Original Research Article

L-Arginine's Glucose Homeostatic Influence in Renal Damaged Wistar Rats is Possibly Mediated by Adiponectin Expression

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Abstract

Hypoglycemia, as a direct result of the glucosuria is usually seen in patients with acute kidney injury hence glucose homeostasis is disturbed. The compensatory effect of adiponectin in the insulin deficient state is documented. We previously demonstrated that L-arginine enhances glucose transport mechanisms in renal-damaged rats. The current study was designed to investigate possible glucose handling synergy between L-arginine and adiponectin in Wistar rats induced with acute kidney injury. Twenty four rats weighing between 120g-150g were divided into 4 groups of six rats each. Group 1 (Control) had normal feed and water; Group 2 (Untreated) was induced with AKI and left untreated. Group 3 and 4 took 50mg/kg and 500mg/kg L-arginine respectively after AKI induction. Acute kidney injury was induced by intra-muscular injection of glycerol (50% solution, 8 ml/kg BW). Oral glucose tolerance test, insulin response test, serum creatinine test and adiponectin assay (ELISA) were carried out. Data was analysed using one way ANOVA and expressed as mean± standard error of mean (SEM) with p ≤ 0.05 considered as significant. L-arginine induced rapid insulin-like action which was effective after thirty minutes of oral glucose loading when compared the control group. Glucose uptake from the blood was also more effective and quicker in the L-arginine treated groups. Adiponectin was significantly expressed across the test groups when compared to the control group and there was insulin resistance in the untreated kidney injured rats 1 hour into the insulin response test. It may be concluded that L-arginine has a homeostatic influence on glucose handling in kidney-damaged rats possibly mediated by increased adiponectin expression.
Keywords: Kidney injury, Glucose metabolism, Adiponectin, Arginine, Glucose tolerance.

1. INTRODUCTION

The kidneys are essential to the maintenance of a fairly constant internal environment via regulation of body fluid volume, maintenance of electrolyte balance, and excretion of waste products of metabolism that are harmful to the body. The kidneys play important roles in glucose and insulin metabolism contributing about a quarter of the systemic glucose production and a fifth of systemic glucose removal (Meyer *et al.,* 1999). Glucose homeostasis in the kidney is regulated by insulin and it is mediated via glucose transporter (GLUT) proteins (Asano *et al.,* 2004). The term acute kidney injury (AKI), otherwise called acute renal failure, is used to describe a sudden but progressive loss in renal function, resulting in an inability to eliminate waste and maintain both electrolyte and water balance. AKI is associated with a high level of mortality (Ricci *et al.,* 2006).

Adiponectin, a fat-derived hormone has attracted substantial attention due to its involvement as a critical messenger for the crosstalk between adipose tissue and other metabolic related organs such as the kidneys. It has been shown that adiponectin strongly suppresses new glucose formation in the liver through some genetic inhibitory mechanism while promoting insulin sensitization (Wang and Scherer, 2016). L-arginine is an amino acid whose biological activity spans across a wide area of biochemical and physiological functions. One such major function is its influence on blood pressure and flow. This is because L-arginine provides the molecular substrate for the generation of nitric oxide (NO) whose bioavailability is a crucial to the maintenance of endothelial function. It has been previously used to enhance blood circulation around the gastrum and other epithelial tissue (Ajiboye *et al.,*

2019) and has also been demonstrated to improve glucose transport mechanism in renal damaged rats in our previous work (Ajiboye and Nkwopara, 2019). This study therefore is aimed at investigating the effects of L-arginine supplementation on adiponectin expression in acute kidney injury in male Wistar rats.

2. METHODOLOGY
Experimental Design
Twenty four rats weighing between 120-150 grams were used. They were procured from the animal holding facility in Babcock University, Nigeria. The animals were housed at ambient temperature and humidity. They had free access to a standard pellet chow diet and treated water ad libitum. After 2 weeks of acclimatization, acute kidney injury was induced with intra-muscular injection of glycerol (50% solution, 8 ml/kg body weight) (Westenfelder *et al.,* 1980). Two days after the induction, AKI was confirmed by checking the serum creatinine (SCr) levels of the rats.

Abnormally high levels of serum creatinine indicate the presence of kidney injury in the rats. After confirmation of AKI, the rats received L-arginine treatment (high dose-500mg/kg, low dose- 50mg/kg) orally via oral cannula for eight (8) days. Glucose tolerance test, adiponectin assay and insulin response test were carried out after which the animals were sacrificed by cervical dislocation. The carcasses were buried appropriately. All animal handling was in line with international standard for animal care and use in research and all experimentation were in accordance with the guidelines laid by the Babcock University Health Research and Ethics Committee, Nigeria for control and supervision of animal experiments.

ANIMAL GROUPING AND TREATMENT SCHEDULE
This study was done with the random distribution of 24 rats into four (4) groups of six (6) rats each, which is seen below:

GROUPS	TREATMENTS
Group 1 (Control)	Normal feed and water
Group 2 (Untreated)	Glycerol-induced AKI- Intra-muscular injection of glycerol (50% solution, 5ml/kg body weight
Group 3 (50 mg/kg L- arginine)	Glycerol-induced AKI + 50 mg/kg L- arginine
Group 4 (500 mg/kg L- arginine)	Glycerol-induced AKI + 500 mg/kg L-arginine

Determination of blood glucose levels
Blood was drawn from the rats' tails to determine their blood glucose levels with the Accu-check glucometer kit, and the values were recorded in milligrams per deciliter (mg/dl).

Acute kidney injury induction
Acute kidney injury was induced by injecting 8 ml/kg of glycerol (50% in tap water) into the muscles of both hind limbs (Baranowski *et al.,* 1978).

Determination of serum creatinine level
Serum creatinine level was determined by spectrophotometry using commercial creatinine kit from Randox laboratories.

Insulin response test
The insulin tolerance test was performed using human insulin (Humulin) and an Accu-check glucometer kit. The animals were fasted for four (4) hours before insulin administration. The rats were restrained using the wooden restrain and blood was drawn from their tails to determine their baseline blood glucose levels in milligrams per deciliter (mg/dl) with the Accu-check glucometer kit, then insulin (0.5units/kg body weight) was administered via subcutaneous injection (Leguisamo *et al.,* 2012). After insulin injection, the blood glucose concentration of the rats were determined at thirty (30) minutes intervals for up to one hundred and twenty (120) minutes and the values were recorded in milligrams per deciliter (mg/dl).

Oral glucose tolerance test
The glucose tolerance test was performed using exogenous glucose. After an overnight, the rats were restrained using the wooden restrain and blood was drawn from their tails to determine their baseline blood glucose levels. Exogenous glucose (2g/kg body weight) was then administered via intra peritoneal injection. Blood glucose levels were again determined at thirty (30) minutes interval over a total period of one hundred and eighty (180) minutes.

Adiponectin elisa assay
Adiponectin ELISA kit was procured from e-labscience and the standard ELISA procedure was followed. The absorbance for the assay was read at a wavelength of 450 nm.

3. STATISTICAL ANALYSIS
Statistical analysis was carried out using Graph-Pad Prism 5.1 software. Comparisons between the groups was performed by one-way analysis of variance (ANOVA), followed by Student Newman-Keuls (SNK) test to determine for any significant difference. Data was expressed as mean± standard error of mean (SEM) with $p \leq 0.05$ considered as significant.
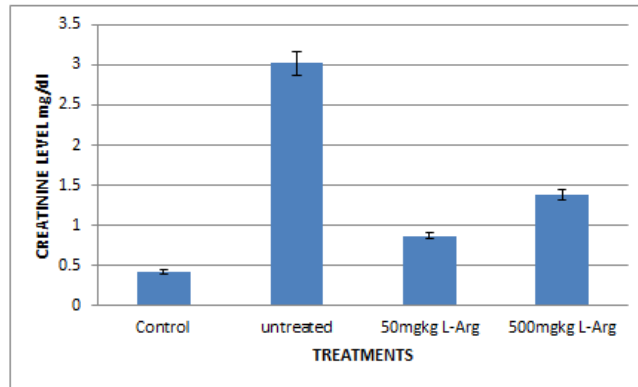
4. RESULTS

Fig-1: Serum creatinine assay.

Figure 1shows the average serum creatinine concentration values in milligrams per deciliter (mg/dl) of plasma. The positive control group had the lowest average of 0.4243 (mg/dl), followed by the group that was administered with a low dose of arginine; 0.8700 (mg/dl), the group administered with a high dose of arginine had an average of 1.3809 (mg/dl) and the negative control group had the highest average of 3.0242 (mg/dl). All groups showed significant difference ($p \leq 0.05$) compared to the control group.

Table-1: Effect of L-arginine supplementation on blood glucose level in acute kidney injury

	Control	500 mg/kg L- arginine		50 mg/kg L- arginine	Untreated
Baseline	85.76 ± 2.76	83.00	± 1.53	89.67 ± 4.48	85.67 ± 2.73
30 minutes	103.68 ± 14.32	97.67	± 6.33	110.67 ± 8.67	110.33 ± 6.84
60 minutes	133.05 ± 35.95	83.00	± 11.50**	102.00 ± 2.08	105.33 ± 5.04
90 minutes	104.61 ± 26.39	87.33	± 15.51**	101.67 ± 6.39	92.33 ± 2.60
120 minutes	79.83 ± 34.51	78.00	± 11.24	90.00 ± 9.07	86.33 ± 2.96
180 minutes	71.34 ± 35.33	81.33	± 4.98	75.67 ± 5.61	70.33 ± 9.28

Values are expressed as mean ± SEM (n=6) and are statistically significant at P<0.05 when compared to Control

Table-2: Effect of L-arginine supplementation on insulin response in acute kidney injury

	Control	500 mg/kg L-arginine	50 mg/kg L-arginine	Untreated
Baseline	86.43 ± 2.43	91.33 ± 4.06**	120.67 ± 3.83	146.33 ± 7.96
30 minutes	68.34 ± 3.34	84.67 ± 5.36**	95.33 ± 7.37**	128.33 ± 5.05
60 minutes	43.05 ± 9.05	87.00 ± 7.06	93.33 ± 5.41	134.33 ± 5.65
90 minutes	34.61 ± 8.61	69.00 ± 7.82**	75.33 ± 5.60	97.33 ± 6.48
120 minutes	28.83 ± 3.83	59.33 ± 3.10	86.00 ± 4.07	103.50 ± 6.50

Values are expressed as mean ± SEM (n=6) and are statistically significant at P<0.05 when compared with Untreated group
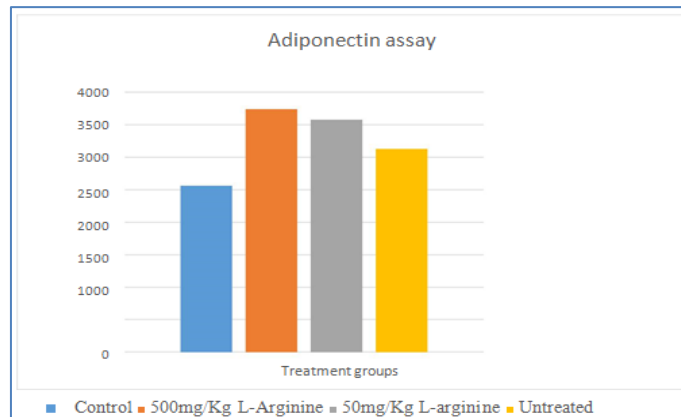

Fig-2: Serum Adiponectin Assay

Figure 2 shows that in the positive control group the average adiponectin concentration is at 2500ng/ml, in high dose arginine average adiponectin concentration is at 3700mg/dl, in low dose arginine group average adiponectin expression is at 3500mg/dl and in negative control group average adiponectin concentration is at 3100 mg/dl. All groups showed significant difference at $p \leq 0.05$ compared to the control group.

5. DISCUSSION AND CONCLUSION

Hypoglycemia as a direct result of glucosuria is a common complication seen in patients with acute kidney injury (AKI). Due to the major role the kidney performs in glucose transport, it is expected that the body's glucose control will be compromised in AKI state. Adiponectin has been shown to compensate for insulin deficiency by increasing sensitization to insulin (Wang and Scherer, 2016). Our previous study already demonstrated that L-arginine enhances glucose transport mechanisms in the damaged kidneys of rats by inducing a short-acting but rapid insulin-like action (Ajiboye and Nkwopara, 2019). The study was thus designed as a preliminary investigation into the possibility of a synergy existing between L-arginine and adiponectin which can serve as novel means to aid glucose handling in acute kidney injury.

Acute kidney injury was induced in the experimental rats using glycerol (50% solution, 8ml/kg body weight) and it was confirmed by the observed derangement in serum creatinine levels in the rats. This study showed that L-arginine induced rapid insulin-like action which was effective after thirty (30) minutes of oral glucose loading in both groups treated with L-arginine when compared the control group. Also glucose uptake from the blood was more effective and quicker in the L-arginine treated groups confirming our finding in our previous study. L-arginine stimulated nitric oxide release is capable of mediating glucose transport through GLUT4 translocation in adipocytes via a mechanism different from the insulin signaling pathway (Balon and Nadler, 1994; Tanaka *et al.*, 2003). This NO-stimulated glucose uptake in adipocytes occurring through a non-insulin pathway mechanism may be via adiponectin action.

Adiponectin was significantly expressed across the test groups when compared to the control group. Adiponectin is known to have an anti-inflammatory effect so its increased expression in kidney damage is reasonable. Ouchi and Walsh (2007) postulated that adiponectin attenuates inflammatory responses to multiple stimuli by modulating signaling pathways in a variety of cell types. Yamamoto *et al.*, (2005) identified adiponectin as an adipocyte specific protein with anti-inflammatory properties.

Glucose level in the untreated group was significantly lower when compared to the control group. This may be as a result of the anti-glucogenic action of adiponectin. Adiponectin inhibit hepatic gluconeogenesis by blocking the expression of genes involved in glucose production (such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) and it helps fatty acid oxidation in skeletal muscle, which contribute to a beneficial metabolic action in whole body energy homeostasis (Phillip and Zhao, 2016). Furthermore, Karbowska and Kochan (2006) postulated that adiponectin plays a key role in glucose metabolism by acting like insulin in promoting insulin sensitivity, inhibiting cell death and inflammation. These studies strengthen the assumption that the expression of adiponectin in kidney injury may have caused insulin-like actions in the regulation of blood glucose.

Insulin tolerance test (ITT) is a test to detect insulin resistance or measure the sensitivity of target organs to insulin (Sin *et al.,* 1996). Baseline plasma glucose in the test groups was significantly higher than in the control group. This supports the fact that glucose handling is compromised in AKI rats. Insulin resistance is characterized by reduced glucose indices such as uptake, metabolism and storage which may be due to inhibited glucose transport in target tissue and also by inhibition of the hepatic glucose output (Samuel and Shulman, 2012). Individuals with acute kidney injury develop insulin resistance, and this is due to loss of kidney function. Evidence from the current study shows that insulin resistance occurred in the untreated kidney injured rats 1 hour into the insulin response test. Also, it was observed that despite evidence to show that there is insulin resistance at about 60-90 min into the test, there is a significant observable insulin-like action which may be responsible for the continued glucose lowering effect in the two groups treated with L-arginine. Thus, comparing the effects of L-arginine in the glucose tolerance and insulin response test it can be concluded that L-arginine confers a homeostatic influence on glucose handling in kidney-damaged rats and this may have been mediated by increased adiponectin expression.

6. CONCLUSION

The study provides evidence that L-arginine up regulates adiponectin expression in acute kidney injury and thus possibly aiding glucose movement and delivery to tissues. Both L-arginine and adiponectin expressed insulin-like behavior in the renal damaged animals and this may have contributed to improved glucose handling observed in L-arginine treated animals. Further studies will be needed to prove the synergy between the two agents and their role(s) in glucose handling in acute kidney injury.

REFERENCES

- Ajiboye, K. I., & Nkwopara, C. (2019). L-Arginine Modulates Glucose Transport Mechanisms in Glycerol-Induced Kidney Injury in Wistar Rats.
- Ajiboye, Kolawole, I., Oluwole, Francis, S., & Ajiboye Oyebimpe, F. (2019). Oral administration of L-Ariginine Ameliorate NSAID-induced Gastric Injury in Wistar Rats. Saudi Journal of Medical and Pharmaceutical Sciences, *5*(7), 637-641.
- Asano, T., Ogihara, T., Katagiri, H., Sakoda, H., Ono, H., Fujishiro, M., ... & Uchijima, Y. (2004). Glucose transporter and Na+/glucose cotransporter as molecular targets of anti-diabetic drugs. *Current medicinal chemistry*, *11*(20), 2717-2724.
- Balon, T. W., & Nadler, J. L. (1994). Nitric oxide release is present from incubated skeletal muscle preparations. *Journal of Applied Physiology*, *77*(6), 2519-2521.
- Baranowski, R. L., Westenfelder, C., & Kurtzman, N. A. (1978). Intrarenal renin and angiotensins in glycerol-induced acute renal failure. *Kidney international*, *14*(6), 576-584.
- Karbowska, J., & Kochan, Z. (2006). Role of adiponectin in the regulation of carbohydrate and lipid metabolism. *Journal of physiology and pharmacology: an official journal of the Polish Physiological Society*, *57*, 103-113.
- Meyer, C., Dostou, J. M., & Gerich, J. E. (1999). Role of the human kidney in glucose counterregulation. *Diabetes*, *48*(5), 943-948.
- Leguisamo, N. M., Lehnen, A. M., Machado, U. F., Okamoto, M. M., Markoski, M. M., Pinto, G. H., &

Schaan, B. D. (2012). GLUT4 content decreases along with insulin resistance and high levels of inflammatory markers in rats with metabolic syndrome. *Cardiovascular diabetology*, *11*(1), 1-10.
- Ouchi, N., & Walsh, K. (2007). Adiponectin as an anti-inflammatory factor. *Clinica chimica acta*, *380*(1-2), 24-30.
- Ricci, Z., Cruz, D. N., & Ronco, C. (2011). Classification and staging of acute kidney injury: beyond the RIFLE and AKIN criteria. *Nature Reviews Nephrology*, *7*(4), 201-208.
- Samuel, V., & Shulman, G. (2012). Mechanisms for Insulin Resistance: Common Threads and Missing Leads. *Cell,* 148, 852-871.
- Sin, T., Castillo, T., Muñoz, S., Candia, M., López, G., & Calvillán, M. (1996). Insulin tolerance test. A useful test to determine insulin resistance in obese hyperandrogenic women. *Revista Medica De Chile*, *124*(8), 931-937.
- Tanaka, T., Nakatani, K., Morioka, K., Urakawa, H., Maruyama, N., Kitagawa, N., ... & Adachi, Y. (2003). Nitric oxide stimulates glucose transport through insulin-independent GLUT4 translocation in 3T3-L1 adipocytes. *European journal of endocrinology*, *149*(1), 61-68.
- Wang, Z. V., & Scherer, P. E (2016): Adiponectin, the past two decades. *Journal of molecular cell biology*, *8*(2), 93–100
- Westenfelder, C., Arevalo, G. J., Crawford, P. W., Zerwer, P., Baranowski, R. L., Birch, F. M., ... & Kurtzman, N. A. (1980). Renal tubular function in glycerol-induced acute renal failure. *Kidney international*, *18*(4), 432-444.
- Yamamoto, K., Kiyohara, T., Murayama, Y., Kihara, S., Okamoto, Y., Funahashi, T., ... & Shinomura, Y. (2005). Production of adiponectin, an anti-inflammatory protein, in mesenteric adipose tissue in Crohn's disease. *Gut*, *54*(6), 789-796.
- Zhao, V., & Phillip, E. (2016). Adiponectin: The Past Two Decades. *Cell Biology, 8*(2), 93-100.