Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/9551
DOI URL: http://dx.doi.org/10.21474/IJAR01/9551

RESEARCH ARTICLE

L-ARGININE SUPPLEMENTATION IMPROVES INSULIN RESISTANCE BY INCREASING THE INSULIN RECEPTOR EXPRESSION IN TYPE II DIABETIC RATS.

Nashwa EL-Khazragy[1] and Dalia A. Saad[2].

1. Department of Clinical Pathology/Hematology, Biomedical Research, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
2. Department of Physiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Manuscript Info

Manuscript History
Received: 08 June 2019
Final Accepted: 10 July 2019
Published: August 2019

Key words:-
Insulin resistance, Type 2 Diabetes, L-arginine, Insulin receptors.

Abstract

Background: L-arginine is a multifactorial compound, indirectly involved in multiple regulatory mechanisms substrate for nitric acid synthesis. It has been documented that it has a beneficial effect on insulin resistance in Type 2 Diabetes (T2D) and visceral obesity. Experimental and clinical trials have illustrated the undergoing mechanistic pathways either on molecular or biochemical levels. However, the relationship between L-arginine supplementation and insulin receptor expression has not elucidated. **Aim:** The study aimed to evaluate the influence of L-arginine supplementation on insulin receptor expression, insulin resistance and weight gain in high fat diet fed rats. **Subjects& Methods:** 48 female albinos with visceral obesity were randomly assigned to either receive a high fat diet for 12 weeks or L- arginine with a dose of 5% in food for the last 8 weeks. Two age groups were enrolled "adult and old", Biochemical analysis was performed including: the concentration of insulin receptor expression in visceral fat, plasma insulin receptor and fasting blood glucose, the insulin resistance was evaluated according to the homeostasis model assessment- insulin resistance (HOMA-IR) protocol. **Results:** The concentration of insulin receptors was significantly higher in L-arginine treated group and associated with significant decrease in HOMA_IR and insulin concentration. In addition, the influence of L-arginine is conversely proportional with age. **Conclusion:** our results supports the hypotheses that dietary L-arginine supplementation results in increase insulin receptors, which could lead to negative feedback on insulin resistance.

Introduction:-

Type two Diabetes mellitus "T2D" is a danger chronic disease; it is caused by different factors such as accumulation of lipids (fats), wrong diet system, damaging life style and aging [20]. The risk of being a diabetic patient is increased by getting older; especially (after reaching the age of 45) as people tend to gain extra weight due to less exercise followed by lose in muscle mass [21].

Corresponding Author: - Nashwa EL-Khazragy
Address:- Department of Clinical Pathology/Hematology, Biomedical Research, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Briefly this metabolic pathway takes place by the accumulation of a cells type known as (Senescent cells) which plays its role in aging, which do elevate the direct impact of pancreatic β-cell function associated with senescence-associated secretory phenotype (SASP)-mediated **[22]**, which are responsible for tissue damaging in addition to damaging the adipose tissues leading to of liver cell damaging **[23].** On the other hand, in case of young age the Senescent cells are accumulated through obesity, less exercises ending up by loss in muscle mass causing prediabetes and consequence of metabolic changes and tissue damage as well [24].

Insulin resistance is the inability of a type 2 diabetic patient to use up his own insulin properly [25]. Initially the amount of insulin produced is insufficient to reduce the glucose concentration in blood therefore; resisting mechanism of insulin binding receptors takes place which are located on cells to bind to insulin to reduce blood glucose levels [25].

L-arginine plays an important role in the metabolic pathway of lipids and insulin resistance; it works as a functional amino acid, maintenance of reproduction and growth in addition to its consideration to be the nitric acid precursor. Moreover, it has an anti-aging property, it do maintain the cells from aging. This property is attributed to the reduction of arterial blood pressure as well as obesity leading dysfunction of the endothelial cells which bring about remission of type 2 diabetes [26]. L-arginine do increase the transportation of the endothelial cells responsible for the production of an excess amount of nitric oxide therefore; increasing the blood amount produced from cardiovascular muscle therefore; increasing the activity of the activated insulin receptors found on cells therefore ending up by cardiovascular diseases [26].

Results:-

2.1. Impact of high fat diet and dietary L-arginine on weight gain and visceral fat

The body weight, length and BMI as well as visceral fat weight were compared in all groups [(control: n=8), (high fat diet model: n=8) and dietary L-arginine treated group]. In order to assess the effect of age on disturbance of lipid metabolism, two age groups were enrolled in this model, an adult and old group. The initial body weight, BMI and visceral fat weight was significantly higher in rats fed by adult high fat diet, as compared with control group and rats fed by a high fat diet supplemented with L-arginine ($p<0.01$).when a post-Hoc test was performed, no significant difference was achieved between rats that fed by a high fat diet supplemented with L-arginine and control group for BMI and visceral fat weight ($p>0.05$). Similar result was obtained for body weight and length in old age model. A significant increase in body weight and length was achieved between high fat diet fed rats and control group as well as high fat diet supplemented with L-arginine ($p<0.01$). The results are presented in Table 1 and Figure 1.

Table 1:-Effect of L-arginine on body weight, length, BMI and Visceral fat weight

Age	group	Body weight(gram) mean±SD (range)	Body Length(Cm) mean±SD (range)	BMI ((Kg/m^2) mean±SD (range)	VFW (gram) mean±SD (range)
Adult	Control (n=8)	190±10.7 (180-200)	20±0.8 (19-20)	0.5±0.05 (0.4-0.5)∗	11±1.6 (8.2-13)∗
	High Fat diet (n=8)	274±25.0 (240-310)	21±1.0 (19-22)	0.7±0.09 (0.6-0.8)	15±3.6 (11-20)
	L-arginine treated	208±7.2 (200-220)	20±0.4 (19-20)	0.5±0.03 (0.5-0.6)∗	8.9±0.8 (8-10)∗
Statistics (ANOVA)		F=59.2 ; p=0.001	F=0.9 ; p=0.4	F=22.9 ; p=0.001	F=15.3 ; p=0.01
Old	Control (n=8)	299±8.3 (290-310)∗	22±1.1 (20-23)∗	0.7±0.07 (0.6-0.8)	21±2.2 (19-26)
	High Fat diet (n=8)	328±16.8 (300-350)	23±0.5 (22-23)	0.6±0.02 (0.4-0.7)	25±3.6 (18-30)
	L-arginine treated	286±27.2 (230-310)∗	22±0.7 (21-23)∗	0.6±0.08 (0.4-0.7)	16±3.2 (11-20)
Statistics (ANOVA)		F=10 ; p=0.001	F=3.4 ; p=0.05	F=1.0 ; p=0.4	F=16.7 ; p=0.001

BMI: Body mass index, VFW: Visceral fat weight, F= ANOVA test value, ANOVA: analysis of Variances, ∗; no significant difference ($p>0.05$) was observed between both groups in the same age group and same column by post-Hoc test.

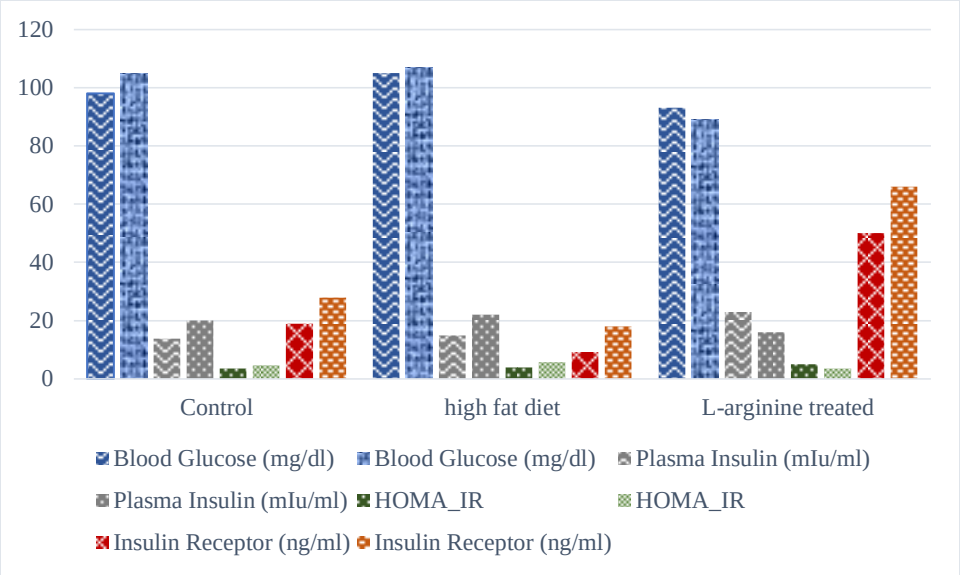

Figure 1:-Boxplot graph illustrating a significant difference ($p<0.01$) between adult control, high fat diet, and dietary L-arginine for the levels of plasma insulin, insulin receptor. Similar results were obtained for old age group. Nerveless, no significant difference was detected between studied adult group for fasting blood glucose and HOMA_IR as well as for plasma insulin in old age group ($p>0.05$). Homeostatic Model Assessment of Insulin Resistance (HOMA_IR)

2.2 Effect of L-arginine on modulation of fasting blood glucose (FBS), plasma insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA_IR) and Insulin Receptors

The results presented in Table 2 and 3 demonstrates that L-arginine diet supplementation is associated with increase in the expression of insulin receptor and plasma insulin levels than in adult high fat diet fed rats with a significant difference ($p<0.01$). On the other hand, in old aged model, a significant association was obtained between L-arginine diet supplementation and blood glucose level, plasma insulin, HOMA_IR and insulin receptor expression ($p<0.01$), L-arginine has a high tendency to decrease blood glucose, HOMA_IR and increase in plasma insulin and insulin receptor levels (Figure 2).

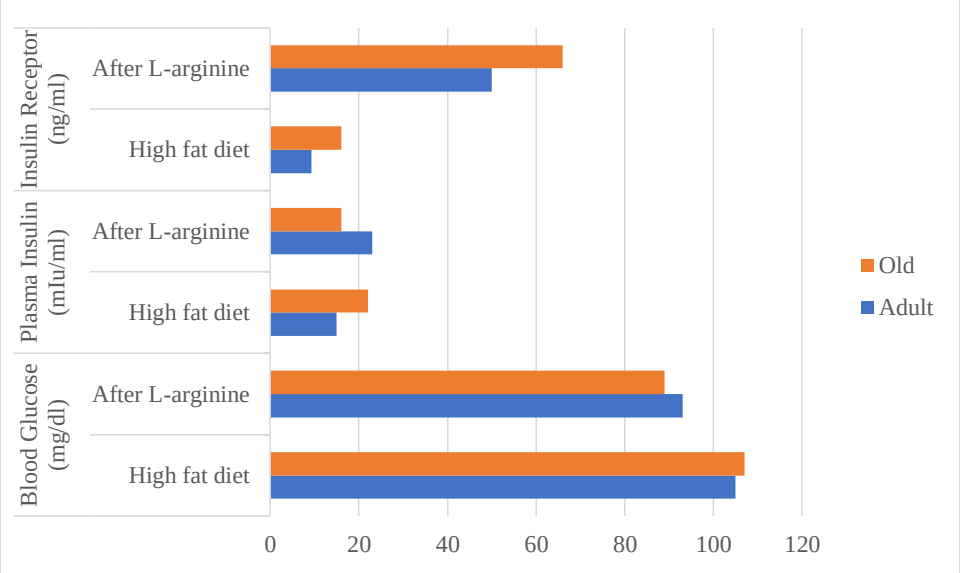

Figure 2:-Boxplot graph illustrating the effect of L-arginine on modulating the level of blood glucose (mg/dl), plasma insulin (mIu/ml) and insulin receptors (ng/ml) in high fat diet model. A comparative analysis was conducted between adult and old models for the fold change in each parameter. A significant association was observed between the fold change and old age ($p<0.01$) for insulin level and insulin receptor expression, however, no significant

association between age and fold change in fasting blood glucose was detected (p>0.05). Therefore, old age group are more sensitive to L-arginine than adult age.

2.3. Comparative analysis between the impact of L-arginine in adult and old aged model

The fold change is calculated for FBS, plasma insulin and visceral fat insulin receptor expression between high fat diet supplemented with L-arginine and high fat diet group for each age group, then a non-parametric Mann-Whitney test was conducted for the fold change in each parameter between adult and old aged groups. As presented in table 3, an apparent significant increase (W: 42; p=0.006) in insulin receptor expression (fold change; 0.4, 0.3); respectively, and a decrease in plasma insulin levels are demonstrated in old aged group compared to adults. This indicates that L-arginine supplementation protect the old aged rats against development of insulin resistance in rats fed a high fat diet. Although, no effect was detected on fasting glucose levels between old aged rats and adults (p>0.05).

Table 3:-Impact of age on the effect of L-arginine for modulating diabetic biochemical markers in high fat diet

Statistics	Fold change (FBS)		Fold change (plasma insulin)		Fold change (Insulin Receptor)	
	Adult	**Old**	**Adult**	**Old**	**Adult**	**Old**
median	-0.1	-0.2	0.4	-0.3	4.0	3.0
Statistics	W: 60 , p=0.4		W: 36; p=0.001		W: 42; p=0.006	

W: Wilcoxon value of Mann-WhitenyTest; the fold change was calculated by comparing the change in mean value of variable in high fat diet group after treatment with dietary L-arginine, FBS: fasting blood glucose.

Discussions:-

L-arginine is a metabolic amino acids that serve as precursor for production of glutamate, agmatine and urea [1]. Several experimental studies have highlighted on pharmacological beneficial effects of exogenous L-arginine[2]. These effects include reduction in atherosclerotic risk and erectile dysfunction, anti-inflammatory response, improvement of gastritis[3]. Moreover, it considered as potent anti-aging drug, its effect is superior to any other previously discovered nutraceutical agent[4].

Based on the evidence of physiological and clinical value of L-arginine, in the current study we aimed to investigate the impact of L-arginine supplemented high fat diet on modulation of body weight and insulin resistance in rats fed with high fat diet, in addition to find out if L-arginine will show different behavior between adults and old rats. Finally, we tried to find out the association between the expression level of insulin receptor and L-arginine supplementation in rats fed on high fat diet. We observed an increase in insulin receptor protein expression in rats fed by HF supplemented with L-arginine, more interesting, the effect of L-arginine on insulin receptor level was more prominent in old aged rats. These results could be explained by the effect of L-arginine on glycolysis. Recently, a study had evaluated the mechanistic pathways at molecular levels, by which L-arginine can regulates glucose metabolism and lipogenesis, the study was conducted on juvenile fish that was fed on different graded L-arginine doses[5]. They demonstrated that L-arginine could improves the synthesis of fatty acids and enhance glycolysis at 1.62% dietary levels, meanwhile; higher levels of L-arginine (2.7%) induce insulin resistance via increasing the expression of gluconeogenesis -related genes and inhibition of Insulin receptor substrate 1 gene (IRS1), thus resulted in increase of plasma glucose level. However, at molecular levels, mammals behave by different mechanisms[6].

Away from molecular mechanism, anther mechanistic pathways have been investigated by researchers. It has been evident that L-arginine supplementation could preserve the renal efficiency in Type 2 Diabetes mellitus rats[7], it has been thought that L-arginine was converted by nitric oxide synthase (NOS) into nitric oxide which furtherly activated to guanylyl cyclase (GC) which will transformed into formed into cyclic guanosine monophosphate (cGMP)[8]. The biosynthesis of cGMP is a potent vascular-dilator that maintains renal function and delay the progress in diabetic nephropathy via improving renal blood flow and increase glomerular filtration rate (GFR)[9].
In this study, a significant improvement was achieved for the diabetic biochemical markers, including FBS, plasma insulin and HOMA-IR in rats fed on HFD supplemented with L-arginine compared with rats fed on high fat diet. Similar observation was detected for weight gain; L-arginine supplementation was associated with apparent reduction in weight gain and visceral fat weight. The interesting finding was the in-significant difference between L-arginine supplemented group and control group, which reflects the efficiency of L-arginine in controlling insulin

resistance in T2D which modulates the biochemical markers levels in rats fed on HF diet to be approximately nearer to the control group, but far away from diabetic rats. In agreement with our results, an increase in insulin resistance was observed in obese patients with high visceral fat[9], however, a supplementation of L-arginine for three months duration showed high tendency to decrease the basal HOMA_IR as well as weight gain[10]. The independent contribution of hyperlipoidemia and insulin resistance in cardiovascular complications have been confirmed by several studies, a significant association was detected between the intima-media in the carotid artery and a decrease in insulin sensitivity[11].

A cross link association have been detected between the reduction in plasma levels of arginine and Diabetes Mellitus, this is contributed to the elevation of ADMA "NOS inhibitor"[12]. Several experimental trials have tested the effect of arginine in diabetes, the results demonstrate that low dose of intravenous arginine improves insulin resistance in T2D patients[13].

Although, several studies have investigated the protective pathway through which L-arginine can regulates insulin resistance in Diabetes, and they went through different mechanistic pathways either on molecular or biochemical levels[6][14], to our knowledge, this is the first study that investigate the effect of L-arginine supplementation on the expression of insulin receptors in T2D rats, we hypnotized that one of the possible logic mechanism through which L-arginine improves insulin resistance in diabetes could be contributed to the bypass of insulin resistance by increasing the expression of the receptors. The results of this study demonstrated an increase in insulin receptor expression with L-arginine supplements which was furtherly associated with improvement of diabetic biomarkers. Unfornatually, the limitation of this study is contributed to the inability to investigate the mechanistic pathway by which this insulin receptors were increased by L-arginine.

Recent studies have demonstrated the relationship between L-arginine and skin elasticity and accelerated wound healing, thus improving its role as an anti-aging substance[15]. In this study, the results suggested that L-arginine supplementation improves the insulin resistance in adult T2D diabetic rats. Detection of visceral fat expression level of insulin receptor expression revealed an increase in receptor expression that was associated with L-arginine supplementation in adult rats compared to old group. This is consistent to results reported by recent studies, researchers have demonstrated that L-arginine plays a crucial role in collagen synthesis as it is the one of metabolic precursors of proline the topical administration of L-arginine increase the resistance to traction force and increases the amount of elastic fibers in skin of female mice > 20 weeks age[16]. Moreover, a hydrophilic character of L-arginine has been proved by researchers, thus suggesting that it could be a promising active antiaging compound for women in post-menopausal period [17].

In Conclusion, we investigated the association between L-arginine supplementation and insulin receptors expression in rats. Our results revealed that L-arginine increases the expression of insulin receptor that was association with reduction in insulin resistance; the effect was more potent in adult rats compared to old group, thus supporting its relationship with aging.

Materials and Methods:-

Experimental Model:

This study was conducted on 48 albino female rats, weighing between 150 – 180 gm. The rats were purchased from Research Institute of Ophthalmology (Giza). They were maintained in animal cages (3 rats/cage) under controlled conditions of temperature (25±2 ℃) and relative humidity (50-70% RH). The rats were allowed standard pelleted chow and tap water *ad libitum* with 12 h duration of light and dark cycle. They were acclimatized to the laboratory conditions for a week before start of experimental procedures to decrease the possible discomfort of animals. Animals were not exposed to unnecessary pain or stress and animal manipulation performed with maximal care and hygiene. All animal experiments performed according to the Ethics Committee of Faculty of Medicine, Ain Shams University and according to the National Institutes of Health guide for the care and use of Laboratory animals '(NIH publication No.8023, revised 1978'[18]. The rats **were randomly allocated into the following groups: Group I**: Adult Control group (n= 8): received standard pellet chow for 12 weeks, **group II**: Adult obese group (n=8): received high fat diet for 12 weeks, **group III**: L- arginine Adult obese group (n=8): received high fat diet for 12 weeks and L- arginine at a dose of 5% in food [19] for the last 8 weeks of the study, **group IV**: Old Control group (n= 8): received standard pellet chow for 12 weeks, **group V**: Old obese group (n=8): received high fat diet for 12 weeks, and **group VI**: L- arginine Old obese group (n=8): received high fat diet for 12 weeks and L- arginine at a dose of 5 % [19] in food for the last 8 weeks of the study. The **high fat diet was prepared as follow:** Each 100 gm

of this diet had 352 Kcal. It was prepared by mixing butter (15 gm; 108 Kcal), bread (60 gm; 145 Kcal), milk (123 ml equivalent to 15 gm dry milk powder; 77 Kcal) and unsalted Karish cheese (10 gm; 10 Kcal). This elevates the fat content to about 16-17%, carbohydrates content 41-42% and protein content to 12 -13 %..

Biochemical Tests: two ml deep venous blood samples were collected and divided by equal volumes into in K3-EDTA and gel vacutainer tubes, both tubes were centrifuged at 10,000 rpm for 20 minutes, then plasma and serum were separated simultaneously and stored in -20^0 C until analyzed. The fasting blood glucose was measured in serum samples within two hours after collection using Glucose Assay Kit (Colorimetric), Cat no: STA-680 (***CELLBIOLABS***), Plasma insulin levels was measured using Enzyme linked immunosorbent assay (ELISA) and insulin receptors expression was measured in visceral fat tissue using ELIZA technique; Insulin (Rat) Elisa Cat no:EIA-2048 (***DRG; International; USA***), and finally the HOMA_IR was calculated according to the homeostasis model assessment- insulin resistance (HOMA-IR) protocol.

Measurement of Insulin Receptor expression in visceral fat

After scarification of rats, visceral fat was weighted and preserved in -80^0 C. At the date of analysis, the tissue samples were homogenized, suspended in a 2.0 mL screw cap tube, containing 200 uL of phosphate buffer saline (PBS), a single 5 mm stainless steel bead (***Qiagen***) was added to each tube and the samples were homogenized at maximum speed (30 Hertz) for 2 min using the Qiagen Tissue-Lyser system. Following homogenization the samples were spun for 1 min at maximum speed to reduce foaming, the homogenate was tested for the expression of insulin receptor using ELISA technique then Insulin Receptor Human ELISA; cat no: RD191041200R (***BioVendor***), The level of Insulin receptor expression was calculated in relation to protein.

Statistical analysis

The statistics analysis was performed with Statistical Package for the Social Sciences software 'SPSS" version 24"; IBM. **The data was presented as mean±SD for normally distributed data and as median values for skewed data; comparative analysis between groups was carried out using Kruskal-Wallis or Mann-Whitney test. A p value of <0.05 was considered statistically significant**

References:-

1. A. Miczke et al., "Effect of L-arginine supplementation on insulin resistance and serum adiponectin concentration in rats with fat diet," Int. J. Clin. Exp. Med., vol. 8, no. 7, pp. 10358–10366, 2015.
2. E. L. Feeney et al., "Patterns of dairy food intake, body composition and markers of metabolic health in Ireland: Results from the National Adult Nutrition Survey," Nutr. Diabetes, vol. 7, no. 2, pp. 3–6, 2017.
3. D. V. T. Da Silva, C. A. Conte, V. M. F. Paschoalin, and T. D. S. Alvares, "Hormonal response to L-arginine supplementation in physically active individuals," Food Nutr. Res., vol. 58, no. April, 2014.
4. M. Z. Gad, "Anti-aging effects of L-arginine," J. Adv. Res., vol. 1, no. 3, pp. 169–177, 2010.
5. H. Liang et al., "Dietary arginine affects the insulin signaling pathway, glucose metabolism and lipogenesis in juvenile blunt snout bream Megalobrama amblycephala," Sci. Rep., vol. 7, no. 1, pp. 1–11, 2017.
6. C. Zhou, X. Ge, B. Liu, J. Xie, R. Chen, and M. Ren, "Effect of high dietary carbohydrate on the growth performance, blood chemistry, hepatic enzyme activities and growth hormone gene expression of Wuchang bream (Megalobrama amblycephala) at two temperatures," Asian-Australasian J. Anim. Sci., vol. 28, no. 2, pp. 207–214, 2015.
7. T. Claybaugh et al., "L-arginine supplementation in type II diabetic rats preserves renal function and improves insulin sensitivity by altering the nitric oxide pathway," Int. J. Endocrinol., vol. 2014, 2014.
8. G. Remuzzi, M. Macia, and P. Ruggenenti, "Prevention and treatment of diabetic renal disease in type 2 diabetes: The BENEDICT study," J. Am. Soc. Nephrol., vol. 17, no. SUPPL. 2, pp. 90–97, 2006.
9. M. J. Fowler, "Microvascular and macrovascular complications of diabetes," Clin. Diabetes, vol. 29, no. 3, pp. 116–122, 2011.
10. J. Sulibursk a, K. Grabanska, K. Musialik, A. Cieslewicz, A. Skoluda, and A. Jablecka, "Effect of 3-month L-arginine supplementation on insulin resistance and tumor necrosis factor," pp. 816–823, 2012.
11. Z. Pausova et al., "Role of tumor necrosis factor-α gene locus in obesity and obesity- associated hypertension in French Canadians," Hypertension, vol. 36, no. 1, pp. 14–19, 2000.
12. B. E. Sansbury and B. G. Hill, "Regulation of obesity and insulin resistance by nitric oxide," Free Radic. Biol. Med., vol. 73, pp. 383–399, 2014.
13. A. Sciacqua, N. Grillo, M. Quero, G. Sesti, and F. Perticone, "Asymmetric dimethylarginine plasma levels and endothelial function in newly diagnosed Type 2 diabetic patients," Int. J. Mol. Sci., vol. 13, no. 11, pp. 13804–

13815, 2012.
14. N. W. Rajapakse, A. L. Chong, W. Z. Zhang, and D. M. Kaye, "Insulin-Mediated Activation of the L-Arginine Nitric Oxide Pathway in Man, and Its Impairment in Diabetes," PLoS One, vol. 8, no. 5, pp. 8–13, 2013.
15. Á. do P. B. de Souza, M. M. R. de Oliveira, R. R. de Andrade, R. F. B. de Amorim, A. L. Bocca, and M. de F. Borin, "The in vivo effect of L-arginine on skin elasticity in mice," Brazilian J. Pharm. Sci., vol. 53, no. 3, 2017.
16. A. Barbul, "Proline Precursors to Sustain Mammalian Collagen Synthesis," J. Nutr., vol. 138, no. 10, pp. 2021S-2024S, 2008.
17. T. Tsuboi, M. Maeda, and T. Hayashi, "Administration of L-arginine plus L-citrulline or L-citrulline alone successfully retarded endothelial senescence," PLoS One, vol. 13, no. 2, pp. 1–14, 2018.
18. N. R. Council., C. for the U. of the G. for the C. Animals., U. of Laboratory, and I. for L. A. Research., Committee for the update of the guide for the care and use of laboratory animals. Guide for the care and use of laboratory animals. 2011.
19. M. A. Alam, K. Kauter, K. Withers, C. Sernia, and L. Brown, "Chronic l-arginine treatment improves metabolic, cardiovascular and liver complications in diet-induced obesity in rats," Food Funct., vol. 4, no. 1, pp. 83–91, 2013.
20. Palmer, A. K., Tchkonia, T., LeBrasseur, N. K., Chini, E. N., Xu, M., & Kirkland, J. L. (2015): "Cellular senescence in type 2 diabetes: a therapeutic opportunity": Diabetes, 64(7), 2289-2298.
21. Bellamy, L., Casas, J. P., Hingorani, A. D., & Williams, D. (2009): "Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis". The Lancet, 373(9677), 1773-1779.
22. Kahn, S. E. (2013). "The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes". Diabetologia, 46(1), 3-19.
23. Aguayo-Mazzucato, C., Andle, J., Lee Jr, T. B., Midha, A., Talemal, L., Chipashvili, V., ... & Bonner-Weir, S. (2019): "Acceleration of β Cell Aging Determines Diabetes and Senolysis Improves Disease Outcomes". Cell metabolism.
24. Taylor, R. (2013): "Type 2 diabetes: etiology and reversibility". Diabetes care, 36(4), 1047-1055.
25. Hu S, Han M, Rezaei A, Li D, Wu G, Ma X. (2017): "L-Arginine Modulates Glucose and Lipid Metabolism in Obesity and Diabetes". Bentham Science, 18(6):599-608. doi: 10.2174/1389203717666160627074017
26. Niwanthi W. Rajapakse, Abigail L. Chong, Wei-Zheng Zhang, David M. Kaye, (2013): "Insulin-Mediated Activation of the L-Arginine Nitric Oxide Pathway in Man, and Its Impairment in Diabetes". PLOS ONE Scientific Journals.