from the study group immediately before and after exercise and after 60 min recovery period. Concentration of growth hormone (GH), insulin-like growth factor (IGF-1) and Testosterone (Testo) were determined by using ELISA. Free fatty acids (FFAs), triglycerides (TGs) and fasting serum glucose (GL) levels were determined by using a Kit. Results show that measurements of cardio respiratory fitness on treadmill for maximum heart rate (HRmax) was 185.44±1.24 b/min, maximum oxygen uptake (VO2max) was 2.35±0.12 L/min, VO2max per/ Kg was 32.14±2.44 $m.kg^{-1}.min^{-1}$, ventilation (VE) was 93.09±1.73 L/min and respiratory exchange ratio (RER) was 1.27±0.5. On the other hand, GH, IGF-1 levels, Testo, FFAs, TGs and glucose were significantly increased ($P<0.005$) immediately after exercise and remained elevated after 1 h recovery period except GL was decreased ($P<0.005$) and IGF was non significant, as compared with that of before exercise. While all parameters, after I h recovery, were decreased ($P<0.005$) except GH was non significant, as compared with that of exercise protocol. It was concluded that a significant increases of fat metabolism and anabolic hormones immediately after exercise seems to be clear at different intensity exercise protocol. The intensity of exercise protocol at which effective on experimental parameters may be depend on some variables such as age, exercise duration, training status, VO2max.

**Key words:** Hormones · Substrate partitioning · Body composition · Exercise · Metabolism · Recovery

## INTRODUCTION

One of the most important factors that determine the rate of fat oxidation during exercise is the intensity. Exercise physiologists often measure and describe exercise intensity in terms of percentage of aerobic power [1]. Maximal aerobic power or Maximum oxygen uptake (VO2max) is internationally accepted parameter to evaluate the cardio respiratory fitness [2].

Several studies have described the relationship between exercise intensity and fat oxidation. However there is substantial evidence showing that aerobic exercise and resistance exercise can improve body composition by increasing lean body mass and/or decreasing fat mass [3-5]. At rest and during exercise, skeletal muscle is the main site of oxidation of fatty acids (FA). In resting conditions and especially after fasting, FAs are the predominant fuel used by skeletal muscle. During low-intensity exercise, metabolism is elevated several fold compared to resting conditions and fat oxidation is increased. When the exercise intensity increases, fat oxidation increases further, until exercise intensities of about 65% VO2max, after which a decline in the rate of fat oxidation is observed Jeukendrup [6].

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During sustained exercise of moderate intensity, the rates of lipolysis (glycerol production) and fatty acid (FA) mobilization (FA production) increase in men [7, 8] although FA mobilization and lipid oxidation decline if the exercise intensity is increased to a particularly high exertion level [10-13]. During moderate-intensity exercise, elevated lipid oxidation is supported not only by the increased rate of FA mobilization, but also by a change in the relative partitioning of plasma FA toward oxidation rather than storage [8, 14-16]. In addition, Jensen [17] suggested that during moderate intensity exercise circulating FFA and intramyocellular TG provide roughly equal portions of fatty acids for oxidation. Exercise is a potent stimulus to GH release and there is some evidence that the acute increase in GH is important in regulating substrate metabolism post-exercise [18]. Pride [19] suggested that exercise-induced local mechanisms in muscle itself activate protein synthesis, rather than systemic increases in anabolic hormones being the activators of muscle protein synthesis.

The aim of this study was to determine the effect of different exercise intensities protocol on fat metabolism (FFAs and TGs) and response of anabolic hormones (GH, IGF-1 and Tests) immediately after exercise and during recovery period.

## MATERIALS AND METHODS

Nine males, active to varying degrees, in different sports were participate in this study. Their descriptive characteristics are shown in Table 1.

**Experimental Protocol:** The test was conducted in Human physical performance Laboratory at Faculty of physical Education, Helwan University, Egypt. Each subject completed a 10-overnight fast and abstained form training or strenuous exercise for at least 36 h prior to test. On arrival at the laboratory a resting blood sample were drawn from the heated hand vein catheter immediately before and after exercise and after 60 min of recovery period to determined response of fat metabolism (FFAs, TGs) and anabolic hormone (GH, IGF, Testo) for exercise protocol. Subjects then underwent a different intensity exercise protocol on treadmill for 24 min. During Treadmill test, speed started at 7 Km/ h for 3 min this was followed by Seven additional consecutive runs of 3-min duration at treadmill speed of 4, 8, 7, 10, 12, 10, 12 Km/ h. Oxygen uptake when the subjects running on a motorized treadmill was measure by a gas analyzer (GmbH, Germany). The participants breathed through a respiratory valve with a mouthpiece. The results were registered both graphically and numerically in a computer (Compac descpro 286e). HR and RER were noted during exercise protocol and specially in the last period. TheVo2 max, HRmax, VE max, RER were determined over the final 1-min of the test.

**Methods of Biochemical Determination:** ELISA for Serum GH: Enzyme-Immunoassay for Quantitative Determination of human GH from (American Laboratory Products Company) using the method of Iranmesh *et al.* [20]. This assay (22-HGH-E022) is a so-called sandwich-assay. It utilizes a specific, high affinity polyclonal rabbit antiserum coated on the wells of a microtiter plate. The hGH in the samples binds quantitatively to the immobilized antiserum. In the following step, the biotinylated antiserum binds in turn hGH. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antiserum and will catalyse in the closing substrate reaction the turn of the color, quantitatively depending on the hGH level of the sample.

**ELISA for Serum IGF-1:** Enzyme linked immunosorbent assay for the determination of IGF-1 in human serum (Rue de l'Industrie 8, B-1400 Nivelles, Belgium) using the method of Breier *et al.*[21]. The ELISA technique uses antibodies with high affinity and specificity for two different epitopes on IGF-1. A first monoclonal anti-IGF-1 antibody bound to a polystyrene well will capture the IGF-1 of the sample in the presence of a second alkaline phosphatase conjugated monoclonal anti-IGF-1 antibody.

Table 1: Physical and physiological characteristic of the subjects

| Variable | Immediately after exercise | (%C) | P-value Age (years) |
|---|---|---|---|
| | 18.56 ±0.23* | | |
| Body height (cm) | 182.22 ±0.39* | | |
| Body Mass Index (BMI) | 25.05 ±0.29* | | |
| Percent Body Fat (PBF%) | 11.63 ±0.48* | | |
| heart rate (HR b/min) | 185.44± 1.24* | + 114.8 | <0.005 |
| VO2max (L/min) | 2.35±0.12* | +473.17 | <0.005 |
| VO2max (ml.kg$^{-1}$.min$^{-1}$) | 32.14±2.44* | +446.6 | <0.005 |
| Respiratory exchange ratio (RER) | 1.27±0.05* | +41.11 | NS |
| Ventilation (VE L/min) | 93.09±1.73* | +528.56 | <0.005 |

means ± SE, %C=percentage of change as compared with that of before exercise ; n = 9

Following the incubation and the one step formation of the solid phase-IGF-1-conjugated monoclonal antibody sandwich, the well is washed to remove excess of unbound conjugated antibody. Then the chromogen/substrate is added, which turns from clear to yellow proportionally to IGF-1 concentration in the patient sample. The intensity of the yellow color is measured using a spectrophotometer with a 405 nm filter. The sample concentrations are read from a calibration curve.

**ELISA for Serum Testo:** The ACE™ competitive immunoassay (Cayman chemical comp) using the method of Pradelles *et al.* [22] for serum testosterone determination. This assay is based on the competition between Testosterone-specific rabbit antiserum binding sites. Because the concentration of the Testo tracer is held constant while the concentration of the Testo varies, the amount of the testosterone tracer is able to bind to the rabbit antiserum will be inversely proportional to the concentration of the Testo in the well. This rabbit antiserum test (either free or tracer) complex bind to the mous monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagent and then Elman's Reagent (which contains the substrate to AchE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm.

**Serum TGs:** TGs content of serum was estimated by using TGs Kit (Sigma-Aldrich,com) using the method of McGowan *et al.*[23]. This procedure involves enzymatic hydrolysis by lipase of the TGs to glycerol and FFAs. The glycerol produced is then measured by coupled enzyme reactions. The absorbance (A) of sample and that of standard were measured against the reagent blank at 540 nm.

**Serum FFAs:** Quantitative colorimetric FAs determination by using Enzy Chrom™ FFAs Assay Kit (EFFA-100) (bioassaysys.com USA) using the method of Okabe *et al.* [24]. In this assay, FFAs are enzymatically converted to acyl-CoA and subsequently to H2O2. The resulting H2O2 reacts with a specific dye to form a pink colored product. The optical density at 570nm or is directly proportional to FFAs concentration in the sample.

**Serum GL:** GL level was estimated by using glucose-kit(Egyptian American comp) using the method of Trinder [25], the intensity of the product color of sample and standard was measured at 510nm.

**Statistical Analysis:** Data are presented as mean ± standard error of the mean. Statistical analyses were performed using Fisher's protected least significant difference test. Statistical significance was set at $\alpha = 0.05$.

## RESULTS AND DISCUSSION

The cardio respiratory characteristics of the subjects data shown in Table 1 revealed that HR max was 185.44±1.24 b/min, VO2max was 2.35±0.12 L/min, VO2max was 32.14±2.44 $m.kg^{-1}.min^{-1}$, VE was 93.09±1.73 L/min and RER was 1.27±0.05.

Table 2: Biochemical assayed of the subjects, n=9

| Anabolic Hormones assayed | | | | | FFAs, TGs and Glucose assayed | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | Statistical analyses | Before exercise | Immediately after exercise | After 1hr recovery. | Variable | Statistical analyses | Before exercise | Immediately after exercise | After 1hr recovery. |
| GH (ng/ml) | Mean | 3.81 | 4.6 | 4.48 | FFAs (mg%) | Mean | 16.33 | 30.56 | 23 |
| | SE | ±0.05 | ±0.073 | ±0.20 | | SE | ±0.27 | ±0.69 | ±0.68 |
| | %C* | | +20.74 | +17.59 | | %C* | | +87.14 | +40.85 |
| | P- | | <0.005 | <0.05 | | P- | | <0.005 | <0.005 |
| | %C** | | | -2.61 | | %C** | | | -24.74 |
| | P- | | | NS | | P- | | | <0.005 |
| IGF-1 (ng/ml) | Mean | 703.56 | 727.89 | 703.67 | Tgs (mg%) | Mean | 116.5 | 139.11 | 127.89 |
| | SE | ±2.57 | ±3.44 | ±4.68 | | SE | ±1.23 | ±1.16 | ±0.91 |
| | %C* | | +3.46 | +0.02 | | %C* | | +19.41 | +9.78 |
| | P- | | < 0.005 | NS | | P- | | <0.005 | <0.005 |
| | %C** | | | -3.31 | | %C** | | | -8.07 |
| | P- | | | 0.005 | | P- | | | <0.005 |
| Testo (pg/ml) | Mean | 3.34 | 8.49 | 4.2 | Glucose (mg%) | Mean | 83.67 | 99.78 | 79.56 |
| | SE | ±0.033 | ±0.15 | ±0.09 | | SE | 0.38 | 0.52 | 0.30 |
| | %C* | | +154.19 | +25.75 | | %C* | | +19.25 | -4.91 |
| | P- | | <0.005 | <0.005 | | P- | | <.005 | <.005 |
| | %C** | | | - 50.53 | | %C** | | | -20.27 |
| | P- | | | <0.005 | | P- | | | <.005 |

NS=Non-significant
%C*=percentage of change as compared with that of before exercise.
%C**=percentage of change as compared with that of immediately after exercise.

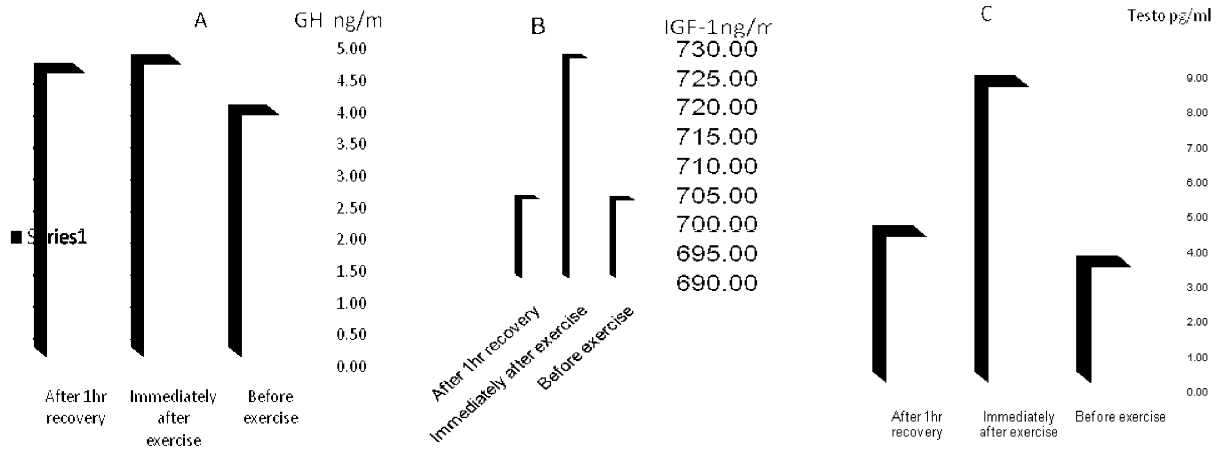


Fig. 1: Anabolic Hormones assayed as in Table 2: GH (A), IGF-1(B) and Testo(C)

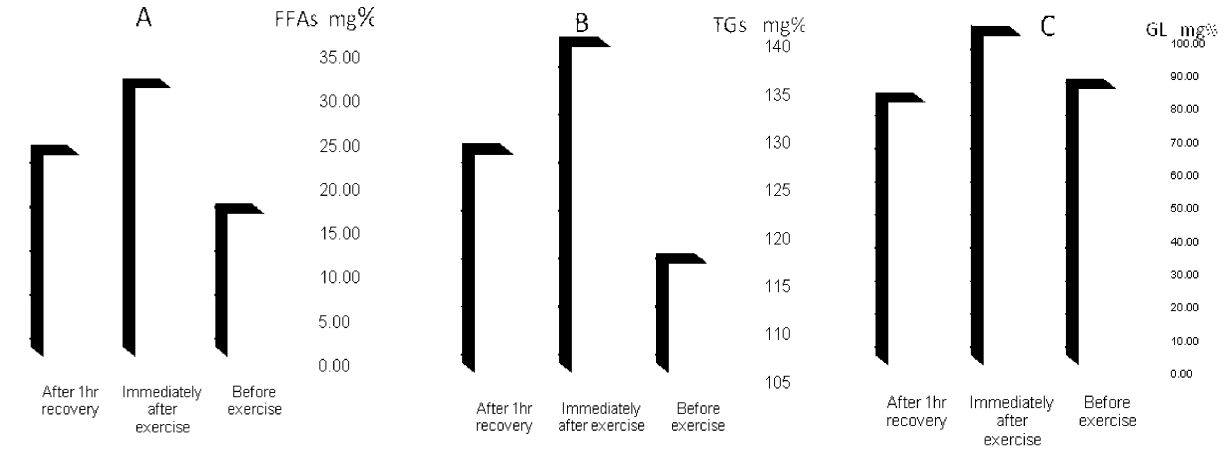


Fig. 2: FAAs(A),TGs(B) and GL(C), assayed in Table 2

Biochemical results of the subjects are listed in Table 2.

As shown in Table 2 and Fig. 1, the values of GH, IGF-1 and Testo exhibits significant elevation immediately after exercise protocol +20.74%, +3.46%, +154.19% respectively. Meanwhile, the elevation still present after1 h recovery in both GH (+17.59%) and Testo (+25.75%) but IGF-1 was non significant as compared with that of before exercise. However, after 1h recovery IGF-1 and Testo showed significant decreased-3.31%,- 50.53% respectively, while GH was non significant, as compared with that of exercise protocol.

As shown in Table 2 and Fig. 2, the levels FFAs, TGs and GL were significantly increase immediately after exercise protocol +87.14%, +19.41%, +19.25% respectively. Moreover, the elevation still present after1 h recovery in both FFAs (+40.85%) and TGs (+9.78%) while significant drop was seen in GL (-4.91%) as compared with that of before exercise. However, the levels FFAs, TGs and GL displayed significant decreased-24.74%,-8.07%, -20.27% respectively as compared with that of exercise protocol.

## DISCUSSION

The finding of the present study revealed significant increases after exercise protocol for all three anabolic hormones parameters which agreement with several previous studies [18, 26, 27]. The elevation still present in GH and Testo after 1 hr recovery. The release of GH is sensitive to many physiologic stimuli, including exercise. Cathy *et al.* [28] reported that exercise at all intensities stimulates greater GH release than that observed at rest. The concentration of GH in blood increases with time for a given work intensity and can increase 10-fold during prolonged moderate exercise. During more intensive exercise (with accumulation of lactateat 70% VO2 max) for a short term period such as (10-20 min) GH will increase by

5-10-fold [27]. Meanwhile, Godfrey *et al.* [26] suggested that exercise intensity above lactate threshold and for a minimum of 10 minutes appears to elicit the greatest stimulus to the secretion of GH. In addition, the acute increase in GH is important in regulating substrate metabolism post-exercise [18].Meanwhile, Godfrey *et al.* [26] concluded that the regulation of selective aspects of metabolic function including increased fat metabolism. However, the GH response to exercise is altered by many factors, including sex steroid concentrations, fitness level and the intensity of previous exercise sessions. Although the exact mechanisms for exercise-induced growth hormone response remain elusive, a number of candidates have been implicated. These include neural input, direct stimulation by catecholamines, lactate and or nitric oxide and changes in acid-base balance [26].

On the other hand, Pride [19] suggested that exercise-induced local mechanisms in muscle itself activate protein synthesis, rather than systemic increases in anabolic hormones being the activators of muscle protein synthesis. Much of the stimulus for protein synthesis has been attributed to insulin-like growth factor-1 with modest contributions from the hGH-GH receptor interaction on the cell membrane [26]. Indeed, the increase in IGF-I occurred essentially coincidentally with the GH increase [29].

The scientific evidence appears to be contradictory with studies showing exercise increases Testo levels [30, 31], others show that it decreases Testo levels [32] and still others showing no effect. Some feel that these small changes are due to reduction in plasma volume, or to a decrease in rate of inactivation and removal of Testo [33]. However, others have concluded on the basis of parallel increase in Luteinizing hormone (LH) concentration that the increase in plasma Testo is due to an increase rate of production [34]. While the Testo response to exercise is small and the concentration returns to resting values two hours after exercise [35]. Furthermore, significant increases in Testo concentration in young female athletes have been recorded immediately after exercise, to be returned to pre-exercise levels ninety minutes after completion [36].

This study displayed significant increases in FFAs,TGs and GL after exercise protocol. This elevation is still significant in FFAs and TGs after 1 h recovery. Fatty acids are a major fuel source for humans both at rest and during exercise. Plasma FFAs, although present only in micromolar concentrations, are the major circulating lipid fuel. FFA availability can increase two-to four-fold with moderate intensity exercise [17]. During low-intensity, prolonged exercise, blood levels of epinephrine (Epi) rise, which increases in lipase activity and thus promotes lipolysis (the breakdown of triglycerides into its glycerol and fatty acid components), this increase in lipolysis results in an increase in blood and muscle levels of FFA and promote fat metabolism [37, 38]. During high intensity exercise, lipolysis is potently stimulated, because catecholamines (fat metabolizing hormones) are highest during this intensity [39]. However, alpha receptors (receptors stimulated by catecholamines) inhibit these fatty acids from being transported by its protein carrier albumen to the musculature that it may be oxidized. Further, Romijn *et al.* [10] found that when intensity is lowered sympathetic tone lowers proportionally and a high rise in plasma fatty acids is seen. For this reason, we suggest that a combination of high intensity with low intensity additionally, the effect period of protocol exercise may be a highly effective technique for fat metabolism. Furthermore, the hormonal environment generated by exercise (increased epinephrine and decreased insulin) promotes lipolysis and mobilization of fatty acids from intramuscular triglycerides and adipose tissue triglycerides. During low-to moderate-intensity exercise (below 65 percent of VO2max), the rate of appearance of plasma free fatty acids closely matches the rate of fat oxidation [40].

However, the significant increases in the concentration of GL and FFA immediately after exercise than before exercise as a results of Lower insulin and higher Glucagon concentration whereas, plasma insulin decreases during moderate-intensity long-term exercise [41]. The lower insulin concentration during exercise favors the mobilization of glucose from the liver and FFA from adipose tissue, both of which are necessary to maintain the plasma glucose concentration [36]. With plasma insulin decreasing with long term exercise, it should be no surprise that the plasma glucagon concentration increase. This increase in plasma glucagon favors the mobilization of FFA from adipose and glucose from the liver [36].

From the results obtained it is plausible that the increase in fat oxidation After 1hr recovery is related to an elevation in GH release at the end of exercise and during recovery from exercise [28].They related the increase in fat oxidation observed during recovery from exercise to Epi and GH release, with GH being a primary correlate of fat expenditure during recovery. In addition to exerting its own lipolytic effect, GH has been reported to potentiate the lipolytic response of adipose tissue to Epi [42].

Additionally, the increased FA utilization in recovery may have been related to depletion of CHO removing energy substrate competition between oxidative glycolysis and β-oxidation [43] or alternatively to stimulation of energy sensing pathways such as activation of AMP-activated protein kinase (AMPK) in tissues such as muscle and liver [44].

Since lipolysis provides the substrate for FA mobilization, lipolysis and FA mobilization would reasonably be expected to follow similar qualitative patterns of change in response to a physiological stimulus. However, FA mobilization could also be affected by a change in intracellular handling of FA at the sites of lipolysis. Both lipolysis and FA mobilization were elevated in the post exercise recovery period in men and plasma FA oxidation was also increased leading to an elevation of total lipid oxidation after exercise. Hence, these data can be interpreted to describe a coordinated sequence of events initiated by lipolysis, leading directly to increased mobilization of FA and subsequently to increased oxidation of FA.

In summary, an increase in exercise intensity from low to moderate would result in increased lipolysis, adipose tissue blood flow and muscle blood flow, which increase the absolute rates of fat oxidation. However, at higher intensity the increase in glycolytic flux increased CHO oxidation whereas a decrease in fat oxidation is observed. There is a correspondingly positive relationship between fat expenditure during recovery and high concentration of GH.

## CONCLUSION

Significance of fat oxidation seems to be clear at different intensity exercise and the intensity of exercise protocol at which effective on fat metabolism may be depending on some variables such as age, exercise duration, training status, VO2max. A combination of high intensity with low intensity additionally, the effect period of exercise protocol may be a highly effective technique for fat metabolism and response of anabolic hormones immediately after exercise, these parameter still elevated after 1 hr recovery period except the GL was significant decreased and IGF-1 was not significant.

Again, understanding the fat metabolism and responses of anabolic hormones immediately after exercise and during recovery period helps in determination the optimal recovery period, especially with difference the type of exercise and in developing training program.

## REFERENCES


1. Berger, C.G., 2004. Understanding Substrate Metabolism during Exercise, the Crossover Concept. Top Clin. Nutr., 19: 130-135.
2. Chatterjee, S., P. Chatterjee and A. Bandyopadhay, 2005. Validity of Queens College test for estimation of maximum uptake in female students. Indian J. Med. Res., 121: 32-35.
3. de Glisezinski, I., C. Moro, F. Pillard, F. Marion-Latard, I. Harant, M. Meste, M. Berlan, F. Crampes and D. Rivière, 2003. Aerobic training improves exercise-induced lipolysis in SCAT and lipid utilization in overweight men. Am. J. Physiol. Endocrinol. Metab., 285: 984-990.
4. Pratley, R., B. Nicklas, M. Rubin, J. Miller, A. Smith, M. Smith, B. Hurley and A. Goldberg, 1994. Strength training increases resting metabolic rate and nor epinephrine levels in healthy 50-to 65-yr-old men. J. Appl. Physiol., 76: 133-137.
5. Treuth, M.S., A.S. Ryan, R.E. Pratley, M.A. Rubin, J.P. Miller, B.J. Nicklas and J. Sorkin, 1994. Effects of strength training on total and regional body composition in older men. J. Appl. Physiol., 77: 614-620.
6. Jeukendrup, A.E., Regulation 2002. of fat metabolism in skeletal muscle. Ann. N.Y. Acad Sci., 967: 217-35.
7. Friedlander, A.L., G.A. Casazza, M.A. Horning, A. Usaj and G.A. Brooks, 1999. Endurance training increases fatty acid turnover, but no fat oxidation, in young men. J. Appl. Physiol., 86: 2097-2105.
8. Friedlander, A.L., K.A. Jacobs, J.A. Fattor, M.A. Horning, T.A. Hagobian, T.A. Bauer, E.E. Wolfel and G.A. Brooks, 2007. Contributions of working muscle to whole body lipid metabolism are altered by exercise intensity and training. Am. J. Physiol. Endocrinol. Metab., 292: 107-116.
9. Romijn, J.A., E.F. Coyle, L.S. Sidossis, A. Gastaldelli, J.F. Horowitz, E. Endert and R.R. Wolfe, 1993. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am. J. Physiol. Endocrinol. Metab, 265: 380-391.
10. Romijn, J.A., E.F. Coyle, L.S. Sidossis, J. Rosenblatt and R.R. Wolfe, 2000. Substrate metabolism during different exercise intensities in endurance-trained women. J. Appl. Physiol., 88: 1707-1714.
11. Brooks, G.A. and J. Mercier, 1994. The balance of Catecholamine release, growth hormone secretion and energy expenditureduring exercise vs. recovery in men. J. Appl. Physiol., 76: 2253-2261.

12. Brooks, G.A., 1997. Importance of the 'crossover' concept in exercise metabolism. Clin. Exp. Pharmacol. Physiol., 24: 889-895.
13. Brooks, G.A., 1998. Mammalian fuel utilization during sustained carbohydrate and lipidutilization during exercise: The 'crossover' concept. J. Appl. Physiol., 76: 2253-2261.
14. Friedlander, A.L., G.A. Casazza, M.A. Horning, T.F. Budinger and G.A. Brooks, 1998. Effects of exercise intensity and training on lipid metabolism in young women. Am. J. Physiol. Endocrinol. Metab., 275: 853-836.
15. Jacobs, K.A., G.A. Casazza, S.H. Suh, M.A. Horning and G.A. Brooks, 2005. Fatty acid reesterification but not oxidation is increased by oral contraceptive use in women. J. Appl. Physiol., 98: 1720-1732.
16. Boon, H., E.E. Blaak, W.H.M. Saris, H.A. Keizer, A.J.M. Wagenmakers and L.J.C. Van Loon, 2007. Substrate source utilisation in long-term diagnosed type 2 diabetes patients at rest and during exercise and subsequent recovery. Diabetologia, 50: 103-112.
17. Jensen, M.D., 2003. Fate of fatty acids at rest and during exercise: regulatory mechanisms. Acta P. Scandinavica, 178: 385-390.
18. Widdowson, W.M., M.L. Healy and P.H. Sönksen, 2009. The physiology of growth hormone and sport. Growth Hormone and IGF Research, 19: 308-319.
19. Pride, H., 2010. Acute Increases In Anabolic Hormones do not Enhance Protein Synthesis. Stalkville, 5: 531.
20. Iranmesh, A., 1991. Human Growth Hormone (hGH) ELISA Kit. J. Clin. Endocrinol. Metab, 73: 1081-1088.
21. Breier, M., B.W. Gallaher and P.D. Gluckman, 1991. Radioimmunoassay for Insulin-Like Growth Factor-I: Solutions to Some Potentiel Problems and Pitfalls. J. Endocrinol., 128: 347-357.
22. Pradelles, P., J. Grassi and J.A. Maclouf, 1985. Enzyme-Immunoassay of eicosanoids using acetyl choline esterase as label an alternative to radio-Immunoassay. Anal. Cemb., 57: 1170-1173.
23. McGowan, M.W., J.D. Artiss, D.R. Strandbergh and B.A. Zak, 1993. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem., 29: 538.
24. Okabe, H., Y. Uji, K. Nagashima and A. Noma, 1980. Enzymic determination of free fatty acids in serum. Clin. Chem., 26: 1540-1543.
25. Trinder, P., 1969. A calorimetric method for determination of glucose. Ann. Clinc. Biochem., 6: 24.
26. Godfrey, R.J., Z. Madgwick and G.P. Whyte, 2003. The exercise-induced growth hormone response in athletes. Sports Med., 33: 599-613.
27. Felsing, N.E., J.A. Brasel and D.M. Cooper, 1992. Effect of low and high intensity on circulating growth hormone in men. J. Clin. Endocrinol. Metab., 75: 157-162.
28. Cathy, J., P. Roy, W. Laurie, Y.W. Judy, A. Rob and L. Mmrgaret, 2002. Gender governs the relationship between exercise intensity and growth hormone release in young adults. J. Appl. Physiol., 92: 2053-2060.
29. Mettel, H., C. Danm, B.J.O. Anne, B. Alina and E.S. Andkathy, 1997. Exercise-induced changes in circulating growth factors with cyclic variation in plasma estradiol in women. Deps of Med. and Ped. Harbor-UCLA, Med Cen, Torrance, California.
30. Remes, K., K. Kuoppasalmi and H. Adlercreutz, 1979. Effect of Long-Term Physical Training on Plasma Testosterone, Androstenedione, Luteinizing Hormone and Sex-Hormone-Binding Globulin Capacity. Scand J. Clin. Lab. Invest, 39: 743.
31. Vogel, R.B., C.A. Books, C. Ketchum, C.W. Zauner and F.T. Murray, 1985. Increase of free and total testosterone during sub maximal exercise in normal males. Medicine and Science in Sports and exercise, 17: 119-123.
32. Hackney, A.C., C.L. Fahrner and T.P. Gulledge, 1998. Basal Reproductive Hormonal Profiles are Altered in Endurance Trained Men. J. Sports Med. PhysbFitness, 38: 138.
33. Terjung, R., 1979. Endocrine response to exercise. In Exercise and Sport Sciences Reviews, R.S. Hutton and D.I. Miller (eds.). Macmillan, seventh volume, New York, pp: 153-179.
34. Cumming, D.C., L.A. Brunsting, G. Strich, A.L. Ries and R.W. Rebar, 1986. Reproductive hormone increase in response to acute exercise in men. Med. Sci. Sports and Exercise, 18: 369-73.
35. Jensen, J., H. Oftebro, B. Breigan, A. Johnsson, K. Ahlin, H.D. Meen, S.B. Stromme and H.A. Dahl, 1991. Comparison of changes in testosterone concentrations after strength andendurance exercise in well trained men. European J. Appl. Physiol. Andoccupational Physiol., 63: 467-71.
36. Banfi, G., M. Marinelli, G.S. Rloi and V. Agape, 1993. Usefulness of free testosterone/cortisol ratio during a season of elite speed skating athletes. J. Sports Med., 14: 373-379.

37. Powers, S.K. and E. Howley, 2001. Exercise Physiology: Theory and Application to fitness and performance. McGraw-Hill, 4th edition, New York.
38. Horowitz, J.F. and S.K. Am, 2000. Lipid metabolism during endurance exercise. J. Clin. Nutr., 72: 558-563.
39. Shepard, P. and O. Astrand, 2000. Endurance in sport.The encyclopedia of sport medicine. Black Well Sceince,second edition, pp: 14.
40. Coleman, E., 2011. Fat Loading for Endurance Sports. www.nutritiondimension.com. Edition Reviewed and Recertified.
41. Gyntelberg, F., M.J. Rennie, R.C. Hickson and J.O. Holloszy, 1977. Effect of training on the response of plasma glucagon to exercise Journal of Applied Physiology. Respiratory, Environ. exercise Physiol., 43: 302-305.
42. Beauville, M., I. Harant, F. Crampes, D. Riviere, M.T. Tauber, J.P. Tauber and M. Garrigues, 1992. Effect of long-term rhGH administration in GH-deficient adults on fat cell epinephrine response. Am. J. Physiol. Endocrinol. Metab, 263: 467-472.
43. Sidossis, L.S. and R.R. Wolfe, 1996. Glucose and insulin-induced inhibition of fatty acid oxidation: the glucose-fatty acid cycle reversed. Am. J. Physiol. Endocrinol. Metab, 270: 733-738.
44. Park, H., V.K. Kaushik, S. Constant, M. Prentki, E. Przybytkowski, N.B. Ruderman and A.K. Saha, 2002. Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphase acyltransferase and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J. Biol. Chem., 277: 32571-32577.