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**Research Article** 

## **REGULATORY ROLE OF L-ARGININE SUPPLEMENTATION ON SOME**

### **BIOCHEMICAL PARAMETERS IN HIGH FAT DIET-FED RATS**

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#### ABSTRACT

High fat diet is a public health problem worldwide and considered as a risk factor for many diseases. This study aimed to investigate the effectiveness of L.arginine in high fat-fed rats. The present study indicate that after high fat diet (25%), there are significant increase in concentration of glucose, triglycerides, total cholesterol, corticosterone and urea as well as the body weights increased. Furthermore liver and adipose tissue differently changed than normal. Supplementation of L.arginine (1.2%) in drinking water markedly reduces glucose, triglycerides, total cholesterol, and Urea levels to more or less than normal. This modulation of metabolic syndrome due to the presence of nitric oxide (NO) is synthesized from L.arginine which simulate glucose uptake as well as glucose and fatty acid oxidation in skeletal muscle, heart, liver and adipose tissue, inhibit the synthesis of glucose, and fat in target tissues (e.g., liver and adipose); and enhance lipolysis in adipocytes. Thus, an inhibition of (NO) synthesis causes hyperlipidemia and fat accretion in rats, whereas dietary arginine supplementation reduces fat mass in high fat-fed rats and improvement metabolic syndrome.

Keywords: *L- Arginine*, High-Fat diet, Glucose, Triglycerides, Male Rats.

#### INTRODUCTION

High fat intake is prevalent in most developed countries and is associated with high incidence of some chronic diseases, especially cardiovascular disease and certain type of cancer (Committee on Diet and Health 1989).

Fat deposition in humans or animals depends on the balance between dietary caloric intake and whole-body energy expenditure. A chronic imbalance in energy metabolism (more energy input than energy output ) due to complex genetic and\or environmental factors result in excess fat accretion or obesity in humans, which is currently a major public health problem world wide (Hill Jo, et al., 2003 and Hill Jo. and Peter 1998). This metabolic disorder has continued to increase an alarming rate in the past and affects both adults and children (Bray and Bellanger 2006).

There are white and brown adipose tissues in animals, the amounts varying with species, developmental stage and environment.( Smith and Carstens 2005).

Studies with high fat fed-rats have demonstrated that oral administration of L-

argenine (Arg) effectively reduces carcass whit fat and enhances whole-body insulin sensitivity (Jobgen., et al., 2008 and Wu et al., 2009).

Using the swine model, Tan et al., (2009), reported that, dietary supplementation with Arg reduced white adipose tissue mass and improved the metabolic profile in the body (He et al., 2009). Many authors observed that intramuscular fat content was increased in Arg-supplemented pigs (Ma x.y., 2010). These results indicate that white adipose tissue and skeletal muscle respond differentially to Arg. treatment possibly due to tissue specific of the expression of lipogenic and lipolytic enzymes by this nutrient.

Adipose tissue accumulation is determined by the balance between lipogenesis and lipolysis. Fatty acid synthesis is regulatory by key enzyme, including acetylco-enzyme-A(CoA) Carboxylase (ACC) and fatty acid synthase (FAS), where as hydrolysis of tri-acyl-glycerols in adipose tissue is catalyzed by hormonesensitive lipase (HSL), (Zou and shao et al., 2008). In addition, Arg. affect multiple metabolic involving fatty acid and glucose syntheses, amino acid degradation, and cellular redox state (Wu, 2009). Specifically, arginine stimulates lipolysis and the expression of key genes responsible for activation of fatty acid oxidation and reduces white adipose tissue in diabetic fatty acid (Tan, et al., 2012)

#### **MATERIALS AND METHODS**

L-arginine was obtained from Egypt Trade Company Dokki, Egypt, added to drinking water (1.2 g %). Male albino rats (Rattus Rattus) weighing (120  $\pm$ 10 g) were purchased from the National Research Centre, Egypt. All rats were maintained ad libitum diet and tap water under good ventilation condition,

Thirty rats were divided into equally three groups as follows:

Group I : served as normal controls.

Group II: randomly feed on 25 % high fat diet, prepared by mixing 250 g butter with 750 g rat shaw diet, and drink tap water, for 12 weeks.

Group III: received in addition to HF-diet, (1.2 g %) L-arginine in drinking water, at 6 wk age, for 12 weeks.

Blood samples were collected from orbital venous plexus on plain tubes and sera obtained after centrifugation at 3000 rpm.

All chemical analyses were determined by using commercial kits derived from Spectrum company, Hannover, Germany.

Serum glucose, cholesterol, triglycerides and were determined calorimetrically urea according to the methods of (Caraway and Watts, 1987), (Ellefason and Caraway 1976), (Bucolo and David 1973), and (Batton and Crouch 1977) respectively. Serum Corticoserone was determined using commercial radioimmunoassay kits derived from IBL. International Co. Germany, according to the method of (Rattner et al., 1980)

The rats body weight for all studied groups was initially determined at the beginning of the experiment and then measured weekly for 12 weeks.

Histological studies: specimens of liver and dorsal adipose tissues were collected and fixed in 10 % formalin saline. The samples were prepared for histological examination by washing with tap water for 12 hours, and then serial alcohols (methyl, ethyl and absolute) was used for dehydration, tissue samples cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned by sledge microtone and collected on glass slide for staining by using hematoxylin and eosin (Banchroft et al., 1996). Statistical analysis: Student t-test was applied

for the statistical analysis of collected data to

determine the probable significance levels. The differences were considered significant at p< 0.05 (Byrkit, 1980).

#### **RESULTS AND DISCUSSION**

Effect of (1.2 %) L-arginine on Glucose (mg/dl) level in all groups, refer to (Table 1) Serum glucose mean level of rats received high fat diet for 6 weeks were significantly increased by 23.06 % more than control, while after Larginine supplementation for another 6 weeks the glucose mean level decreased to reach about 2.32 % only more than control. These improvements of glucose level are in agreement with (Wenjiag, et al., 2005) who found that, in arginine-supplemented diabetic rats (1.51%), serum glucose level were 23% lower after 10 weeks. Also Wenjuan et al., 2009 reported that, dietary arginine supplementation reduced serum concentration of glucose and improved its tolerance in high fat diet rats.

#### Effect of (1.2 %) L-arginine on cholesterol (mg/dl) level in all groups, refer to (Table 2) Effect of L-arginine on triglyceride (mg/dl) level in all groups; refer to (Table 3)

Data presented in both tables 2 and 3 indicated decreased cholesterol and triglycerides concentrations in high fat diet rats after the Larginine supplementation. The change percent reached to (-2.4 and 19.3 %) for both cholesterol and triglycerides compared to control(13.7 and 31.6 %) respectively. These results are in accordance with that of Wenjuan et al., (2009) who reported that the dietary arginine supplementation shifts nutrient partitioning to promote muscle over fat gain and may provide a useful treatment for improving the metabolic profile and reducing body white fat in diet-induced obese rats.

Ahmed et al., (2011) stated, high relative percentage of lipid levels in rats fed high fat diet than those fed on high fat diet with L-arginine. In addition L-arginine enhances AMP-activated protein kinase expression and activity, there by modulating lipid metabolism and energy balance towards the loss of triacylglycerols.( Tan et al.,2012).

# Effect of L-arginine on urea (mg/dl) level in all groups, refer to (Table 4)

Table (4) revealed increased level of serum urea in high fat diet rats by 46.94 % compared with control. The elevation of blood urea is an indicator for kidney disorders, whereas urea is the principle end product of protein catabolism and accelerated amino acid deamination for gluconeogenesis is possible and acceptable postulate to interpret the elevated levels of urea (Bishop et al., 2005). As a result of L-arginine intake, there was noticed that the serum urea level decreased compared to high fat diet rats. These data were confirmed the regulating role of L-arginine in metabolic pathway, involved in the amino acid and protein metabolism. The present data agree with that of Bie et al.,(2012) who reported that, as a nitrogenous precursor of nitric oxide (NO), L-arginine regulates multiple metabolic pathways involved in metabolism of fatty acids, glucose, amino acids and proteins through cell signaling and gene expression.

Effect of L-arginine on corticosterone (ng/ml) level in all groups, refer to (Table 5) Corticosterone has a wide range of activities in rodents. It regulates carbohydrate, protein and fat metabolism. It has also an influence on the hem-biotic system. Cotricosterone level in nocturnal animals like rats exhibit a distinct circadian variations with peak values in the latter portion of the day, followed by a nadir in the morning (D'Agostino et al., 1982) and is believed to play an important role in sleep-wake cvcles. Plasma corticosterone in rats is often used in connection with ACTH measurement as a stress indicator (Vazquez - et al., 2001). Regarding to serum corticosterone levels, there was insignificant increase in high fat diet rats with arginnine supplementation compared to both high fat diet rat and control. These result is well visualized the benefit role of L-arginin to prevent the corticosterone rises. As well as it has ameliorative affect in high fat diet rats metabolism. Wenjuan et al., (2009) stated that concentration of insulin, growth serum hormone, T3, T4, and corticosterone hormones were not affected by either high fat feeding or dietary L-arginine supplementation.

As in figure (1), initial rat body weights (at 6 wk of age) were  $145 \pm 2.5$  and  $136 \pm 4.2$  g, and final body weights (at 12 wks of age) were  $310 \pm 6.3$ and 220 ± 3.6 g for high fat-diet and L-argininetreated rats respectively. The analysis of body weight changes during the experimental period indicates a significant decrease (p<0.01) in weight of high fat diet with L-arginine supplementation rats compared to high fat diet rats. Body weights differed (P< 0.05) between high-fat diet and high-fat diet -arginine rats, with control. Compared with HF-diet rats, arginine-supplemented rats had 5.0, 10.8 14.3, 15.5, 24.4 and 29.0 % lower ( P< 0.01 ) body weights at wk 7, 8, 9, 10, 11, and 12, respectively after the treatment initiation (6 wk age) (fig 1). These results are in accordance with Wenjiang et al., (2005) who reported that, dietary arginine supplementation to (Zucker Diabetic Fatty rats) did no affect food or water intake, but resulted in a 16% loss of body weight.

Therefore the effects of oral arginine administration were independent of intakes of energy and nutrients from enteral diet, but resulted from an increased intake of L-arginine via the drinking water. Similary Bie et al. (2011) reported that, dietary arginine supplementation decreased whole-body fat mass and serum levels of very low-density lipoprotein in growing-finishing pigs. Also Bie et al., (2012) postulated that, arginine can be used to prevent and treat adiposity in fatty rats. They discovered that arginine differentially regulates expression of fat-metabolic genes in skeletal muscle but lipolysis in white adipose tissue. Specifically, arginine down-regulates expression of lipogenic genes, such as lipoprotein lipase (LPL) acetyl-CoA carboxylase (ACC) alpha but up-regulates tissue, including hormone-sensitive lipase (HSL).

#### Histological observation

- ✓ Liver section of control rats showed normal hepatocytes radiating in cords and normal histological structure of hepatic lobule, refer to (Fig 2).
- ✓ Fig (3) demonstrated that, rats received high fat-diet showing fatty change and vacuolization of Centro lobular hepatocytes.
- ✓ Fig (4), demonstrated that, dilatation of hepatic sinusoids and sinusoidal leucocytosis in high fat diet rats, noted inflammatory cells infiltration in hepatic sinusoids.
- ✓ Fig (5), liver of high fat diet rats supplemented 1.2% g L-arginine showing hydropic degeneration of focal hepatocytes and individual numbers of fatty cells. These results cleared that, Larginine play a role in histopathological improvement of liver cells induced by high fat-diet.
- ✓ The histopathological examination of adipose tissues of control revealed the normal architecture of fat cells and normal adipocytes size with hexagonal structure refer to (Fig 6 and 7).
- ✓ Fig (8), demonstrated the moderate changes in the form of large size of hexagonal shape of adipocytes in high fat diet rats. As well as adipose tissue showing very large size of hexagonal shape of adipocytes (Fig 9).
- ✓ On the other hand, rat treated with Larginine had normal size of fats cells and normal hexagonal shape of adipocytes tissue (Fig 10 and 11).

#### CONCLUSION

The present study indicated that L-arginine supplementation markedly reduced body weight as well as the improvement of liver and adipose tissues. This is consistent with an increase in the oxidation of both glucose and fatty acid in adipose tissue of arginine-treated rats, an indication of an overall increase in energy expenditure rather than a specific effect of lipid metabolism. The study revealed the beneficial roles of L-arginine treatment in enhanced glucose oxidation and reduced serum

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levels of glucose, cholesterol and triglycerides. Thus L-arginine is a potentially novel antiobesity amino acid and dietary supplementation may be help in the treatment of adiposity and its associated metabolic disorders.

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0.05

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	Control	Fat	Fat + L-Arginine
Mean ± S.D.	79.20 ± 4.19	98.10 ± 2.04	81.04 ± 5.05
± S.E.	1.30	0.64	1.58
% Change		23.06	2 32

0.001

Table 1: Effect of (	1.2 %) L	-arginine on	Glucose	(mg/dl)
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Significant at p<0.05 .Highly significant at p<0.01

#### Table 2: Effect of (1.2 %) L-arginine on cholesterol (mg/dl) level in all groups

	Control	Fat	Fat + L-arginine
Mean ± S.D.	78.75 ± 10.36	89.50 ± 10.01	76.80 ± 5.45
± S.E.	3.20	3.10	1.70
Change %		13.7	-2.4
P <		0.05	n.s.

Significant at p<0.05. Highly significant at p<0.01

#### Table 3: Effect of L-arginine on triglyceride (mg/dl) level in all groups

	Control	HF-diet	HF-diet + arginin
Mean ± S.D.	107.30 ± 3.95	141.20 ± 8.97	128.0 ± 5.45
± S.E.	1.20	2.80	1.70
Change %		31.6	19.3
P<		0.001	0.01

Significant at p<0.05 .Highly significant at p<0.01

#### Table 4: Effect of L-arginine on urea (mg/dl) level in all groups

	Control	HF-diet	HF-diet + arginin
Mean ± S.D.	34.81 ± 3.14	51.15 ± 4.64	36.40 ± 4.35
± S.E.	0.98	1.45	1.35
Change %		46.94	4.6
P<		0.001	0.01

Significant at p<0.05 .Highly significant at p<0.01

#### Table 5: Effect of L-arginine on corticosterone (ng/ml) level in all groups

	Control	HF-diet	HF-diet + arginine
Mean ± S.D.	9.66 ± 1.27	10.20 ± 1.19	$9.80 \pm 1.04$
± S.E.	0.39	0.37	0.33
Change %		5.6	1.5
P<		n.s.	n.s.

Significant at p<0.05 .Highly significant at p<0.01



Fig. 1:



**Fig. 2: normal liver section control** Liver section of control rats showed normal hepatocytes radiating in cords and normal histological structure of hepatic lobule (Fig2).



Fig. 3: Liver of High fat diet-fed rats Fig (3), demonstrated that, rats received high fat-diet showing fatty change and vacuolization of Centro lobular hepatocytes



Fig. 4: Liver of High fat diet-fed rats Fig. 4: demonstrated that, dilatation of hepatic sinusoids and sinusoidal leucocytosis in high fat diet rats, noted inflammatory cells infiltration in hepatic sinusoids



Fig. 5: Liver of High fat diet- fed rats treated with L-arginin

Fig. (5), liver of high fat diet rats supplemented 1.2% g L-arginine showing hydropic degeneration of focal hepatocytes and individual numbers of fatty cells. These results cleared that, L-arginine play a role in histopathological improvement of liver cells induced by high fat-diet



The histopathological examination of adipose tissues of control revealed the normal architecture of fat cells and normal adipocytes size with hexagonal structure.(Fig, 6 and 7), respectively



Fig. 8: High fat diet-fed rat adipose tissues (X 100)









with L-argenine (X 400) with L-argenine (X 100) On the other hand, rat treated with L-arginine had normal size of fats cells and normal hexagonal shape of adipocytes tissue (fig 10)

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