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The Effect of Creatine Supplementation on Weight Loss and Physiological Status in Obese Laboratory Rats (*Rattus norvegicus*)

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Abstract:

This study was conducted at the University of Basra, College of Science - Department of Biology. The study included the evaluation and comparison of the physiological and histological effects of the aqueous solution of the dietary supplement creatine on laboratory rats. The results revealed a significant decrease in weights ($p < 0.05$) in the group of animals treated with the T3 creatine solution, where a notable drop in weights was observed. Meanwhile, the control group (C). The results revealed a significant increase ($p < 0.05$) in the levels of both albumin and globulin, as well as total protein, in the animals treated with the T3 creatine solution compared to the control group. The results revealed a significant decrease ($p < 0.05$) in leptin levels compared to the control group C. The results revealed a significant decrease ($p < 0.05$) in the levels of thyroid hormones T3 and T4 in the treated animals compared to the control group C. The results revealed a significant decrease ($p < 0.05$) in glucose and insulin levels, as well as insulin resistance, compared to the control group C. The results revealed a significant decrease ($p < 0.05$) in the levels of liver enzymes

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AST and ALT compared to the control group C. The results revealed a significant decrease ($p < 0.05$) in levels of triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) compared to the control group. Additionally, there was a significant increase ($p < 0.05$) in high-density lipoproteins (HDL) levels when compared to the control group.

Keywords: Obesity, Creatine, Dietary supplements, Fats, Cholesterol, Obesity disease.

Introduction :

Obesity is one of the pressing issues of our time, with the number of affected individuals soaring in recent years across vast regions of the globe. Factors such as age, gender, and ethnicity play a secondary role. At the same time, the primary culprit lies in diets rich in high-fat foods, alongside genetic or environmental influences (1). Many individuals struggling with obesity turn to artificial dietary supplements and various medications as a remedy, leading to a significant surge in demand for these products across all sectors. This has given rise to new varieties, such as, minerals, fatty acids, omega-3, and bodybuilding supplements (2).

Creatine, often embraced by athletes to amplify the strength and size of skeletal muscles while enhancing their performance, it serves as a natural energy source, providing a steady supply to the muscles, allowing athletes to persist in their workouts for extended periods without succumbing to hunger or fatigue within the kidneys, liver, and pancreas (3).

Creatine :

Creatine is a nitrogenous compound that is not a protein, synthesized in the muscle structure with the help of enzymes that extract water molecules from creatine phosphate. It can be found in milk, red meats, and many fish. Testing its levels in the blood is one of the most crucial assessments for evaluating kidney function (4). This compound is made up of the amino acids Methionine, Glycine, and Arginine, which together constitute 1% of the blood volume in the body. It is produced in the liver, pancreas, and kidneys. It is present in small amounts in the blood of healthy individuals, approximately 1g daily (3) .Creatine levels increase in conjunction with kidney diseases, both acute and chronic inflammation,

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arteriosclerosis, or in cases of urinary tract obstruction for various reasons. Its presence is noted in intestinal juices, blood, and urine (5) .

Creatine testing is a more precise method for assessing kidney function. Conversely, in cases of muscle atrophy, heart failure, hyperthyroidism, or various advanced liver diseases, creatine levels significantly decrease, with 95% of the body's creatine reserves found in skeletal muscles (6).

Creatine as a Dietary Supplement :

Dietary supplements are substances that complement our diets, aimed at enhancing both physical and mental capabilities. They can be vitamins, minerals, or plant and herbal extracts, all of which contribute positively to human health. Yet, they cannot replace a well-rounded meal (7). Athletes, in particular, have turned to creatine as a dietary supplement, available in various forms such as bars, powders, drinks, capsules, gummies, or gels, to boost physical and muscular performance and enhance their sense of vitality by promoting ATP (adenosine triphosphate), the energy source for muscle contraction and response, while also increasing muscle mass (8). However, excessive use can lead to nausea, stomach pain, and diarrhea accompanied by intestinal cramps and weight loss. In severe cases, it may result in facial swelling, difficulty breathing, rapid heartbeat, and trouble urinating due to dehydration and electrolyte imbalance (9).

Materials and Methods

After providing the animals with the nutrient-rich standard diet and inducing obesity over 8 weeks, the animals were weighed. Fat images were measured to confirm the onset of obesity and increased fat levels compared to the standard random sample in the negative control group, which consisted of twenty animals fed the regular standard diet. The animals that developed obesity were then divided into two equal groups as follows :

Group One (C-) - The negative control group during the obesity induction period, consisting of 20 animals fed a balanced standard diet for eight weeks during the obesity induction phase, was neglected after the onset of obesity .

Group Two (C+) - The positive control group, consisting of 20 animals, continued to be fed the high-fat diet throughout the experiment without any additional dosing .

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Group Three (T3) - This group includes 20 animals fed a high-fat diet. They administered an oral dose using a 1 ml medical syringe and a special curved dosing device with a solution of creatine monohydrate at a dose of 1000 mg/kg of body weight, given only once a day. The daily therapeutic dose of creatine was calculated by multiplying the dose concentration by the animal's weight, which ranges between 380-420 g (10). The methods and weights for dosing the animals used in the experiment with the creatine supplement at 1000 mg/kg were followed according to (11).

Preparation of the Creatine Solution :

The solution was prepared in the lab by adding 5 grams of creatine to 75 ml of water to ensure complete dissolution and optimal absorption by the body during administration to the animal. It's essential to shake the bottle well before use, as the solute tends to settle at the bottom.

Measuring Weight Gain Rates :

The weights of the test animals were compared at the beginning and end of the experiment, and the weight gain rate was calculated using the equation below.

The weight gain rate of the animal (g) = final weight (g) - initial weight (g)

Sample Collection :

Once the experiment concluded and the designated study period had elapsed, the animals were weighed to determine their final weight. The experimental animals were then sacrificed by placing them in a glass container filled with cotton soaked in chloroform (the closed method) for anesthesia.

Collection of Blood Samples

Blood is drawn from the heart using a heart puncture with a sterile 5ml syringe. Two milliliters are placed in pre-labeled plastic tubes containing EDTA, an anticoagulant, for the purpose of conducting blood tests on the plasma. The remaining three milliliters of blood are placed in tubes containing gel and immediately centrifuged at 3000 RPM for fifteen minutes. After this, the serum, free of red blood cells, is separated using a pipette and transferred into smaller Eppendorf tubes, which are labeled and stored in a deep freezer at -20°C for future biochemical testing.

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Devices and Materials :

In the experiment, several devices were employed, including a centrifuge, a gas mass spectrometer, a liquid separation funnel, and a compound light photo microscope. All the equipment and the number of tests used to detect fat levels, hormones, and blood parameters were sourced from reputable global companies. The materials were of high purity and well-known origin (kc20 Kubota, Mass Hunter, Gosonic, Stuart, VWR) and came from esteemed manufacturers in China, the USA, and Germany.

Results :

The weight gain rate of the groups after the treatments.

The results, as shown in Table 1, indicated a significant decrease in weights ($p < 0.05$) in the group of animals treated with the T3 creatine solution at a significant level ($p < 0.05$), where a notable reduction in weights was observed compared to the control group C. It is worth noting that the control treatment continued to show an increase in weight compared to the weight at the beginning of the experiment.

Table (1): Effect of Creatine Solution on Body Weights of Obese Experimental Animals

Group	Final Body Weight (g)	Weight Loss (g)
C	411.25 ± 5.82^{c}	33.50 ± 22.13^{a}
T3	333.00 ± 30.27^{ab}	-53.25 ± 31.63^{bc}
LSD	11.59	14.51

Values represent Mean ± Standard Error

Different lowercase letters within the same column indicate significant differences between groups.

C: Obese control group.

T3: Third treatment group administered creatine solution at a concentration of 1000 mg/kg body weight for 30 days.

Blood Images :

The levels of fats were measured, including cholesterol, triglycerides, low-density lipoproteins, high-density lipoproteins, and very low-density lipoproteins, in the negative

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control group. The results aligned with the established standards for the relative limits set for healthy, disease-free animals.

Table No. (2) illustrates the level of blood fats in healthy animals C-

Standards					groups
VLDL mg\dl	LDL mg\dl	HDL mg\dl	TC mg\dl	TG mg\dl	
8.63±0.23b	18.19±0.13b	43.91±1.09a	62.98±0.13b	44.73±0.43b	C-

The impact of obesity on globulin, albumin, and total protein (g/100ml) and its treatment with creatine solution :

The results presented in Table 3 revealed that treating obese rats led to a significant increase ($p < 0.05$) in the levels of both albumin and globulin, as well as total protein in the animals treated with creatine solution T3, compared to the control group C.

Table 3 illustrates the therapeutic effect of creatine solution on globulin, albumin, and total protein levels in the blood of obese animals.

Total protein	Albumin	Globulin	groups
5.41± 0.54a	2.52±0.23a	2.89±0.49a	C
7.89±0.80b	3.55±0.78b	4.34±0.19b	T3
0.57	0.40	0.30	LSD

The numbers represent the mean ± standard error.

The presence of different lowercase letters among the groups indicates significant differences. C represents the control treatment.

T3 (the third treatment group) was administered a creatine solution at a concentration of 1000 mg/kg of body weight for a duration of thirty days.

Leptin Hormone :

The results shown in Table 4 revealed that treating obese animals with T3 creatine solution led to a significant decrease ($p < 0.05$) in leptin levels compared to the control group C.

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Table No. (4) illustrates the therapeutic effect of creatine solution on leptin hormone levels in the blood of obese animals.

Leptin	groups
5.56±0.28b	**C**
3.03±0.68a	**T3**
0.30	**LSD**

The numbers represent the mean ± standard error: the presence of different lowercase letters among the groups indicates significant differences.

C represents the control treatment, while

T3 (the third treatment group) was administered a creatine solution at a concentration of 1000 mg/kg of body weight for a duration of thirty days.

Thyroid Hormones :

The results shown in Table 5 revealed that treating obese rats with a creatine solution led to a significant decrease ($p < 0.05$) in the levels of T3 and T4 in the animals compared to the control group.

Table 5 illustrates the therapeutic impact of creatine solution on the thyroid hormones T3 and T4 in obese experimental animals.

standards		groups
T4	**T3**	
56.05±5.10c	0.55±0.23c	**C**
27.29±3.02a	0.40±0.02a	**T3**
2.52	0.025	**LSD**

The numbers represent the mean ± standard error–

the presence of different lowercase letters among the groups indicates significant differences.

C represents the control group, while

T3 (the third treatment group) was administered a creatine solution at a concentration of 1000 mg/kg of body weight for a duration of thirty days.

Glucose and Insulin :

The results presented in Table 6 revealed that treating obese rats with T3 creatine solution led to a significant decrease ($p < 0.05$) in glucose and insulin levels, as well as insulin resistance, compared to the control group C.

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Table number (6) illustrates the therapeutic effect of creatine solution on glucose, insulin, and insulin resistance in obese experimental animals.

Standards			Groups
Iinsulin resistance	insulin	glucose	
2.75±0.65b	0.32±0.05c	193.13±30.16b	C
0.36±0.24a	0.14±0.05a	97.94±7a	T3
0.25	0.036	10.06	LSD

The numbers represent the mean ± standard error.

The presence of different lowercase letters among the groups indicates significant differences .

C represents the control group, while

T3 (third treatment group) was administered a creatine solution at a concentration of 1000 mg/kg of body weight for a duration of thirty days.

Liver Enzymes ALT & AST (International Units/Liter) :

The results presented in Table (7) reveal that treating obese rats with T3 creatine solution led to a significant decrease ($p < 0.05$) in the levels of liver enzymes AST and ALT compared to the control group C .

Table (7) illustrates the therapeutic effect of the creatine solution on liver enzymes AST and ALT in obese experimental animals.

Standards		Groups
ALT u\l	AST ul	
478.11±56.94c	544.83±111.52b	C
138.28±54.52ab	129.20±50.43a	T3
28.71	65.8	LSD

The numbers represent the mean ± standard error .

-The presence of different lowercase letters among the groups indicates significant differences .

-C represents the control group .

T3 (the third treatment group) was administered a creatine solution at a concentration of 1000 mg/kg of body weight for thirty days .

Fat images:

The results, as shown in Table (), revealed that treating obese rats with T3 creatine solution led to a significant decrease ($p < 0.05$) in triglycerides, cholesterol, low-density lipoproteins, and very low-density lipoproteins compared to the control group C, along

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with a significant increase ($p < 0.05$) in high-density lipoproteins when compared to the control group.

Table 8 illustrates the therapeutic effect of creatine solution on blood lipids in obese experimental animals.

Standards					Groups
VLDL mg\dl	LDL mg\dl	HDL mg\dl	TC mg\dl	TG mg\dl	
11.82±3.03b	17.19±5.93b	41.48±5.75a	55.02±2.52b	50.71±0.87b	**C**
6.51±0.74a	3.91±1.40 a	58.24±4.95b	44.57±2.97a	35.10±5.30 a	**T3**
1.049	1.942	3.453	1.938	3.234	**LSD**

The numbers represent the mean ± standard error .

-The presence of different lowercase letters among the groups indicates significant differences .

-C represents the control group .

T3 (the third treatment group) was administered a creatine solution at a concentration of 1000 mg/kg of body weight for a duration of thirty days.

Discussion:

Body Weight Gain and Induction of Obesity in Experimental Animals:

The present findings demonstrated that the administration of a high-fat diet resulted in a significant increase in body weight among the male rat groups (C+, T1, T2, and T3) during the first month of the experiment compared with the negative control group (C−). A statistically significant difference ($p < 0.05$) was observed between animals fed the high-fat diet and those receiving the standard diet. These results indicate that the high-fat diet effectively induced obesity in the experimental animals. High dietary fat intake is known to increase total body mass, promote adipose tissue accumulation, and induce metabolic alterations associated with impaired metabolic regulation.

These findings are consistent with those reported by (12), who observed that animals fed a high-fat diet exhibited significant body weight gain accompanied by increased abdominal and adipose tissue fat deposition compared with animals maintained on a standard diet. This confirms the reliability of the high-fat diet model in inducing experimental obesity .Moreover, no significant differences in body weight were detected among the four high-fat-fed groups (C+, T1, T2, and T3) during the early stage of the experiment, suggesting that the potential effects of the administered extracts or dietary

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supplements on body weight had not yet manifested. This observation aligns with previous reports indicating that early physiological responses to high-fat feeding are generally comparable among treated groups before the onset of intervention-specific effects.

Effect of Creatine Solution on Body Weight Gain in Obese Animals:

The study indicated that the group of obese animals treated with creatine solution (T3) exhibited a significant reduction in body weight ($p < 0.05$) compared with the control group. This effect may be attributed to the role of creatine in reducing fat accumulation in obese rats through the enhancement of energy metabolic performance and increased ATP turnover, thereby contributing to body weight reduction .Furthermore, creatine may promote lipid metabolism and enhance fat digestion and utilization, which contributes to a decrease in total body weight through the activation of lipid metabolic pathways (13).

Effect of Creatine Solution on Albumin, Globulin, and Total Protein Levels in Obese Animals:

The results of the present study demonstrated a statistically significant increase ($p < 0.05$) in serum albumin, globulin, and total protein levels in the obese experimental animals treated with creatine solution (T3) compared with the control group. This improvement may be attributed to the potential role of creatine solution in enhancing protein status in obese animals, which is likely associated with its metabolic and antioxidant effects .The observed elevation in protein levels may be related to creatine's role in stimulating muscular and hepatic protein synthesis through increased ATP production and improved protein metabolism. Enhanced cellular energy availability may support anabolic processes and promote the maintenance of plasma protein concentrations. These findings are consistent with those reported by (14), who indicated that improvement in plasma protein status reflects the capacity of such treatments to support physiological and hepatic functions in obese animals treated with creatine solution, as well as to mitigate obesity-related damage.

Effect of Creatine Solution on Leptin Levels:

The observed reduction in leptin levels in the obese animals treated with creatine solution (T3) may be attributed to the role of creatine in improving body composition. Creatine does not promote fat accumulation; rather, it enhances lean body mass while reducing adipose tissue. This shift in body composition contributes to an increase in basal metabolic rate,

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leading to enhanced fat oxidation and, consequently, decreased leptin secretion from adipose tissue compared with the obese control group (C). These findings are consistent with those reported by (15), who suggested that creatine supplementation improves physical performance and promotes skeletal muscle development without increasing fat mass, thereby supporting mechanisms associated with improved metabolic efficiency and reduced adiposity.

Effect of Creatine Solution on Thyroid Hormones (T3 and T4) in Obese Animals:

The significant reduction in serum thyroid hormones (T3 and T4) observed in obese experimental animals treated with creatine solution (T3) suggests that creatine may enhance cellular energy production independently of thyroid hormone stimulation. Creatine supplementation increases intracellular ATP availability, thereby supporting energy metabolism without requiring elevated thyroid hormone activity .These findings are consistent with those reported by (16), who indicated that creatine supplementation may reduce the peripheral conversion of T4 to T3 in the liver, leading to decreased circulating levels of both hormones. This mechanism may reflect a reduced physiological need to stimulate metabolic rate via thyroid activation when cellular energy availability is improved .Furthermore, experimentally induced obesity in animals may itself contribute to decreased thyroid hormone levels due to chronic inflammation and leptin resistance, factors known to influence hypothalamic–pituitary–thyroid axis regulation. This may explain the greater decline observed following creatine supplementation, as suggested by .(17)

Effect of Creatine Solution on Glucose, Insulin, and Insulin Resistance (HOMA-IR) in Obese Animals:

The marked reduction in serum glucose and insulin levels, along with improved insulin sensitivity (as indicated by decreased HOMA-IR values), observed in the obese animals treated with creatine solution (T3), may be attributed to the metabolic role of creatine in enhancing muscular function and glucose utilization. Although creatine is primarily recognized for its role in improving muscle performance, it also exerts beneficial effects on insulin sensitivity. Creatine supplementation appears to facilitate glucose uptake into skeletal muscle, thereby reducing circulating glucose levels and decreasing the demand for insulin secretion in obese animals .These findings are consistent with those

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reported by (18), who demonstrated that creatine enhances the activity of the glucose transporter (GLUT4) in muscle tissue, promoting increased cellular glucose uptake and lowering blood glucose concentrations.

Moreover, creatine supplementation, whether combined with exercise or administered alone, has been shown to improve insulin sensitivity and reduce circulating insulin levels, particularly in obese subjects. This effect may be mediated through reduced inflammation and enhanced intramuscular glucose utilization, thereby alleviating insulin resistance, as reported by (19)

Effect of Creatine Solution on Liver Enzymes (ALT and AST) in Obese Animals:

The present findings demonstrated that obese animals treated with creatine solution (T3) exhibited a significant reduction in liver enzyme levels (ALT and AST). This decrease may be attributed to the antioxidant properties of creatine, as creatine has the capacity to protect hepatocytes from oxidative stress and reduce cellular damage by enhancing intracellular ATP production (18) .These results are consistent with those reported by (20), who indicated that creatine supplementation may improve liver health under conditions associated with metabolic syndrome by reducing inflammation and enhancing cellular energy efficiency. The observed decline in ALT and AST levels therefore suggests a hepatoprotective effect of creatine in obese animals.

Effect of Creatine Solution on Serum Lipid Profile in Obese Animals:

The significant reduction in serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), accompanied by an increase in high-density lipoprotein cholesterol (HDL-C), in the obese animals treated with creatine solution (T3) compared with the obese control group, reflects a marked improvement in lipid profile parameters .This improvement may be explained by the role of creatine in enhancing insulin sensitivity and reducing adipose tissue accumulation, thereby positively influencing lipid metabolism in obese animals. Furthermore, the antioxidant effect of creatine may protect hepatic tissue from oxidative damage, consequently limiting excessive lipid synthesis and improving overall lipid homeostasis (13).

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