

## **Effect of Post-Training Meal Timing on Insulin Resistance, Fat Mass and Muscle Oxidative Stress in Relation to Serum Leptin in Male Albino Rat**

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### **Abstract**

This study was designed to investigate whether the post exercise immediate or delayed feeding would result in greater skeletal muscle mass, glycogen and antioxidant status. In addition, the effect on insulin resistance, plasma leptin and its relation to some gastrocnemius muscle parameters. Thirty adult male albino rats were divided into three equal groups; none exercised group, the immediate group receives their meals immediately after exercise and the delayed one receive their meals three hours later. Rats were exercised by swimming 1 h/day, 4 times/week for 12 weeks. Blood samples were taken for plasma leptin, glucose and plasma insulin. Insulin resistance was estimated by HOMA-IR formula. Gastrocnemius muscle was taken for determination of glycogen concentration, malondialdehyde (MDA) content and total antioxidant capacity (TAC). Data of the present study showed that muscle bulk, its glycogen content and TAC were greater with lower fat tissue weight and plasma leptin in the immediate feeding group versus the delayed. There was a significant decrease in blood glucose and insulin resistance levels of the exercised groups with their pre exercised values with insignificant changes in immediate and delayed group.

**Keywords:** Meal Timing; Insulin Resistance; Leptin; Oxidative Stress

### **Introduction**

Meal timing and composition before, during, and after exercise can assist in improving athletic performance. There are several purposes to fueling the body appropriately before exercise includes maintaining blood glucose levels, avoiding inadequate hydration and improve muscle performance [1].

The supplementation of protein or amino acids following a resistance training bout results in a net positive protein balance that enables skeletal muscle hypertrophy to take place. Carbohydrates play a limited role protein synthesis. However, carbohydrates are vital to replenish glycogen stores diminished from prolonged or high intensity exercise [2]. However, the effect of mixed standard diet in post exercise meal timing in rats remains unclear.

Adipose tissue, especially in the visceral compartment, has been considered both as a simple energy depository tissue and an active endocrine organ releasing adipokines including leptin. The interplay between adipokines and obesity is characterized by chronic low grade inflammation with oxidative stress. Over-expression of oxidative stress damages cellular structures together with under-production of anti-oxidant mechanisms, leading to the development of obesity-related complications [3].

Leptin is a hormone mainly secreted by adipocytes suggests a type of peripheral control during the development of obesity and other metabolic disorders. It has anorexigenic action, as it enters by diffusion into the central nervous system, causes satiety. Nevertheless, obesity is associated with increased leptin levels and it has been postulated that the apparent decrease in anorexigenic effects and weight loss are the result of a mechanism of resistance to it [4]. Both diet composition and exercise influence serum leptin; however, the effect of post exercise meal timing on leptin is unclear.

Skeletal muscles produce reactive oxygen species (ROS) during contraction and at rest. Exercise is associated with an increase in oxygen uptake by whole body and particularly by skeletal muscle, activation of oxidative mitochondrial metabolism and, thus, increased mitochondrial production of ROS with minor contributions from other cellular sources [5].

Acute exercise induces an increase (ROS) production dependent on exercise intensity with highest ROS in strenuous exercise this results in severe oxidative damage, including muscle weakness and fatigue, DNA mutations, lipid peroxidation, mitochondrial dysfunction and apoptosis/necrosis [6]. However, chronic exercise training may reduce oxidative stress, or even remain unchanged [7]. Due to these discrepancies, this study was conducted on a trial to:

1. Demonstrate the effect of post exercise meal timing on BMI, fat accumulation, gastrocnemius parameters (ms mass-glycogen- oxidant state)
2. Evaluate the effect of post exercise meal timing on leptin level and insulin resistance.
3. Demonstrate the effect of 12 weeks swimming exercise on gastrocnemius ms oxidative state.
4. In addition, an important question to this discussion is: "is there relation between plasma leptin, oxidant state in gastrocnemius muscle, fat accumulation and HOM-IR or not?"

## **Material and Methods**

### **Animals**

Thirty Adult male albino (Sprague dawley strain) rats were used throughout the present study. Rats were purchased from the National Research Center, Cairo, Egypt. All animals were housed in stainless steel cages offering individual housing. Each rat had a number. They were left freely wandering in their cage for two weeks with normal hour's dark: light cycle for acclimatization before starting the experiment. The ethics protocol was approved by The Laboratory Animals Maintenance and Usage Committee of Faculty of Medicine in Minia University. The rats were randomly classified into the following three groups.

1. Control non-exercised groups (CN).
2. Exercised rats exercise every other day and receive their meal immediately after exercise (EI).
3. Exercised rats exercise every other day and receive their meal three hours after exercise (ED).

### **Diet protocol**

Composition of the experimental diet was according to the formula [8]. It included the standard diet (Fat 5% [corn oil 5%], carbohydrates 65% [corn starch 15% and sucrose 50%], proteins 20.3% [casein 20% with DL-Methionine 0.3%], fiber 5%, salt mixture 3.7%, and vitamin mixture 1%). This was purchased from El-Gomhoria Company, Cairo, Egypt. HFD was left at refrigerator 4°C until needed. Obesity was induced in 12 weeks.

### **Exercise protocol**

At the beginning of the program, the rats were given the chance to stay in water bath for a short time in few numbers. After becoming familiar with water, rats were put in water bath in large numbers and were urged to swim actively all the time. We did not use any sinker to increase exercise intensity as we found in pilot experiments that animals swam continuously to stay on the water surface. Rats in trained groups were exercised by swimming 1h/day, 4times/week for 12 weeks. The swimming exercise was performed in plastic barrel (50cm) diameter filled with water (50 cm deep) maintained at 32-36°C [9].

### **Body mass index (BMI)**

Nose-to-anus length was measured in all rats. The measurements were done in anaesthetized rats with light ether. Using a ruler to measure body length, this is considered to be the distance between the bottoms of the lower incisors to the anus from ventral surface. Rats were weighed using electronic balance (FY 2000).

All rats were weighed and their nose to tail length was measured and BMI was calculated weekly.

1. The body weight and length were used to determine BMI according to the following formula: -Body mass index (BMI) = body weight (g)/length<sup>2</sup> (cm<sup>2</sup>).

### **Sample collection**

Samples of blood were taken from all rats at the beginning (by retro- orbital route) and at the end of experiment by decapitation. Fasting blood samples were withdrawn through the Retro-orbital route using heparinized capillary tubes inserted in medial canthus medial to eye globe. At the end of the experiment, all rats were sacrificed and blood samples were prepared. Sera were separated and stored in aliquots at -80°C.

Serum insulin was measured by Glory Science Insulin Enzyme-Linked Immunosorbent assay (ELISA), serum glucose by enzymatic colorimetric method using commercial kits (Biodiagnostic, Egypt) and serum leptin by CUSABIO Rat Leptin Enzyme-Linked Immunosorbent assay (ELISA). Insulin resistance was measured by the homeostasis model assessment insulin resistance (HOMA-IR) [10] HOMA-IR= fasting insulin (μIU/ml) x fasting glucose (mmol/l)/22.5.

### **Tissue samples**

#### **Fat tissue mass**

Rats were opened via ventral abdominal incision. Mesenteric adipose tissue was removed by elevating the intestines and cutting the fat free, starting at the distal end close to the appendix. When adipose tissue was divided into smaller parts, these parts were dissected piece by piece from the animal, starting at the proximal end. Samples were immediately frozen on liquid nitrogen and stored at 80°C [11].

The epididymal fat pads and testes were removed from the border of the epididymis. The retroperitoneal are found along the dorsal wall of the abdomen, surrounding the kidney, and when massive extend into the pelvis. Retroperitoneal and epididymal adipose tissue were immediately removed and weighed. This present work concentrates on visceral fat mainly, because visceral fat is more important than the subcutaneous fat in the development of obesity-induced insulin resistance [12].

#### **Gastrocnemius Muscle Glycogen Content**

Skin was removed from the hindlimb, and scissor was inserted under the Achilles's tendon; the blade was used to separate the gastrocnemius muscle

Muscle tissue was weighed, homogenized in 5 ml TCA reagent and transferred to a covered centrifuge tube, placed in a boiling water bath for 15 minutes then, cooled and centrifuged for 5 minutes. 1 ml of the supernatant was delivered to a dry test tube. 3 ml of conc. Sulphuric acid were added. Then, the tubes were heated in boiling water bath for 5 minutes then cooled again. The intensity of the pink color was read spectrophotometrically at 520 nm [13].

Specimens from gastrocnemius muscle were weighed and homogenized separately in potassium phosphate buffer 10 mM pH (7.4) in ratio 1:10. The homogenates were centrifuged at 8000 rpm for 15 min at 4°C. Determination of MDA according to the method of [14] and TAC using colorimetric assay kit according to the manufacturer's instructions (Biodiagnostic, Egypt).

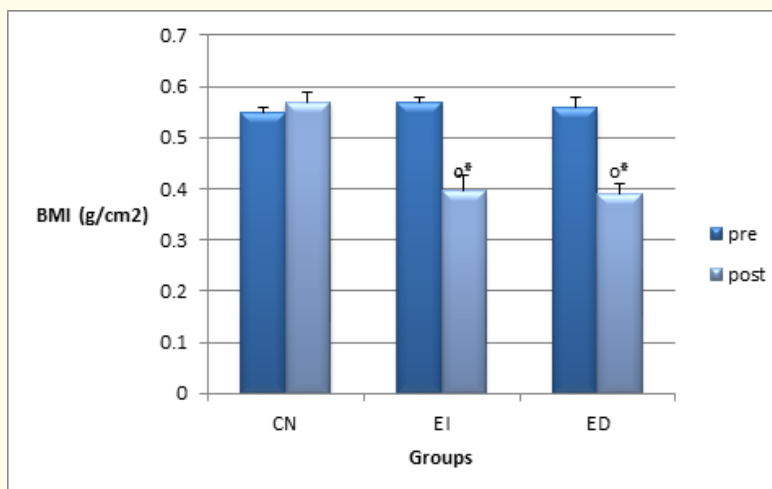
#### **Statistical Evaluation of Experimental Data**

Data were presented as means ± SE (Standard Error). The Student's t-test was utilized to determine if differences existed between the trained and sedentary and immediate and delayed animals at the beginning and end of the study. Two-way analysis of variance (ANOVA) followed by a Student Newman-Keuls post hoc analysis was used to assess the effects of obesity, training and meal timing on the dependent variables. The level of significance considered when P < 0.05. Pearson's correlation coefficients were used to evaluate the correlations between HOMA-IR, fat mass, gastrocnemius ms. Oxidative state and plasma leptin. A P-value of < 0.05 was considered to be statistically significant.

Results

Changes in Body Mass Index (BMI) in different groups

Exercised groups showed significant lower BMI than their corresponding non exercised groups. Immediate feeding groups showed no significant difference in BMI with their corresponding delayed feeding groups respectively. There was a significant decrease in BMI of the exercised groups with their pre exercised values (Figure 1) (Table 1).



**Figure 1:** Changes in Body Mass Index (BMI) in different studied groups. CN= control non exercised, EI= Exercised immediate feeding, ED= Exercised delayed feeding, BMI= Body mass index. \*: Significant from corresponding control group, □: Significant with the corresponding delayed exercised group, o: Significant difference for pre and post-intervention within each group, P < 0.05. Values are expressed as mean ± S.E.M. of 10 rats in each group.

Groups	CN	EI	ED
<b>Parameters</b>			
Fat tissue wt. (gm)	12.83 ± 0.72	4.4 ± 0.28*□	6.3 ± 0.5*
Gastronecmiusms. Wt. (gm)	3 ± 0.23	6 ± 0.5*□	4.31 ± 0.4*
Ms. Glycogen (mg/gm tissue)	285.4 ± 34.4	735.5 ± 83.66*□	505.5 ± 43.65*
Gastronecmius MDA (pg/mg tissue)	25.69 ± 3.59	20.42 ± 1.8*□	23 ± 2*
Gastronecmius. TAC(UM/mg tissue)	15.85 ± 2.1	20.5 ± 3.5*□	17 ± 2.5*

**Table 1:** Changes in Body Mass Index (BMI) in different studied groups.

CN= control non exercised, EI=Exercised immediate feeding, ED=Exercised delayed feeding.

\*: Significant from corresponding control group, □: Significant with the corresponding delayed exercised group, P < 0.05. Values are expressed as mean ± S.E.M. of 10 rats in each group.

Changes in fat mass and gastrocnemius muscle parameters in different groups

Exercised groups showed significant lower fat weight and gastrocnemius MDA with significantly higher values in the gastrocnemius muscle weight, its TAC and glycogen content than that of the corresponding non exercised group. In addition, the immediate groups, there were significantly lower values in the fat tissue weight and gastrocnemius MDA that accompanied with significantly higher values of the gastrocnemius weight, its glycogen content and TAC when compared with the delayed (Table 2).

	CN		EI		ED	
	Pre interventional	post interventional	Pre interventional	post interventional	Pre interventional	post interventional
Glucose (mg/dL)	77.2 ± 7.7	69.5 ± 2.3	81.67 ± 3	70.5 ± 5.5 <sup>o</sup>	85 ± 5.7	69.48 ± 9.2 <sup>o</sup>
Insulin (uIU/mL)	3.9 ± 0.59	3.97 ± 0.35	4.1 ± 0.3	3.9 ± 0.2	4 ± 0.6	3.9 ± 0.5
HOMA-IR	19.3 ± 3.4	17.9 ± 0.7	22.6 ± 4.3	16.1 ± 1.1 <sup>o</sup>	23.3 ± 1.9	18.94 ± 1.6 <sup>o</sup>
leptin (ng/mL)	6.4 ± 0.16	6.5 ± 0.20	6.5 ± 0.1	4.5 ± 0.13 <sup>o*□</sup>	6.2 ± 0.3	5.2 ± 0.2 <sup>o*</sup>

**Table 2:** Changes in plasma glucose, insulin, HOMI-IR and leptin in different groups.

CN= control non exercised, EI= Exercised immediate feeding, ED= Exercised delayed feeding, HOMA-IR= insulin resistance as assessed by homeostasis model, \*: Significant from corresponding control group, □ Significant with the corresponding delayed exercised group, o:Significant difference for pre and post-intervention within each group, P < 0.05. Values are expressed as mean ± S.E.M. of 10 rats in each group.

### Changes in plasma glucose, HOMI-IR and leptin in different groups

Exercised groups showed significantly lower plasma leptin values than control group. Additionally, the immediate groups showed significantly lower plasma leptin levels as compared with the delayed groups (Table 3). There was a significant decrease in blood glucose, insulin resistance and plasma leptin levels of the exercised groups with their pre exercised values.

Variables	Plasma leptin r	p
HOMA-IR	0.59	0.2
Fat mass	0.8	0.03
Gastrocnemiusms. MDA	0.8	0.04
Gastrocnemiusms. TAC	-0.9	0.01

**Table 3:** Pearson Correlation Coefficients between plasma leptin and others parameters (HOMA-IR, body fat and gastrocnemius ms oxidative state), HOMA-IR = insulin resistance as assessed by homeostasis model.

### Correlation analysis between plasma leptin and others parameters (HOMA-IR, body fat and gastrocnemius ms oxidative state)

There were strong positive correlations between (plasma leptin& fat mass) and (leptin and gastrocnemius ms.MDA). This correlations were significant [P = 0.03, 0.04 respectively]. There were strong negative correlations between leptin and gastrocnemius ms TAC. This correlations was significant [P = 0.01]. There were moderate positive correlations between plasma leptin and HOMA-IR. This correlations was insignificant [P = 0.2].

### Discussion

Regular physical exercise has been shown to be one of the most important lifestyle influences improving functional performance, decreasing morbidity and all causes of mortality [15].

Swimming exercise reduced significantly BMI and fat mass which is in agreement with [16]. Exercise training produced this effect by reduced inflammatory cytokine levels, increased lipolytic responses to increasing catecholamine, improved mitochondrial biogenesis and increased the activity of enzymes involved in β-oxidation [17,18]. Also, adaptation to endurance training increases the energy expenditure helping body weight loss [19].

In the present work, immediate feeding showed non- significant change in BMI than the delayed group respectively. This can be explained by the increase in muscle weight in the immediate group than the delayed one. Similar results by [20].

The immediate group showed significantly lower fat mass weight with significantly higher gastrocnemius ms weight than their corresponding delayed group this agreed with [21-22]. This could be explained by [23] who reported that the increase in lipoprotein lipase (LPL) in immediate group Thus more energy after exercise would be directed away from fat stores and toward muscle store facilitating muscle protein and glycogen synthesis and decrease fat storage in the immediate group.

In the present study, the trained groups showed higher muscle glycogen than the sedentary one respectively. This in agreements with [24]. Training increases PI3-kinase activity [25]. That enhances insulin sensitivity, glycogen synthase expression [26].

Exercised groups showed significant decrease in blood glucose, insulin resistance than their corresponding pre experimental values. This is agreed with [27]. Various mechanisms have been suggested for a decrease in insulin resistance with swimming exercise. These include: (1) Increased muscle mass that is an insulin sensitive tissue [28]; (2) Muscles release myokines that are involved in anti-inflammatory responses. IL-6 is the primary myokine released in response to exercise and has been shown to increase levels of the anti-inflammatory IL-10 and decrease levels of the pro-inflammatory TNF- $\alpha$  that reduced the intramyocellular fat content and improve IR [29]; (3) Increased expression of hexokinase, translocation of glucose transporter 4 (GLUT4) to the cell surface and expressing higher levels of insulin receptors in the adipocytes [12].

Much controversy exists concerning the effects of endurance training on the oxidative status and antioxidant defense systems of skeletal muscle, which may decrease, increase, or even remain unchanged [30-31]. Some controversy might arise from the different methodology used for determinations, differences in duration of exercise and differences in the models employed (running vs. swimming). Training can play positive or negative effects on oxidative stress, depending on training load and specificity [15].

Increased ROS with exercised could be due to the Increased metabolic rate and consumption of oxygen by muscle fibers, increased temperature and decreased PH of cellular muscle during the exercise [32]. Major sites of ROS generation in active muscles during exercise are mitochondria, xanthine oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, phospholipase A2 dependent processes, and some immune cells including macrophages, monocytes, eosinophils and neutrophils [33]. Exercise-induced increase generation of ROS [34].

Exercise produces ROS and whether they are beneficial or dangerous to health is dependent upon the ROS concentration, duration of ROS exposure and training status. A single exhaustive exercise increases ROS, which cannot be buffered by endogenous antioxidants, in untrained individuals. This results in severe oxidative damage, including muscle weakness and fatigue, DNA mutations, lipid peroxidation, mitochondrial dysfunction and apoptosis/necrosis [35]. Regular exercise training induces the endogenous antioxidative system and protects the body against adverse effects of oxidative damage [36].

Skeletal muscle contractions during exercise lead to elevated levels of (ROS) in skeletal muscle and this lead to a reduction of force generation. Accumulated ROS produced during exercise also have positive effects by influencing cellular processes that lead to increased expression of antioxidants by activation of two important redox-sensitive signaling pathways including nuclear factor  $\kappa$  B (NF- $\kappa$ B) and mitogen activated protein kinase (MAPK). These molecules are particularly elevated in regularly exercising muscle to prevent the negative effects of ROS by neutralizing the free radicals [37].

In the present study, exercise training decreased MDA levels and augmented TAC as compared to control groups explained by positive effect of training on production antioxidant. Immediate feeding rats increased TAC and lowered MDA compared to delayed group. This effect is largely due to the lower fat mass in immediate group.

Leptin is a hormone secreted from adipose tissue. Leptin correlates with the fat content in our bodies [38-39]. This correlation explained the decreased of plasma leptin in lean, trained and immediate groups in compared to obese, none trained and delayed groups respectively in the present study.

Numerous endocrine factors contribute to the regulation of leptin which affect with change body fat content. Some studies have explained that any change in leptin levels after endurance training is related to change in insulin levels or sensitivity [40-41]. Moreover, with the increase in adipose tissue, not only serum leptin levels tend to increase, but also the ability of insulin to stimulate glucose transport and metabolism in adipocytes and skeletal muscle is impaired resulting in peripheral insulin resistance and hyperglycemia [42].

In the present study, there were moderate positive correlations between plasma leptin and HOMA-IR which are in agreement with [43].

This correlation was insignificant [ $P = 0.2$ ] after exercise by improving fat accumulation. This data suggesting that the size of the adipose tissue is a key regulator of plasma leptin.

There were strong positive significant correlation between plasma leptin and gastrocnemius ms.MDA and strong negative correlation between leptin and gastrocnemius ms TAC. This could explained that leptin promotes oxidative stress, increasing phagocytic activity of macrophages, inducing pro-inflammatory cytokine synthesis (Tumour necrosis factor- $\alpha$ , Interleukins -6), and interferon-gamma, exerting its effect on several cells (e.g., T-cells, monocytes, neutrophils, and endothelial cells) [44] and also increasing levels of markers of endothelial cell dysfunction [45]. Pro-inflammatory effects of leptin are related to structural and functional similarities with the IL-6 family of cytokines [46].

## Conclusion

Immediate feeding after the exercise bout produced greater increase in muscle bulk and muscle glycogen, with decreased fat tissue weight and plasma leptin level in comparison with that of the corresponding delayed feeding either on obese and lean groups.

Adaptive responses to exercise training are crucial in maintaining physiological homeostasis and health span. Exercise-induced aerobic bioenergetic reactions in mitochondria and cytosol increase production of (ROS), where excess of ROS can be scavenged by enzymatic as well as non-enzymatic antioxidants to protect against deleterious oxidative stress.

There were positive correlation between (plasma leptin& body fat content),(plasma leptin& HOMA-IR) and(plasma leptin and oxidative stress).

## Recommendations

Sport nutritionist should advise training subjects to receive their meals immediately after exercise.

Future studies under different physiological conditions studying the effect of post exercise meal timing with gender difference and measure muscle contractility, plasma ghrelin and plasma adiponectin in relation to meal timing.

## Authors' Conflict of Interest Disclosure

The authors stated that there are no conflicts of interest regarding the publication of this article.

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