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ORIGINAL ARTICLES

Determination of Plasma Nitric Oxide and Sodium in type II Diabetic Saudi Patients: Early Biomarkers of Hypertension

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ABSTRACT

Background: Diabetes is now one of the most common non-communicable diseases globally. It is the fifth leading cause of death in most high-income countries.Diabetes has several complications of which one is hypertension.The presence of high blood pressure in diabetes is associated with a 4 fold increase in death chiefly from heart disease and strokes. Nitric Oxide (NO) is an important cellular signaling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short half-life for a few seconds in the blood. **Objective:** The target of the present study was to estimate the level of nitric oxide, sodium and potassium as indicators of hypertension incidence in diabetic Saudi patients. **Methodology:** Thirty patients with type II diabetes mellitus matched in age and sex with thirty normal control subjects enrolled from Prince Maged Ben Abdel Aziz Diabetic Center, Al Madina, S.A. were studied. Nitrate level in the plasma was measured using Colorimetric Nitric Oxide Assay Kit. Sodium level in the plasma was measured using photometric determination of serum sodium Mg-Uranylacetate method, colour test. Potassium level in the plasma was measured using photometric turbidimetric test. Fasting blood glucose was measured using an enzymatic colorimetric test for glucose determination without deproteinisation-glucose oxidase peroxidase method. **Results:** Plasma potassium and sodium levels show significant increase in diabetic patients, while, that of nitrate decreases in comparison with normal healthy control subject ($p<0.01$). Sodium level shows significant negative correlation with fasting blood glucose levels in diabetic patients ($r = -0.696$, $p<0.01$). **Conclusion:** In type II diabetes mellitus altered NO pathway is a central defect causing hypertension. Also, results show that blood pressure in diabetic patients is sodium sensitive, and revealed that: in diabetes mellitus a relatively high sodium intake may be a factor that predisposes to the development of diabetic vascular disease.

Key words: Diabetes, hypertension, Nitric Oxide.

Introduction

Diabetes is now one of the most common non-communicable diseases globally. It is the fifth leading cause of death in most high-income countries and there are substantial evidences that it is epidemic in many low- and middle-income countries. Diabetes is certain to be one of the most challenging health problems in the 21st century. (Sicree *et al.*, 2008).

Surveys in Middle Eastern Countries have shown that 23.7% of adult Saudi are in the age of 30 – 70 years have diabetes, and 14.1% have impaired fasting glucose. It is worrying that the prevalence seems to be doubling every two decades, and in some countries like Saudi Arabia this could soon reach 50% in those over 50 years of age (Elhadd, 2012).

Diabetes has several complications of which hypertension or high blood pressure is one of them. Data indicate that at least 60-80% of individuals whom developed diabetes will eventually develop high blood pressure. The high blood pressure is gradual at early stages and may take at least 10–15 years to fully develop. Besides diabetes, other factors that may also increase high blood pressure include: obesity, insulin resistance and high cholesterol levels. In general, fewer than 25% of diabetics have good control of their blood pressure. The presence of high blood pressure in diabetes is associated with a 4 fold increase in death chiefly from heart disease and strokes (Urbina *et al.*, 2008).

The chief reason why people with diabetes develop high blood pressure is hardening of their arteries. Diabetes tends to speed up the process of atherosclerosis. The other fact about diabetes is that, it

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affects both large and small blood vessels in the body. Over time, blood vessels become clogged with fatty depots, become non-compliant and lose their elasticity. The process of atherosclerosis is a lot faster in diabetic individuals whom do not have good control of their blood sugars. The high blood pressure eventually leads to heart failure, strokes, heart attacks, blindness, kidney failure, loss of libido and poor circulation of blood in the legs. When the blood supply to the feet is compromised, the chances of infections and amputations also increase. All diabetics should know that even mild elevations in blood pressure can be detrimental to health. Studies have shown that diabetics with even a slight elevation in blood pressure have 2-3 times the risk of heart disease compared to individuals without diabetes (Karen,2007 ; Bakris, 2011).

NO is an important cellular signaling molecule involved in many physiological and pathological processes. It is a powerful vasodilator with a short half-life of a few seconds in the blood. Long-known pharmaceuticals such as nitroglycerine and amyl nitrite were discovered, more than a century after their first use in medicine, to be active through the mechanism of being precursors to nitric oxide. Low levels of nitric oxide production are important in protecting organs such as the liver from ischemic damage (Dessy and Ferron 2004).

Nitric oxide, known as the 'endothelium-derived relaxing factor' (EDRF), is biosynthesized endogenously from L-arginine, oxygen, and NADPH by various nitric oxide synthase (NOS) enzymes. Reduction of inorganic nitrate may also serve to make nitric oxide. The endothelium (inner lining) of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow. Nitric oxide is highly reactive (having a life-time of a few seconds), yet diffuses freely across membranes. These attributes make nitric oxide ideal for a transient paracrine (between adjacent cells) and autocrine (within a single cell) signaling molecule **(**Pál Pacher et al., 2007**).**

Nitric oxide (NO) contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction and growth, platelet aggregation, and leukocyte adhesion to the endothelium. Humans with atherosclerosis, diabetes, or hypertension often show impaired NO pathways (Dessy and Ferron, 2004). A high salt intake was demonstrated to attenuate NO production in patients with essential hypertension, although bioavailability remains unregulated (Osanai *et al.,* 2002).

Decreased NO bioavailability and endothelial dysfunction develop at an early stage, prior to carbohydrate-intolerance, and may constitute an early link not only to insulin resistance and hyperglycemia but also to the cardio-metabolic patho-physiologic sequelae of the metabolic syndrome (Duplain *et al.,* 2001).

The aim of the present work was to estimate the level of nitric oxide, sodium and potassium as indicators of hypertension incidence in diabetic Saudi patients.

Subjects and methods:

Subjects:

Thirty patients with type 2 diabetes mellitus matched in age and sex with thirty normal control subjects enrolled from Prince Maged Ben Abdel Aziz diabetic center-Al Madina, S.A. were studied. Exclusion criteria for both diabetic and control subjects included any of the following: hypertension (defined as blood pressure. 150/90 mmHg), hypercholesterolemia (defined as LDL-cholesterol . 75th percentile for age and sex), tobacco use within the past 5 years, current use of insulin, anti-oxidants or hormone replacement therapy and laboratory evidence of renal, hepatic, or hematological abnormalities. Consent was taken from every patient before conducted the research.

Methods:

Thoroughly, all patients and control examined clinically and about 2 ml of whole blood was drawn from each subject. Plasma was isolated by centrifugation (15 min at 13,000 rpm) and then stored at –80°C till further analysis. Nitrate levels in the plasma samples were measured using Colorimetric Nitric Oxide Assay Kit (Oxford Biomedical Research Company). Nitric oxide can be spectro-photometrically assayed by measuring the accumulation of its stable degradation products, nitrate and nitrite. The ratio of these two products in biological fluids, tissue culture media, etc. may vary substantially. Hence, for accurate assessment of the total nitric oxide generated, one must monitor both nitrate and nitrite. An excellent solution to this problem is the enzymatic conversion of nitrate to nitrite by the enzyme nitrate reductase (NR), followed by quantitation of nitrite using Griess Reagent. In addition to providing all necessary components in a micro-titer format, this kit employs affinity purified nitrate reductase and NADH, there by circumventing some of the potential problems reported for nitric oxide measurement using NADPH dependent nitrate reductase.

Sodium level in the plasma samples was measured using (photometric determination of serum sodium Mg-Uranylacetate method, colour test (Human Company).

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Potassium level in the plasma samples was measured using (photometric turbidimetric test) (Human company)**.** Fasting blood glucose was measured using an enzymatic colorimetric test for glucose determination without deproteinisation-glucose oxidase peroxidase method (Human Company).

Statistical methods:

The entire data was statistically analyzed using SPSS program. Data were expressed as mean ± SD and were compared by analysis of variance.*P* < 0.05 was considered statistically significant. Given the small sample size, Spearman correlation coefficients were used to look for an association between Na^+ or K^+ and Nitric Oxide in diabetic group.

Results:

The plasma nitrate level in diabetic patients was 30.7±4.16µmol/L, which showed significant decrement difference compared to the healthy control group (58.7±4.5µmol/L) (p<0.01). Patients having diabetes had a plasma sodium level of 138.86 ±1.77 mmol/L, which showed significant increment difference compared to the healthy control group (132.2±2.45 mmol/L) (p<0.01). The average plasma potassium level in diabetic patients was 4.21±0.08mmol/L, which showed also significant decrement difference compared to the healthy group (4.8±0.15mmol/L) (p<0.01) (Table 1 and Figure1). A negative correlation existed between fasting blood glucose levels and sodium levels in diabetic patients (r= -0.696, P<0.01) (Table, 2 and Figure, 2).

Table 1: Represents mean plasma levels ± SD for nitrate, sodium, potassium and fasting glucose in diabetic patients and healthy control subjects, ** = P< 0.01.

Variable	**Control subjects (n=30)** mean plasma levels ± SD	**Diabetic patients (n=30)** mean plasma levels ± SD
Nitrate level µmol/L	58.7±4.5	30.7±4.16**
Sodium level mmol/L	132.2±2.45	138.86±1.77**
Potassium level mmol/L	4.8±0.15	4.21±0.08**
Glucose level mmol/L	5.02±0.01	10.72± 3.25**

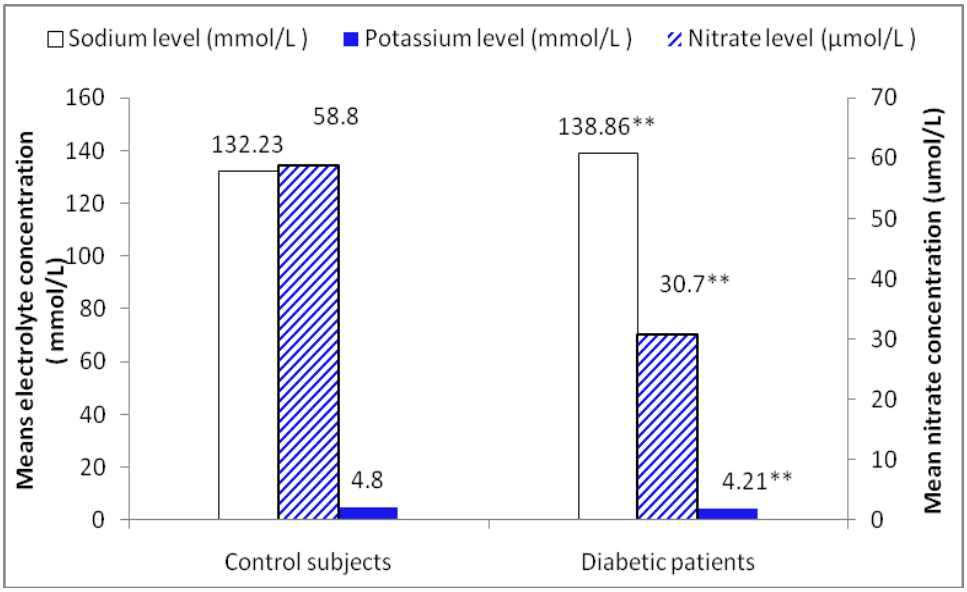

Fig. 1: Represents mean concentration of nitrate (µmol/L), sodium and potassium (mmol/L) in patients with diabetes and control subjects.**P< 0.01.

Table 2: Represents correlation between Nitrate, sodium, potassium and fasting glucose levels in diabetic patients. ** P < 0.01

Variable	Sodium level	Potassium level	Nitrate level
Nitrate level	r= -0.252	r= 0.306	----------
Sodium level	----------	----------	----------
Potassium level	r= -0.303	----------	----------
Glucose level	**r= -0.696 ** **	r= 0.160	r= -0.071

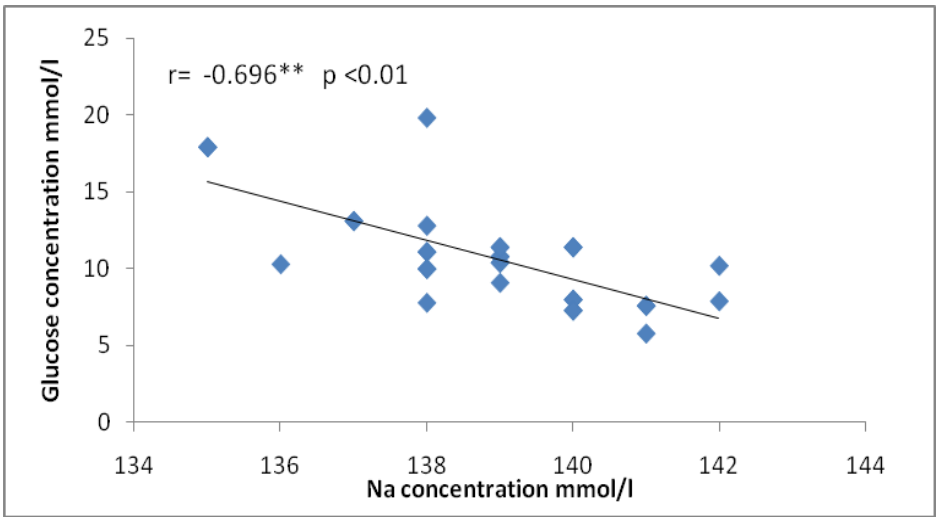

Fig. 2: Represents a negative correlation between glucose concentration (mmol/L), and sodium (mmol/L) in patients with diabetes. **P< 0.01.

Discussion:

NO is a potent signaling molecule, a key determinant of endothelial function, metabolic and vascular health, also affecting the nervous and immune systems. Protective effects occur at pico- to nano-molar NO concentrations. At higher concentrations, NO and its derivatives become cytotoxic. NO stimulates glucose oxidation in skeletal, cardiac muscle, liver and adipose tissue via cGMP-dependent mechanisms. NO mediates flow-mediated vasodilation and opposes vasoconstrictor effects. It counteracts vascular stiffness and lowers blood pressure. NO is a critical modulator of blood flow, vascular tone and blood pressure (Hayden and Tyagi 2003).

Our study is in agreement with other study done by Afridi and his colleagues (Afridi *et al.*,2008) which revealed that: there is a significant increase in sodium level and a significant decrease in potassium level in diabetic patients when compared to control subjects. The disorder may be based on the movement of electrolytes between intra- and extracellular spaces, dependent on the impaired insulin action as well as hyper osmolarity (Nugent, 2005). Also, a condition of polyuria and thirst found in diabetic patients confirm our result of increasing sodium level.

It is important to emphasize that an increment in the plasma insulin concentration of as little as 30-40 U/ml is capable of eliciting this anti-natriuretic effect (Fleming and Busse, 2003). For the compensatory hyperinsulinemia to induce kidney for sodium retention, expansion of the extracellular fluid volume, and ultimately hypertension, it is necessary that the kidneys of obese, diabetic, and hypertensive subjects maintain normal sensitivity to the anti-natriuretic effect of insulin, even though severe resistance exists regarding carbohydrate metabolism (Swasti Tiwari, et al., 2007).

Our results demonstrated inverse correlation between fasting glucose levels and sodium concentrations. This result attributed to hyperglycaemia and glycosuria which lead to osmotic diuresis and further loss of water, excretion of partly neutralized ketoacids via the kidney with loss of cations (Na+ and K+). The water loss usually exceeds that of the sodium chloride. Eventually, the loss of water from the intracellular and extracellular compartments will become quantitatively similar. Because of the osmotic shift of water, plasma sodium concentrations are usually low or normal in diabetic ketoacidosis (DKA) and can be slightly increased in hyperglycemic hyperosmolar state (HHS), despite extensive water loss (American Diabetes Association, 2004). In this context, the plasma sodium concentration should be corrected for hyperglycemia by adding 1.6 mmol to the reported sodium level for every 5.6 mmol/L increase in glucose above 5.6 mmol/L (Stoner, 2005).

Our results showed inverse correlation between NO level and diabetic state and this fact clearly underlines that hyperglycemia is a major determinant factor in serum NO levels. It is widely recognized that hyperglycemia induces impairment of the endothelial function via increased oxidative stress (Aydin *et al.*, 2001), which is a characteristic feature of diabetic individuals. The hyperglycemic state stimulates the production of advanced glycosylated end products (Candido *et al.*, 2003)., enhances the poly-pathway (Vikramadithyan *et al.*, 2005) and activates protein kinase C leading to oxidative stress (Geraldes and King, 2010) and (Hink *et al.*, 2001) A reduced content of glutathione, an important antioxidant in erythrocytes, has been demonstrated in diabetic patients (Chung *et al.*, 2003). Also, reduced radical-trapping antioxidant parameter (TRAP) and increased lipid peroxidation levels support the *in vivo* presence of increased oxidative stress in diabetes.

Diminished NO bioactivity reflects an imbalance between its synthesis and degradation. There may also be impaired vascular smooth muscle cell relaxation responsivity to NO. The patho-physiological mechanisms involved are multifactorial and differ with diverse etiologies. Arginases hydrolyze *L*-arginine, thus lowering nitrous oxide synthtase(NOS) activity by competing for *L*-arginine. Arginase plays an important role in the pathogenesis of reduced NO and endothelial dysfunction with pro-inflammatory conditions, aging and diseases like diabetes mellitus (Romero *et al.,* 2008). Decreased co-factor availability also may play a role as tetra-hydrofolate (BH_4) which is very susceptible to oxidation. BH_4 deficiency uncouples NOS, thus lowering NO output, increasing reactive oxygen species (ROS) production and engendering endothelial dysfunction (Munzel *et al.,*2003).

Reduced NO availability may not only be of relevance to the development of atherosclerotic complications in diabetes, but also interfere with insulin-mediated postprandial glucose disposal and possibly contribute to the development of insulin resistance (Gazalla *et al.,*2011).

Various studies have reported a significant decrease of plasma nitric oxides in patients with type 2 diabetes mellitus without any complications (Vanizor*et al.,* 2001) and (Mikiwa*et al.,*2002). Decreased NO bioavailability to smooth muscle cells in non complicated group has also been demonstrated in different studies (Hink*et al.,*2001). Our results coincide with these reports, and we presumed that the cascade of NO bioactivity and availability on smooth muscle cells was impaired in early affected stage of diabetes mellitus and followed the decrease of endothelial NO production.

Reduced NO availability in diabetes mellitus is relevant to the development of secondary complications in these clinical conditions. Alteration of NO metabolism and increased oxidant stress, previously demonstrated in diabetic patients, have been demonstrated to be involved in the pathogenesis of macro-vascular events, which are increased in hypertensive as well as diabetic patients (Mohamed *et al.,*1999; Bulent *et al.,* 1998; Kinlay *et al.,* 2001; Tretjakovs *et al.,* 2003 and GazallaAyubShiekh *et al.,* 2011).

Our hypothesis that sodium and potassium have a role in the development of hypertension in diabetic patients through decreasing NO is in accordance with previous work by (Büssemaker *et al.,* 2010) who stated that Sodium and potassium homeostasis has an important role in endothelium-dependent vasodilation, which is defective in primary hypertension. Sodium retention decreases the synthesis of NO, one of the main arteriolar vasodilators produced by endothelial cells, and increases plasma level of asymmetric dimethyl L-arginine (ADMA), an endogenous inhibitor of NO production (Fujiwara *et al.,* 2000). Sodium restriction has the opposite effects. A high-potassium diet and increases in serum potassium levels even within the physiologic range cause endothelium-dependent vasodilation by hyperpolarizing the endothelial cell through stimulation of the sodium pump and activation of plasma membrane potassium channels (Amberg *et al.,* 2003; Haddy *et al.,* 2006). Endothelial hyperpolarization is transmitted to vascular smooth muscle cells, resulting in decreased cytosolic calcium, which in turn promotes vasodilation. Experimental potassium depletion inhibits endothelium-dependent vasodilatation (Haddy *et al.,* 2006).

However, increased NO release by high extracellular potassium/low sodium functionally links endothelial cell stiffness, at least in part, to arterial stiffness. Similarly, as reported for endothelial cells, a gradual increase in extracellular potassium has hyperpolarized and relaxed vascular smooth muscle cells by stimulating the adenosine triphosphatase sodium-potassium pump (Na-K-ATPase) and/or activating inwardly rectifying potassium channels (Büssemaker*et al.,* 2002 ; Dwivedi*et al.,* 2005).

Humans exposed to a high salt diet may develop hypertension (Appel*et al.,*2006). In such individuals, the kidney has limited ability to excrete the daily load of sodium and tends to retain the salt, most likely osmotically inactive, in skin and other extracellular compartments (Titze *et al.,*2003). This internal sodium “escape” buffer, which likely is inadequate in humans with high blood pressure, suggests that extra-renal sodium balance has an important role in blood pressure control (Titze *et al.,*2002). Salt and water balance is regulated by a multitude of factors/ mediators. It is beyond doubt that one of the key factors is aldosterone. This mineralo-corticoid hormone controls the activity of epithelial sodium channels in the renal collecting duct. It also acts on epithelial sodium channels in endothelial cells (Golestaneh*et al.,* 2001; Schiffrin, 2006),where it may cause the cell to swell, stiffen and alter its release of nitric oxide (Nagata *et al.,*2006).

Little is known about how dietary salt increases blood pressure. Salt consumption increases thirst and fluid uptake. As a consequence, there is a transient increase in plasma volume and a subsequent increase in arterial blood pressure before extracellular volume returns to normal. Obviously, the body tries to regain the original extracellular volume (e.g. by decreasing volume through vascular smooth muscle contraction) at the expense of increased arterial blood pressure. This view agrees that diuretics decrease blood pressure. However, this well-known effect of diuretics cannot be explained exclusively by a decrease in extracellular volume. Other components of extracellular fluid, for example, electrolyte concentrations, could be important determinants (Daniel, 2004).

Recently, plasma sodium concentration has been suggested to have a direct role in the control of blood pressure because a small increase in plasma sodium level (1-3mmol/L) was found in individuals with hypertension (De Wardener *et al.,* 2004; He *et al.,* 2005).This observation is consistent with the finding that

primary hypertension is virtually absent in populations that consume less than50 mmol/d of sodium chloride(Kaplan, 2006).

There is abundant evidence to suggest that a potassium deficit has a critical role in the development of hypertension and its cardiovascular sequelae. Another study of patients with resistant (treated) hypertension showed the important effect of salt restriction on blood pressure. Low-salt (≤50 mmol/d) compared with high-salt (250 mmol/d) diets decreased systolic and diastolic blood pressures by 22.7 and 9.1 mm Hg, respectively (Pimenta *et al.,* 2009). In numerous studies, increased potassium intake had a beneficial effect on the cardiovascular system. Taken together, a diet low in sodium and rich in potassium is most favorable (Adrogue and Madias, 2007; Kido *et al.,* 2008).

High sodium intake was associated positively with pulse pressure, whereas high potassium intake was associated negatively with systolic, diastolic, and mean blood pressure. The positive association between dietary sodium intake and pulse pressure observed in this study provides further evidence for the current concept linking sodium to an increase in blood pressure through modification and stiffening of the arterial wall, whereas a negative correlation between dietary potassium intake and both systolic and diastolic blood pressure support the view that potassium is a vasodilating electrolyte in our nutrients (Buyck *et al.,* 2009).

In conclusion, our findings indicate that NO concentrations were significantly lower in patients with type II diabetes, supporting the hypothesis that altered NO pathway is a central defect leading to hypertension. Also, these results show that blood pressure in diabetes mellitus is sodium sensitive, and suggest that in diabetes mellitus a relatively high sodium intake may be a factor that predisposes to the development of diabetic vascular disease.

Based on clinical, cellular, and molecular evidence, it is recommended to maintain plasma sodium levels in the low and potassium levels in the high physiologic range. Restriction in sodium intake accompanied by increased potassium intake should profoundly improve the prevalence of hypertension and cardiovascular disease in these groups of patients.

References

Adrogue, H. and N. Madias, 2007. Sodium and potassium in the pathogenesis of hypertension. N Engl J Med. 356(19): 1966-1978.

Afridi, H.I., T.G. Kazi, N. Kazi, M.K. Jamali, M.B. Arain, N. Jalbani, R.A. Sarfaraz, A. Shah, G.A. Kandhro, A.Q. Shah, J.A. Baig, 2008. Potassium, calcium, magnesium, and sodium levels in biological samples of hypertensive and nonhypertensive diabetes mellitus patients. Biol Trace Elem Res., 124(3): 206-24.

Amberg, G., A. Bonev, C. Rossow, M. Nelson and L. Santana, 2003. Modulation of the molecular composition of large conductance, Ca(2+) activated K(+) channels in vascular smooth muscle during hypertension. J Clin. Invest., 112(5): 717-724.

American Diabetes Association, 2004. Hyperglycemic Crises in Diabetes. Diabetes Care, 27(1): s94-s102.

Appel, L., M. Brands, S. Daniels, N. Karanja and P. Elmer, 2006. Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. Hypertension, 47(2): 296-308.

Aydin, A., H. Orhan, A. Sayal, M. Ozata, G. Sahin, A. Işimer, 2001. Oxidative stress and nitric oxide related parameters in type II diabetes mellitus: Effects of glycemic control. Clin. Biochem., 34: 65-70.

Bakris, G., 2011. "Lower Blood Pressure Goals for Patients with Diabetes." Journal of Clinical Hypertension". 2:6(2000): 369-371.

Büssemaker, E., C. Wallner, B. Fisslthaler and I. Fleming, 2002. The Na-K-ATPase is a target for an EDHF displaying characteristics similar to potassium ions in the porcine renal interlobar artery. Br J Pharmacol. 137(5): 647-654.

Büssemaker, E., U. Hillebrand, M. Hausberg, H. Pavenstädt, H. Oberleithner, 2010. Pathogenesis of hypertension: interactions among sodium, potassium, and aldosterone. Am J Kidney Dis., 55(6): 1111-20.

Buyck, J., J. Blacher and E. Kesse-Guyot, 2009. Differential associations of dietary sodium and potassium intake with blood pressure: a focus on pulse pressure. J Hypertens., 27(6): 1158-1164.

Candido, R., J.M. Forbes, M.C. Thomas, V. Thallas, R.G. Dean, W.C. Burns, C. Tikellis, R.H. Ritchie, S.M. Twigg, M.E. Cooper, L.M. Burrell, 2003. A breaker of advanced glycation end products attenuates diabetes-induced myocardial structural changes. Circ Res., 92: 785-792.

Chung, S.S., E.C. Ho, K.S. Lam, S.K. Chung, 2003. Contribution of polyol pathway to diabetes-induced oxidative stress. J Am Soc Nephrol., 14: S233-S236.

Daniel W. Jones, 2004. Hypertension Highlights; Dietary Sodium and Blood Pressure. Hypertension, 43: 932-935.

DeFronzo, R.A., M. Goldberg, Z.S. Agus, 1976. The effects of glucose and insulin on renal electrolyte transport. *J ClinInvest.*, 58: 83-90.

Dessy, C., O. Ferron, 2004. "Pathophysiological Roles of Nitric Oxide: In the Heart and the Coronary Vasculature". Current Medical Chemistry – Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry; 3(3): 207-216.

De Wardener, H., F. He and G. MacGregor, 2004. Plasma sodium and hypertension. Kidney Int., 66(6): 2454-2466.

Duplain, H., R. Burcelin, C. Sartori, S. Cook, M. Egli, M. Lepori, P. Vollenweider, T. Pedrazzini, P. Nicod, B. Thorens, U. Scherrer, 2001. Insulin resistance, hyperlipidemia, and hypertensionin mice lacking endothelial nitric oxide synthase. Circulation, 104: 342-345.

Dwivedi, R., S. Saha, P. Chowienczyk and J. Ritter, 2005. Block of inward rectifying K_ channels (KIR) inhibits bradykinin induced vasodilatation in human forearm resistance vasculature. Arterioscler. Thromb. Vasc. Biol., 25(2): e7-9.

Elhadd, T.A., 2012. The rising menace of diabetes in the Middle East: Time for action. Saudi Journal for Health Science, 1: 44-45.

Fleming, I., R. Busse, 2003. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am J Physiol Regul Integr Comp Physiol., 284: R1-R12.

Fujiwara, N., T. Osanai, T. Kamada, T. Katoh, K. Takahashi and K. Okumura, 2000. Study on the relationship between plasma nitrite and nitrate level and salt sensitivity in human hypertension: modulation of nitric oxide synthesis by salt intake. Circulation, 101(8): 856-861.

Gazalla Ayub Shiekh, Taha Ayub and Khurshid Iqbal Andrabi, 2011. Reduced nitrate level in individuals with hypertension and diabetes JCardio.vasc. Dis. Res., 2(3): 172-176.

Geraldes, P., G.L. King, 2010. Activation of protein kinase C isoforms and its impact on diabetic complications. Circ Res., 106: 1319-1331.

Golestaneh, N., C. Klein, F. Valamanesh, G. Suarez, M. Agarwal and M. Mirshahi, 2001. Mineralocorticoid receptormediated signaling regulates the ion gated sodium channel in vascular endothelial cells and requires an intact cytoskeleton. BiochemBiophys Res Commun., 280(5): 1300-1306.

Haddy, F., P. Vanhoutte and M. Feletou, 2006. Role of potassium in regulating blood flow and blood pressure. Am. J. Physio.lRegulIntegr Comp Physiol., 290(3): R546-552.

Hayden, M.R., S.C. Tyagi, 2003. Is type 2 diabetes mellitus a vascular disease (atherosclerop athy) with hyperglycemia a late manifestation?The role of NOS, NO, and redox stress. Cardiovasc Diabetol., 2: 2.

He, F., N. Markandu, G. Sagnella, H. de Wardener and G. MacGregor, 2005. Plasma sodium: ignored and underestimated. Hypertension, 45(1): 98-102.

Hink, U., H. Li, H. Mollnau, M. Oelze, E. Matheis, M. Hartmann, 2001. Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ Res., 88: 14-22.

Kaplan, N., 2006. Primary hypertension: pathogenesis. In: Kaplan NM, ed. Kaplan's Clinical Hypertension. Philadelphia, PA: Lippincott Williams & Wilkins, 50-121.

Karen, C., 2007. "Diabetes - The Medical Perspective." Diabetes Life Lines, University of Illinois Extension.

Kido, M., K. Ando and M. Onozato, 2008. Protective effect of dietary potassium against vascular injury in salt-sensitive hypertension. Hypertension, 51(2): 225-231.

Kinlay, S., P. Libby, P. Ganz, 2001. Endothelial function and coronary artery disease. Curr. Opin. Lipidol., 12: 383-9.

Mikiwa, K., I. Tadashi, K. Kayoko, N. Atsushi, H. Masahito, H. Hiroshi, 2002. Plasma nitrate/nitrite concentration in healthy population and patients with diabetes mellitus- relationships with gender, aging and diabetic complications. Bull Osaka Med Coll., 48: 1-6.

Mohamed, A.K., A. Bierhaus, S. Schiekofer, 1999. The role of oxidative stress and NF-kappa B activation in late diabetic complications. Biofactors, 10: 157-67.

Munzel, T., R. Feil, A. Mulsch, S.M. Lohmann, F. Hofmann, U. Walter, 2003. Physiology and pathophysiology of vascular signaling controlled by guanosine-3, 5-cyclic monophosphate dependent protein kinase. Circulation, 108: 2172-2183.

Nagata, D., M. Takahashi and K. Sawai, 2006. Molecular mechanism of the inhibitory effect of aldosterone on endothelial NO synthase activity. Hypertension, 48(1): 165-171.

Nugent, B.W., 2005. Hyperosmolar hyperglycemic state. *Emerg Med Clin North Am.*, 23(3): 629-48.

Osanai, T., N. Fujiwara, M. Saitoh, S. Sasaki, H. Tomita, M. Nakamura, H. Osawa, H. Yamabe, *et al.*, 2002. "Relationship between salt intake, nitric oxide, and asymmetric dimethylarginine and its relevance to patients with end-stage renal disease". Blood purification, 20(5): 466-8.

Pál Pacher, Joseph S. Beckman and Lucas Liaudet, 2007. Nitric oxide and peroxynitrite in health and disease. Physiol Rev., 87(1): 315-424.

Pimenta, E., K. Gaddam and S. Oparil, 2009. Effects of dietary sodium reduction on blood pressure in subjects with resistant hypertension: results from a randomized trial. Hypertension, 54(3): 475-481.

Romero, M.J., D.H. Platt, H.E. Tawfik, M. Labazi, A.B. El-Remessy, M. Bartoli, R.B. Caldwell, R.W. Caldwell, 2008. Diabetes-induced coronary vascular dysfunction involves increased arginase activity. Circ. Res., 102: 95-102.

Saito, T., S. Ishikawa, M. Higashiyama, T. Nakamura, K. Rokkaku, H. Hayashi, I. Kusaka, S. Nagasaka, T. Saito, 1999. Inverse distribution of serum sodium and potassium in uncontrolled inpatients with diabetes mellitus. Endocr J. Feb., 46(1): 75-80.

Schiffrin, E., 2006. Effects of aldosterone on the vasculature. Hypertension, 47(3): 312-318.

Sicree, R., J. Shaw and P. Zimmet, 2008. IDF Diabetes Atlas fourth edition, The Global Burden. Diabetes and Impaired Glucose Tolerance. Baker IDI Heart and Diabetes Institute, 5: 69-86.

Stoner, G.D., 2005.Hyperosmolar hyperglycemic state. Am Fam Physician., 71(9): 1723-30.

Swasti Tiwari, Shahla Riazi, Carolyn A. Ecelbarger, 2007. Insulin's impact on renal sodium transport and blood pressure in health, obesity, and diabetes. American Journal of Physiology - Renal Physiology, 293: F974-F984.

Titze, J., R. Lang and C. Ilies, 2003. Osmotically inactive skin Na_ storage in rats. Am J Physiol Renal Physiol., 285(6): F1108-1117.

Titze, J., H. Krause and H. Hecht, 2002. Reduced osmotically inactive Na storage capacity and hypertension in the Dahl model. Am J Physiol Renal Physiol., 283(1): F134-141.

Tretjakovs, P., U. Kalnins, I. Dabina, A. Erglis, I. Dinne, A. Jurka, 2003. Nitric oxide production and arachidonic acid metabolism in platelet membranes of coronary heart disease patients with and without diabetes. Med Princ Pract., 12: 10-6.

Urbina, E., B. Alpert, J. Flynn, L. Hayman, G.A. Harshfield and M. Jacobson, 2008. Ambulatory blood pressure monitoring in children and adolescents: recommendations for standard assessment: a scientific statement from the American Heart Association Atherosclerosis, Hypertension, and Obesity in Youth Committee of the council on cardiovascular disease in the young and the council for high blood pressure research. Hypertension, 52(3): 433-51.

Vanizor, B., A. Orem, S.C. Karahan, E. Kiran, C. Erem, R. Aliyazicioğlu, 2001. Decreased nitric oxide end-products and its relationship with high density lipoprotein and oxidative stress in people with type 2 diabetes without complications. Diabetes Res Clin. Pract., 54: 33-9.

Vikramadithyan, R.K., Y. Hu, H.L. Noh, C.P. Liang, K. Hallam, A.R. Tall, R. Ramasamy, I.J. Goldberg, 2005. Human aldose reductase expression accelerates diabetic atherosclerosis in transgenic mice. J Clin Invest., 2434-2443.