THE EFFECTS OF FRUCTOSE-INDUCED METABOLIC SYNDROME ON RENAL HAEMODYNAMIC AND EXCRETORY FUNCTION IN RAT

By

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LIST OF ABBREVIATIONS

Abbreviation	Details
µg	microgram
µl	microliter
5-MU	5-methylurapidil
ACE	angiotensin converting enzyme
ADP	adenosine diphosphate
AMP	adenosine monophosphate
Ang II	angiotensin II
ANOVA	analysis of variance
ARB	angiotensin receptor blocker
AT_1	angiotensin II (type 1) receptor
ATP	adenosine triphosphate
BMI	body mass index
bpm	beat per minute
bpu	blood perfusion unit
BUN	blood urea nitrogen
Ca^{2+}	calcium
CBF	cortical blood flow
CEC	chloroethylclonidine
Ch	cholesterol
ClCr	creatinine clearance
CVD	cardiovascular diseases
DBP	diastolic blood pressure
dl	deciliter
DNA	deoxyribonucleic acid
DOCA	deoxycorticosterone acetate
ELISA	enzyme-linked immunosorbent assay
g	gram
GFR	glomerular filtration rate
GTP	guanosine triphosphate
H_2O_2	hydrogen peroxide
HDL	high density lipoprotein
HFCS	high fructose corn syrup
HR	heart rate
hr	hour
i.p.	intraperitoneal
i.u.	international unit
IP_3	inositol triphosphate
IVIGTT	intravenous insulin glucose tolerance test
K^+	potassium
kcal	kilocalorie
kg	kilogram
L	liter
m	meter
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase
MBF	medullary blood flow
MDA	malonyldialdehyde

ME	methoxamine
mg	milligram
min	minute
ml	milliliter
mmHg	millimeter mercury
mmol	millimole
NA	noradrenaline
Na^+	sodium
NaCl	sodium chloride
NADPH	nicotinamide adenine dinucleotide phosphate
NFBG	non-fasting blood glucose
ng	nanogram
NIBP	non-invasive blood pressure
nm	nanometer
NO	nitric oxide
$O_2^{-\bullet}$	superoxide anion
ºC	degree Celsius
OGTT	oral glucose tolerance test
P_{Cr}	plasma creatinine
PE	phenylephrine
PGE2	prostaglandin E2
PLC	phospholipase C
RAP	renal arterial pressure
RAS	renin-angiotensin system
RBF	renal blood flow
ROS	reactive oxygen species
rpm	revolutions per minute
SBP	systolic blood pressure
SD	Sprague-Dawley
SEM	standard error of mean
SHR	spontaneously hypertensive rats
SNS	sympathetic nervous system
SOD	superoxide dismutase
TG	triglycerides
TMB	3,3',5,5'-tetramethylbenzidine
U	unit
U_{Cr}	urine creatinine
UFR	urine flow rate
VLDL	very low density lipoprotein
VSMC	vascular smooth muscle cell
w/v	weight by volume
WKY	Wistar-Kyoto
α	alpha
β	beta
γ	gamma
δ	delta
ε	epsilon
ζ	zeta

KESAN SINDROM METABOLIK DIARUH FRUKTOSA TERHADAP HEMODINAMIK DAN FUNGSI EKSKRETORI GINJAL PADA TIKUS

ABSTRAK

Kajian ini menyelidek sama ada keresponsifan α_1-adrenoseptor pada vaskulatur ginjal terubah semasa sindrom metabolik yang disebabkan pemberian fruktosa yang tinggi. Tikus Sprague-Dawley jantan dirawat selama 8 minggu dengan 20% fruktosa dalam air minuman (F), sementara tikus kawalan diberi minum air paip (C) ad libitum. Parameter metabolik, fungsian dan hemodinamik dinilai setiap minggu dan pada akhir kajian. Pada set yang lain, tikus F diberikan sama ada carvedilol (FCV) atau losartan (FL) pada (10mg/kg/hari po) selama 3 minggu bermula dari minggu kelima eksperimen. Kumpulan tikus yang lain diberikan tempol, suatu superoksida dismutase mimetik (FT) pada (1 mmol/L) dengan 20% fruktos dalam air minuman selama 8 minggu. Pada akhir tempoh rawatan, suatu ujian tolerans glukosa insulin intervena dijalankan untuk menilai kesensitifan insulin. Selanjutnya, tikus dibius dengan pentobarbiton dan pengurangan aliran darah kortikal renal diaruh melalui pemberian-secara- intrarenal noradrenalina (NA), fenilefrina (PE), metoksiamina (ME) dan angiotensin II (Ang II) yang ditentukan dalam kehadiran dan ketidakhadiran 5-metilurapidil (5-MU), kloroetilklonidina (CEC) atau BMY 7378. Data, min±SEM tertakluk pada ANOVA dengan kesignifikanan pada $P<0.05$. Pada akhir minggu kelapan, didapati bahawa F mempunyai paras sistemik yang tinggi bagi tekanan darah, glukosa plasma, trigliserida dan insulin, tetapi secara signifikannya pecahan natrium dan ekskresi kalium adalah rendah jika dibandingkan dengan C. Tikus F menunjukkan pengurangan ($P<0.05$) respons vaskular renal terhadap NA, PE, ME dan Ang II jika dibandingkan dengan C. Respons terhadap Ang II secara signifikannya adalah berkurangan atau merosot oleh 5-MU, CEC dan BMY 7378,

dan juga selepas rawatan dengan carvedilol, losartan atau tempol pada tikus F dan C. Respons adrenergik semakin berkurangan oleh 5-MU dan ditingkatkan oleh CEC atau BMY 7378 pada tikus F. Rawatan dengan tempol atau losartan meningkatkan respons konstriktor terhadap NA, PE dan ME dibandingkan dengan F. Data ini mencadangkan bahawa pengambilan fruktos tinggi kronik akan mengurangkan kesensitifan vaskular terhadap agonis adrenergik dan Ang II. Ia juga menghasilkan retensi natrium yang boleh menjelaskan tekanan darah tinggi pada tikus-tikus ini. Di samping itu, α_{1A}-adrenoseptor merupakan fungsi subjenis yang boleh menyederhanakan respons vasokonstriksi dalam tikus yang diberi fruktosa. Ang II memainkan peranan penting dalam mengawal atur hemodinamik renal dan perkaitan interaktif yang wujud di antara Ang II dan neurotransmisi adrenergik pada tikus-tikus ini. Radikal superoksida memainkan peranan penting dalam mengawal respons vascular renal terhadap Ang II dan agonis adrenergik keadaan berintangan insulin. Tambahan pula, rawatan dengan carvedilol, losartan dan tempol boleh meningkatkan kesensitifan insulin dalam model ini.

THE EFFECTS OF FRUCTOSE-INDUCED METABOLIC SYNDROME ON RENAL HAEMODYNAMIC AND EXCRETORY FUNCTION IN RAT

ABSTRACT

This study investigated whether the α_1-adrenoceptor responsiveness of the renal vasculature was altered in a metabolic syndrome state due to high-fructose feeding. Male Sprague-Dawley rats were fed for 8 weeks with 20% fructose in the drinking water (F), while their controls received tap water (C) to drink *ad libitum*. Metabolic, functional and haemodynamic parameters were assessed weekly and at the end of the study. In another set of rats, F received either carvedilol (FCV) or losartan (FL) at (10mg/kg/day po) for 3 weeks starting from week 5 of the experiment. Another group of rats received tempol, a superoxide dismutase mimetic (FT) at (1 mmol/L) with 20% fructose in drinking water for 8 weeks. At the end of the treatment period, an intravenous insulin glucose tolerance test was performed to assess insulin sensitivity. Moreover, rats were pentobarbitone anaesthetized and the reductions in renal cortical blood flow induced by intrarenal administration of noradrenaline (NA), phenylephrine (PE), methoxamine (ME) and angiotensin II (Ang II) were determined in the presence and absence of 5-methylurapidil (5-MU), chloroethylclonidine (CEC) or BMY 7378. Data, mean±SEM were subjected to ANOVA with significance at $P<0.05$. At the end of the 8 weeks, F had higher systemic blood pressure, plasma glucose, triglycerides and insulin levels but significantly lower absolute and fractional sodium and potassium excretion as compared to C. The F rats expressed reduced ($P<0.05$) renal vascular responses to NA, PE, ME and Ang II compared to C. The response to Ang II was significantly attenuated by 5-MU, CEC and BMY 7378, and also following carvedilol, losartan or tempol treatments in the F and C rats. The adrenergic responses were blunted by 5-MU and enhanced by CEC or BMY

7378 in the F. Tempol or losartan treatment enhanced the constrictor responses to NA, PE and ME compared to F. These findings suggest that chronic high-fructose intake blunts vascular sensitivity to adrenergic agonists and Ang II. It also produced sodium retention which may explain high blood pressure in these rats. In addition, α_{1A}-adrenoceptor is the functional subtype that mediates renal vasoconstriction response in the fructose-fed rats. Ang II plays an important role in regulating renal haemodynamics and an interactive relationship exists between Ang II and adrenergic neurotransmission in these rats. Superoxide radicals play crucial role in controlling renal vascular responses to Ang II and adrenergic agonists in insulin resistant state. Moreover, carvedilol, losartan and tempol treatments improved insulin sensitivity in this model.

CHAPTER 1

INTRODUCTION

1.1 The metabolic syndrome

Recently we have witnessed the appearance of the metabolic syndrome with its terrifying impacts on human health worldwide. This epidemic syndrome occurs as a result of adapting new life style that is characterized by lack of activity and bad eating habits. A group of pathologies including hyperlipidemia, hypertriglyceridemia, impaired glucose tolerance, insulin resistance and hypertension have been collectively reported to be associated with the metabolic syndrome (also known as syndrome X or insulin resistance syndrome) (Reaven, 1988, Wajchenberg et al., 1994) (Figure 1.1). The metabolic syndrome has been found in approximately 20% to 30% of a middle-aged population in highly industrialized countries (Hansen, 1999). For example, it is suggested to present in about 25 to 50% of the population of the United States (Keller and Lemberg, 2003) making the metabolic syndrome one of the most common diseases. It has been suggested that there is a relationship between the metabolic syndrome manifestations and the risk for developing cardiovascular disease, type 2 diabetes mellitus and renal disease (Lorenzo et al., 2003, Lakka et al., 2002, Malik et al., 2004). Although not all people with the metabolic syndrome necessarily have diabetes but it is suggested to be a strong prediabetic condition as impaired glucose tolerance can readily be converted to type 2 diabetes mellitus (LeRoith et al., 2003). In addition, there is a link between the metabolic syndrome and premature morbidity and mortality (Keller and Lemberg, 2003, Songer, 1992). In

relation to this, recent clinical studies showed that the metabolic syndrome is associated with higher risk of proteinuria and chronic kidney disease (Chen et al., 2004, Kurella et al., 2005). It is also agreed that insulin resistance is the joint etiologic factor in the metabolic syndrome (Reaven, 1988).

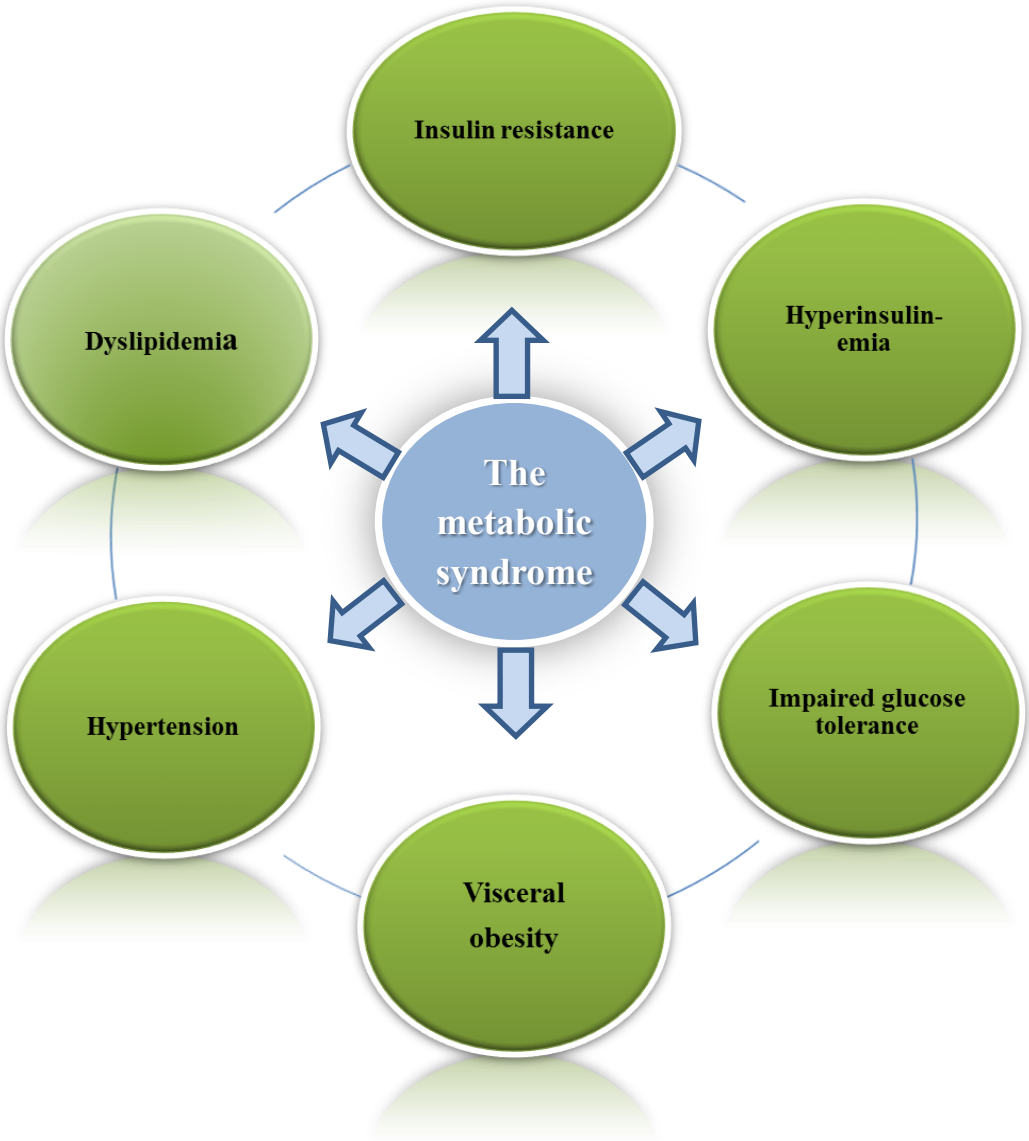

Figure 1.1: The metabolic syndrome or syndrome X: a cluster of pathologies.

There are two pathophysiological components related to the metabolic syndrome: genetic and environmental factors (LeRoith et al., 2003). It has been found that there is a strong family history of type 2 diabetes mellitus and hypertension in individuals who will develop the metabolic syndrome. Environmental influences play an important role in developing the metabolic syndrome (Reaven, 2003). These may include sedentary lifestyle, smoking, and progressive weight gain which can lead to the metabolic syndrome, however, they are associated with a very little genetic influence (LeRoith et al., 2003). In addition, one of the elements of the lifestyle component of the metabolic syndrome which is controllable is nutrition which has the importance of directly influencing humans' health. The increased availability of food rich in calories and the "Westernization" of diets have contributed significantly to the epidemic of metabolic syndrome (Basciano et al., 2005). Diets rich in fat or carbohydrate have been linked to the metabolic syndrome whereby weight gain, insulin resistance, and hyperlipidemia were detected in humans and animals (Hill et al., 1992, Kromhout et al., 1995, Kromhout, 2001).

In order to allow accurate diagnosis of the metabolic syndrome, a precise definition of the metabolic syndrome has been introduced. Since insulin resistance was highlighted as the major component of this syndrome, therefore, it has been suggested that the presence of insulin resistance is required along with two additional parameters for the diagnosis of the metabolic syndrome. For example, the National Cholesterol Education Program (NCEP), Adult Treatment Panel (ATP III) adapted a standard guideline for the metabolic syndrome to be considered (2001). According to this guideline, if three of the five following conditions are present in patients, they are diagnosed as having the metabolic syndrome.

These conditions are:

- The blood pressure should be greater than or equal to 140/90 mmHg.
- The fasting plasma glucose level should be greater than or equal to 6.1 mmol/L.
- The fasting plasma triglycerides should be greater than or equal to 175 mg/dl.
- The high density lipoprotein (HDL) cholesterol should be less than or equal to 45 mg/dl.
- Central obesity, defined as a body mass index (BMI) of 30 kg/m^2.

However, it has the disadvantage of carrying the diagnosis without direct measure of insulin resistance (2001). Therefore, the American Association of Clinical Endocrinologists (AACE) has adapted another criterion to define the insulin resistance syndrome based on the NCEP ATP III guideline. In this definition the AACE focused on insulin resistance but excluded central obesity and insulin values from the definition (Einhorn et al., 2003). Moreover, other criteria, such as a family history of type 2 diabetes, hyperuricemia and polycystic ovary syndrome have also been included in this definition. Further to this, definitions have been formulated to ease the diagnosis of the metabolic syndrome with the inclusion of abdominal obesity due to its relation to insulin resistance such as the definition of International Diabetes Federation (IDF) (Alberti et al., 2005) and the definition of the American Heart Association/National Heart, Lung and Blood Institute (AHA/NHLBI) (Grundy et al., 2005). However, according to Grundy et al., (2005) it seems currently that the updated NCEP ATP III guideline is the definition which is used worldwide with minor modifications.

1.2 Fructose and the metabolic syndrome

In order to study the metabolic syndrome in an animal model where similar metabolic profile to human is provided, the fructose-fed rat model has been utilized. This model develops insulin-resistance, hyperinsulinemia and hypertriglyceridemia in a similar fashion to human metabolic syndrome (Hwang et al., 1989b, Miller et al., 1999). In addition, it is agreed that a high consumption of diet rich in carbohydrates is associated with a higher risk of insulin resistance (Liu and Manson, 2001, Jenkins et al., 1981). Accordingly, the use of a fructose-rich diet was for a long time used as a successful approach to explore the mechanism of the metabolic syndrome development (Rayssiguier et al., 2006). Since then, diets specifically high in fructose have been shown to induce a metabolic disturbance in animal models with a subsequent increase in weight gain (Kanarek and Orthen-Gambill, 1982, Kasim-Karakas et al., 1996), hyperlipidemia (Kasim-Karakas et al., 1996), and hypertension (Sanchez-Lozada et al., 2008b, Dai and McNeill, 1995). Commercially, fructose is used to substitute other sugars like glucose or sucrose as a sweetener for desserts, condiments and beverages (Daly et al., 1997). Fructose is a monosaccharide that resembles glucose in its chemical formula but differs in its chemical structure and is sweeter than glucose. It also exists as a disaccharide sugar known as sucrose which composed of one glucose molecule attached to a fructose molecule (Figure 1.2).

Previous studies have investigated on the relation between long-term ingestion of high calorie diet such as diet rich in fat and fructose and the occurrence of diabetes in human (Gross et al., 2004). Interestingly, the study showed that there is a significant association between type 2 diabetes mellitus and the high intake of high fructose corn syrup (HFCS).

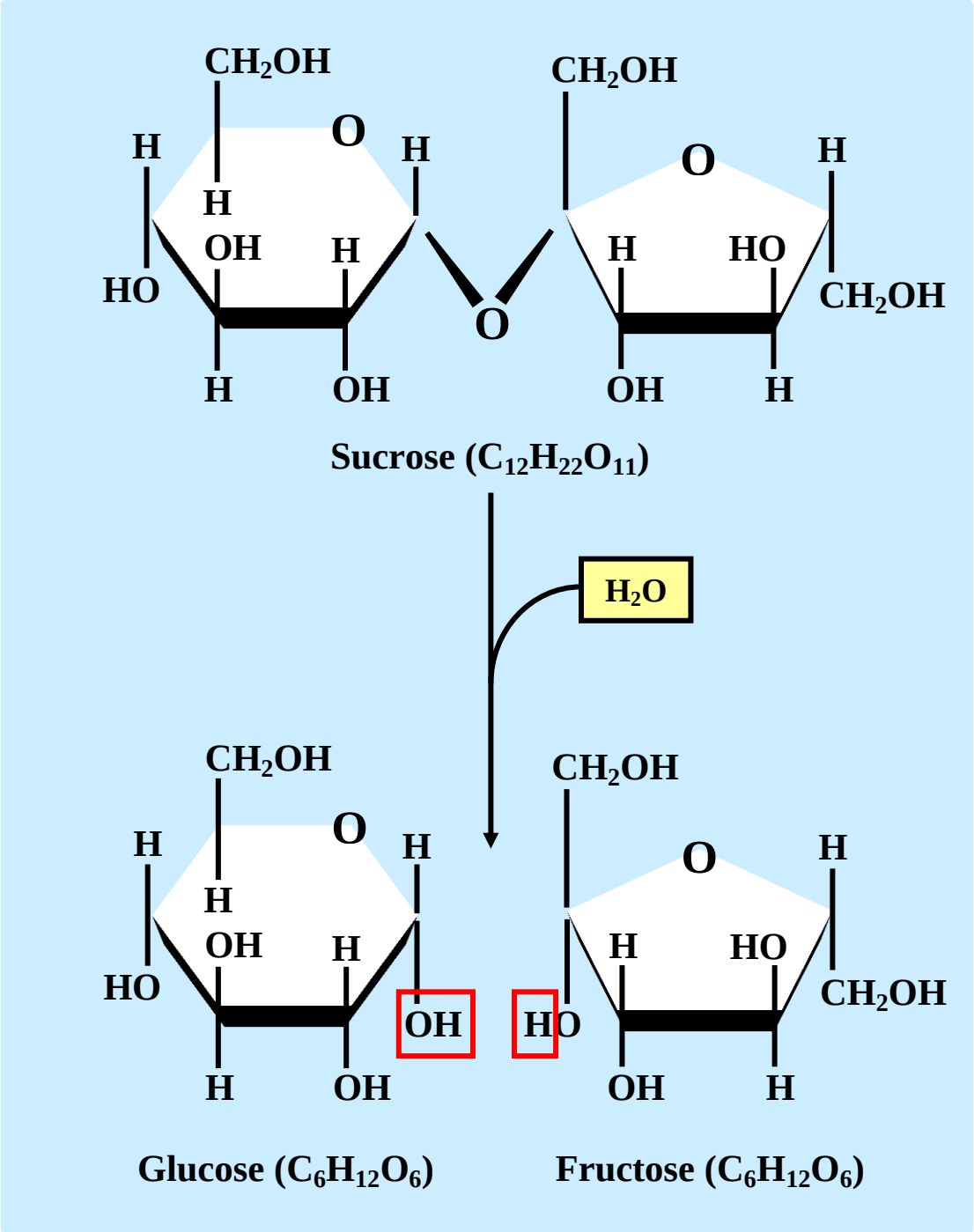

Figure 1.2: Chemical structures of fructose, glucose and sucrose: Sucrose composed of one glucose molecule attached to one fructose molecule through a 1-2 glycoside bond.

This syrup is reported to contain up to 90% of fructose (Guzman-Maldonado and Paredes-Lopez, 1995) and is used to be a constituent of sweetened soft drinks, baked goods, candies, jams, dairy products and carbonated beverages in addition to many types of food product (Bray et al., 2004). Most importantly, fructose-sweetened soft drinks are now one of the most popular drinks worldwide (Park and Yetley, 1993). The increase in HFCS consumption had far exceeded the intake of any other food or food group (Gaby, 2005). As reviewed by Basciano et al., (2005), HFCS becomes problematic due to the increase in its intake and the fact that these high-calorie component when consumed are not actually balanced by lowering calories from the rest of the diet and this results in energy imbalance (Wharton and Hampl, 2004). Collectively, it is obvious that there is a need for a better evaluation and understanding of the hazardous effects of high-calorie rich diet on human life and the necessity for the proper ways to exclude them.

Different modes of fructose administration have been described using different amounts of fructose which result in variable features of the metabolic syndrome (Table 1.1). The administration of fructose either as drinking form or as a dietary component was reported to induce insulin resistance, hypertension and hypertriglyceridemia (Sanchez-Lozada et al., 2007, Abdulla et al., 2010b, 2011c). The use of either a 10% or 20% fructose solution in hamsters was reported to produce hyperglycemia and hyperinsulinemia (Barros et al., 2007). Moreover, a 5-week consumption of 10% fructose solution in rat was found to induce hypertension and insulin resistance (Xu et al., 2010). However, as the amount of fructose ingested increased, the manifestations of the metabolic syndrome will also increase.

Table 1.1: The metabolic effects of variable forms of fructose administration in different species with different lengths of the feeding periods.

Study	Mode	Species	Amount	Length of study	Effect
Huang et al., (2006)	Drink	Mouse	10%	3 weeks	-Hypertension, hyperinsulinemia
Sanchez-Lozada et al., (2007)	Drink	Rat	10%	8 weeks	-Hypertension, hypertriglyceridemia
Xu et al., (2010)				5 weeks	-Hypertension, insulin resistance
Bar-On and Stein, (1968)				6-19 days	-Hypertriglyceridemia
Abdulla et al., (2010b)	Drink	Rat	20%	8 weeks	-Hypertension, hyperglycemia, hyperinsulinemia
Motoyama et al., (2010)				30 days	-Impaired glucose tolerance, dyslipidemia
Wong and Johns, (1999)	Drink		60%	2 weeks	Hyperglycemia
Barros et al., (2007)	Drink	Hamster	10%, 20%	2, 4 & 6 months	Hyperglycemia, hyperinsulinemia
Bar-On and Stein, (1968)	Drink	guinea pig	10%	6-19 days	Hypertriglyceridemia
Feletou et al., (2003)				18 weeks	Hyperglycemia
Farah et al., (2006)	Food	Mouse	60%	8 weeks	-Hypertension, impaired glucose tolerance, dyslipidemia
Sanchez-Lozada et al., (2007)	Food	Rat	60%	8 weeks 6 weeks	-Hypertension, hypertriglyceridemia
Gersch et al., (2007)	Food	Rat	66%	4 weeks	-Hyperinsulinemia, hypertriglyceridemia
Jordan et al., (2003)				2 weeks	-Hyperinsulinemia
Hwang et al., (1987)				4 weeks	-Hypertension, hyperinsulinemia, hypertriglyceridemia
Wong and Johns, (1999)				4 weeks	-Hyperglycemia
Taghibiglou et al., (2000)	Food	Hamster	60%	2 weeks	-Hyperinsulinemia, hypertriglyceridemia
Martinez et al., (1994)	Food	Dog	60%	20-28 days	-Hypertension, insulin resistance, hyperinsulinemia, hypertriglyceridemia
Hallfrisch et al., (1983)	Food	Human	15%	5 weeks	-Hyperinsulinemia, hyperglycemia
Reiser et al., (1989)			20%		-Hypertriglyceridemia

Therefore, it is suggested that the diet form of fructose is associated with increased metabolic disturbance and organ damage compared to drinking form (Sanchez-Lozada et al., 2007). Furthermore, Dai and McNeill (1995) have shown that hypertension in this model of fructose feeding is concentration and time dependent. Therefore, the fructose model can be used as a useful approach for exploring various aspects of the metabolic syndrome through manipulation of its route of administration, amount ingested and length of feeding which may have applicability to the human situation as the metabolic syndrome develops.

1.2.1 Fructose metabolism

Fructose metabolism has gained recent research attention due to its undesirable effects and due to its impact on both glucose and lipid metabolism (Elliott et al., 2002). As reviewed by Basciano et al., (2005), this sugar is readily absorbed from diet and rapidly metabolized by the liver and is considered as a potent regulator of glycogen synthesis and liver glucose uptake. Therefore it produces catalytic improvements due to facilitation of hepatic glucokinase and glucose uptake, however, the positive effects of fructose do not continue with chronic fructose utilization (McGuinness and Cherrington, 2003). The higher amount of fructose in diet produces glucose and fructose malabsorption due to its lipogenic properties in addition to elevation in triglycerides (TG) and cholesterol (Ch) levels compared to other carbohydrates (Hallfrisch, 1990).

Fructose metabolism is shown in Figure 1.3. As previously reported (Elliott et al., 2002, Tran et al., 2009b, Basciano et al., 2005), fructose goes through a

phosphorylation step in the liver by adenosine triphosphate to fructose 1-phosphate in the presence of highly expressed fructose-specific fructokinase enzyme. Then, fructose 1-phosphate undergoes splitting by aldolase enzyme into three-carbon molecules: glyceraldehyde and dihydroxyacetone phosphate. These specific fructose end products can be converted to glyceraldehyde 3-phosphate. Therefore, the fructose molecule undergoes metabolism into two triose phosphates that bypass the main rate-limiting step in glycolysis which is the formation of fructose 1,6-bisphosphate from fructose 6-phosphate in the presence of phosphofructokinase.

On the other hand, hepatic glucose metabolism is limited by the ability to store glucose as glycogen. Moreover, it can also be prevented by the inhibition of glycolysis and additional glucose utilization resulting from the effects of citrate and ATP to limit the action of phosphofructokinase (Elliott et al., 2002). In the liver, fructose metabolism through the glycolytic pathway results in products such as glucose, lactate, pyruvate and glycogen. It is therefore suggested that this is the reason for considering high-fructose intake as a risk factor for cardiovascular diseases (CVD) (Abdullah et al., 2009). Fructose-derived intermediates enter the pathway downstream of the phosphofructokinase enzyme with a resultant formation of triglycerides and glycerol 3-phosphate. It is evident that fructose metabolism is a highly lipogenic pathway that results in hepatic accumulation of triglyceride and consequently hepatic insulin resistance (Basciano et al., 2005).

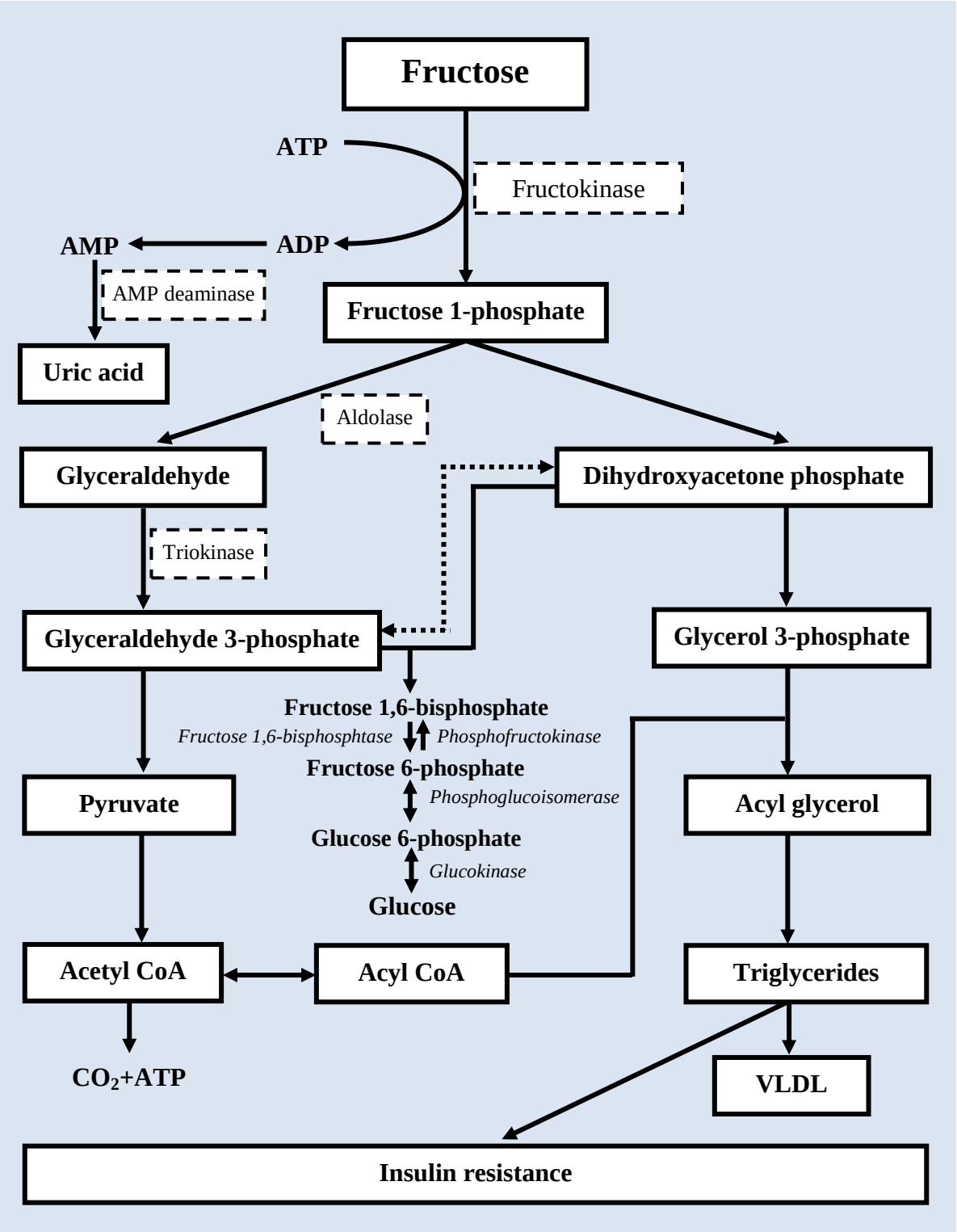

Figure 1.3: Hepatic fructose metabolism (*Redrawn with modification from* Tran et al., 2009b *and* Basciano et al., 2005).

1.2.2 Fructose consumption and energy intake

Fructose is a natural sugar which is found in many fruits and honey and it is safe when consumed by human from its natural sources in a range of 16-20 g per day. However, the daily intake has increased significantly due to added fructose in diet to around four to five folds resulting in increased weight gain and energy consumption (Basciano et al., 2005). This disturbance in energy intake due to fructose is found to be accompanied by alterations in the actions of insulin and leptin (Bray et al., 2004), a hormone that produces the sensation of satiety. These two hormones are suggested to play a key role in the long-term control of energy homeostasis and body adiposity (Havel, 2001, 2002). Therefore, it is suggested that fructose promotes obesity more than glucose because it is not associated with thermogenesis (Levine, 1986). However, it is also reported that dietary fructose intake does not result in significant weight gain in a number of studies in rat (Bezerra et al., 2000, Lingelbach and McDonald, 2000, D'Angelo et al., 2005, Iyer and Katovich, 1994) and dog (Martinez et al., 1994) and this makes this model a proper approach to investigate the correlation between the metabolic syndrome and the development of hypertension without interference from other factors like obesity or genetic predispositions.

In contrast, many other reports have shown that this model is associated with weight gain in rodents (Abdulla et al., 2010b, Kanarek and Orthen-Gambill, 1982, Kasim-Karakas et al., 1996). The reason for this controversy in result from these studies is that fructose was introduced at different ages and therefore had different impacts on body weight. In relation to that, the introduction of fructose in diet during the adulthood period did not stimulate excessive weight gain compared to childhood (Huynh et al., 2008). This suggests that the timing of the fructose consumption

affects weight gain differently. Interestingly, the increase in energy expenditure in rodents was suggested to be a result of activation of the sympathetic nervous system (SNS) (Haynes et al., 1997). Furthermore, fructose was suggested to suppress the action of leptin and therefore results in weight gain (Teff et al., 2004). The resistance to leptin action is associated with an increase in its fasting plasma level (Lee et al., 2006, Huang et al., 2004, Mooradian et al., 2000, Scarpace and Zhang, 2007). However, leptin resistance has also been reported in rats with unchanged plasma leptin levels (Shapiro et al., 2008). These reports therefore illustrate an important relationship between energy consumption, appetite control and weight gain in this model of the metabolic syndrome.

1.2.3 Fructose consumption and insulin resistance

Insulin resistance is considered to be the earlier and major feature in the development of type 2 diabetes mellitus (McGarry, 2002). In addition, it is also associated with other manifestations of the metabolic syndrome like dyslipidemia, hypertension, oxidative stress, endothelial dysfunction and cardiovascular disease (Chisholm et al., 1997). It is known that insulin resistance is associated with compensatory hyperinsulinemia which is reported to be the sum of two mechanisms, one is primary hypersecretion and the other is adaptive hypersecretion (LeRoith et al., 2003). The adaptive hypersecretion is secondary to insulin resistance because of the inadequate stimulation of glucose uptake by insulin in target tissues which through a feedback mechanism can stimulate the β-cell of the pancreas to enhance the rate of insulin production and release (LeRoith et al., 2003). It has been previously shown that the intake of fructose-rich diet produced insulin resistance, a

condition whereby insulin is unable to produce its effects to suppress hepatic glucose production and stimulate glucose uptake in rodents (Hallfrisch et al., 1979, Zavaroni et al., 1980, Higashiura et al., 2000), dogs (Martinez et al., 1994) and also in humans (Wei et al., 2007, Beck-Nielsen et al., 1980). After short term exposure, it has been found that fructose does not stimulate insulin secretion (Curry, 1989) rather the insulin resistance and compensatory hyperinsulinemia was induced after long term fructose feeding in experimental animals (Blakely et al., 1981, Thorburn et al., 1989). Fructose-fed rats have been shown to have impaired whole-body insulin sensitivity (Thorburn et al., 1989, Higashiura et al., 2000). Different approaches have been utilized to assess *in vivo* insulin sensitivity. For example, the oral glucose tolerance test (OGTT) (Abdulla et al., 2010b, Song et al., 2004a), the intravenous glucose tolerance test (IVGTT) (Iyer and Katovich, 1996a), intravenous insulin glucose tolerance test (IVIGTT) (Viswanad et al., 2006) and hyperinsulinemic-euglycemic clamp (Einstein et al., 2008, Gupta et al., 2000). Moreover, insulin resistance in this model is usually linked to hypertension (Bhanot and McNeill, 1996, Reaven, 1988). It has been suggested that the downregultion of the insulin receptors and a lower mRNA level of insulin receptors in skeletal muscles is associated with a decreased insulin-stimulated glucose utilization and a reduction in insulin sensitivity (Catena et al., 2003a). Litherland et al., (2004) have shown that GLUT5 receptors which act as fructose transporters have a possible role in the pathology of the metabolic syndrome associated with fructose feeding and insulin resistance due to the notion that the activity of these receptors is compromised in diabetic rats. It is therefore evident that the insulin resistance associated with hyperinsulinemia is considered as the most important manifestation of the metabolic syndrome. In relation to this, the metabolic syndrome is known previously as insulin resistance

syndrome. This may be due to the association of insulin resistance with other features of the metabolic syndrome especially hypertension. However, it may be that other features yet to be identified are also involved.

1.2.4 Fructose consumption and hypertension

The fructose-fed rat model is usually associated with systolic hypertension and has been reported in several studies (Sanchez-Lozada et al., 2008b, Hwang et al., 1987, Dai and McNeill, 1995, Abdullah et al., 2009). Insulin resistance associated with hyperinsulinemia may play an important role in the development of hypertension possibly due to stimulation of the SNS (Reaven et al., 1996). Therefore, various interventions utilized to enhance insulin sensitivity in this model were also able to normalize blood pressure (Iyer and Katovich, 1994, Higashiura et al., 2000, Navarro-Cid et al., 1995). However, blood pressure lowering agents do not necessarily ameliorate glucose intolerance and hyperinsulinemia (Elliott et al., 2002). It has been reported that the development of high blood pressure may occur as early as one week (Dai and McNeill, 1995), others however have shown increased blood pressure within 4 to 8 weeks (Verma et al., 1994, Abdulla et al., 2010b, 2011c, Hsieh et al., 2005), but interestingly no differences in blood pressure have also been reported (D'Angelo et al., 2005). This variation may be attributed to different diet composition used by these studies (Johnson et al., 1993), animal age or strain (Kotchen et al., 1997) and the techniques utilized to monitor blood pressure (D'Angelo et al., 2005, Ferrari et al., 1990). Therefore, it is suggested based on these studies that this effect of fructose is time and concentration dependent. The mechanism by which fructose induces the development of hypertension is not fully

elucidated, however, several factors have been suggested such as hyperinsulinemia (Daly et al., 1997), enhanced activity of SNS (Verma et al., 1999, Farah et al., 2006, Rosen et al., 1997) or renin-angiotensin system (RAS) (Tran et al., 2009a, Kobayashi et al., 1993, Abdulla et al., 2011c), high circulating level of catecholamines (Tran et al., 2009a) and endothelin-1 (ET-1) (Juan et al., 1998, Verma et al., 1997b), high uric acid production (Reiser, 1985), sodium and fluid retention (DeFronzo, 1981, Reaven, 1988) and altered vascular activity (Verma et al., 1996b, Miller et al., 1999, Verma et al., 1996a). The proposed effect of hyperinsulinemia on blood pressure has been supported by the notion that chronic insulin infusion in rat is associated with elevated blood pressure (Brands et al., 1991a, Meehan et al., 1994). Moreover, fructose feeding has been associated with impaired vascular relaxation (Takagawa et al., 2001) and an increased expression of the angiotensin II (Ang II) type 1 receptor in adipose tissue (Giacchetti et al., 2000). Albeit many studies have explored the mechanisms by which fructose intake is associated with hypertension, there is still controversy on whether this model produces a significant increase in blood pressure or not. Figure 1.4 elucidates proposed mechanisms that may explain fructose induced hypertension.

1.2.5 Fructose consumption and lipids

In addition to hyperinsulinemia and hypertension, fructose intake has been reported to produce hyperlipidemia in rodents (Inoue et al., 1995, Okazaki et al., 1994, Motoyama et al., 2010). High fructose diet is suggested to upregulate the lipogenesis pathway and results in increased triglycerides production (Kok et al., 1996).

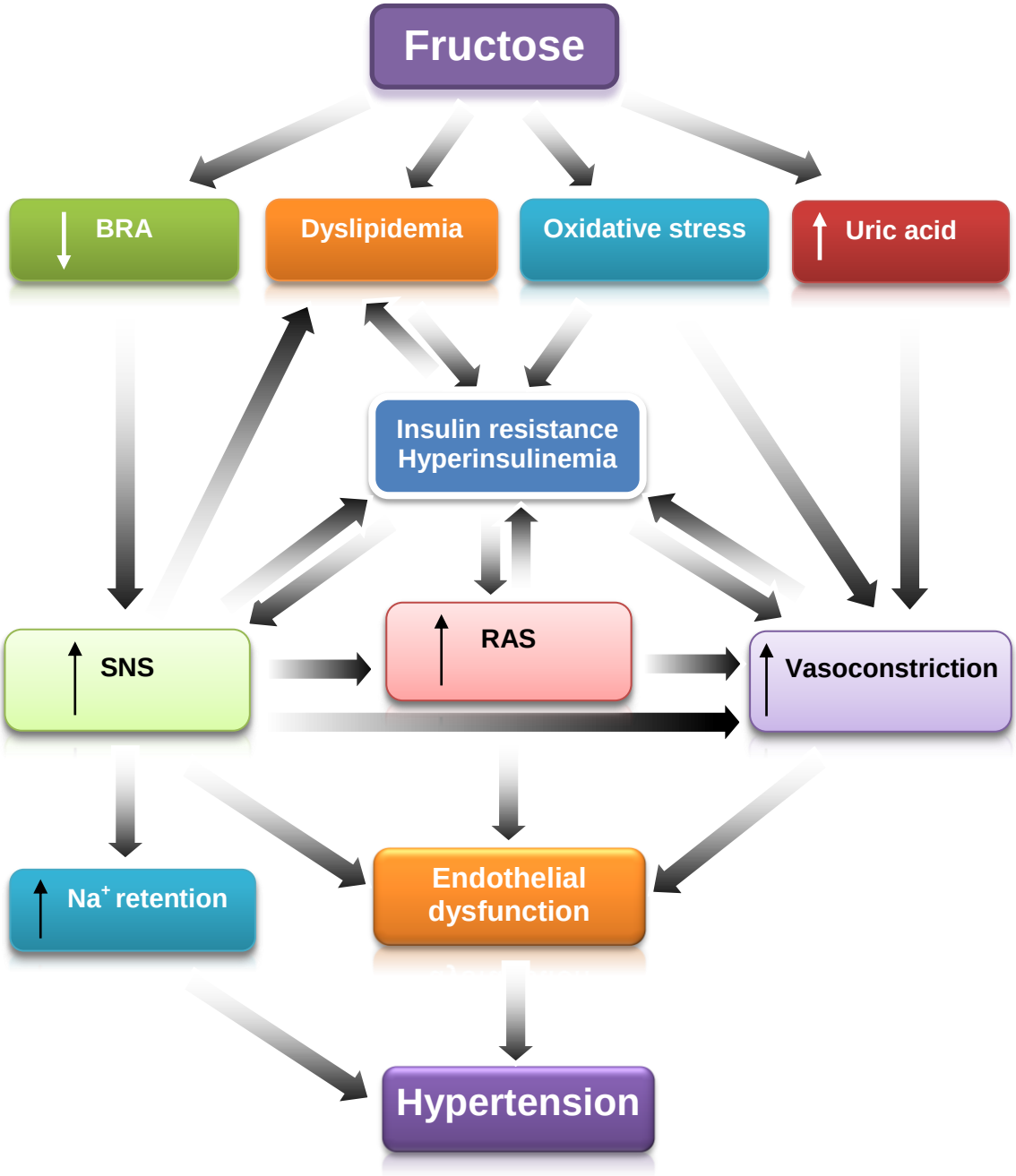

Figure 1.4: Proposed mechanisms that may explain hypertension and decreased vascular responses to vasoactive compounds in the vascular system of fructose-fed rats. SNS, sympathetic nervous system; RAS, renin-angiotensin system.

Therefore, higher circulating level of triglycerides was shown to occur due to fructose feeding in rat (Herman et al., 1970, Abdulla et al., 2010b, Sanchez-Lozada et al., 2007, Gersch et al., 2007). Interestingly, this effect of fructose is also concentration dependent (Elliott et al., 2002). Moreover, it has been shown that the high plasma level of triglycerides in fructose-fed rats is causally related to the development of hypertension in this model (Si et al., 1999). In relation to that, Damiano et al., (1999) reported the development of hypertriglyceridemia and hypertension but not hyperinsulinemia after 2 weeks of fructose feeding. The mechanism by which fructose feeding induces hyperlipidemia is through its metabolic pathway whereby glycerol combines with free fatty acids to form triglycerides (Jurgens et al., 2005). On the other hand, increased free fatty acids level is suggested to alter glucose metabolism and promote the development of hyperinsulinemia and insulin resistance (Abdullah et al., 2009). It has been reported that insulin resistance may be a factor in causing dyslipidemia in this model (Mykkanen et al., 1994, Garg, 1996, Garg et al., 1988) through compensatory hyperinsulinemia, increased very low density lipoprotein (VLDL) secretion from the liver, and hypertriglyceridemia (Reaven, 1992). The increase in VLDL production from the liver during insulin resistance is suggested to be caused by a direct effect of insulin in addition to other effects such as the availability of free fatty acids for triglycerides formation (Lewis et al., 1995). Moreover, previous studies have shown that visceral fat mass is significantly increased in fructose-fed rats (Abdullah et al., 2009, Abdulla et al., 2010b). Thus the lipid lowering agents such as statins have been shown to improve the cardiovascular outcomes through their ability to improve lipid profiles, including the reduction of triglycerides. However, this was considered to be their indirect effect (Miller et al., 2004). It is evident from the literature that lipid

abnormality in insulin resistance state with hypertriglyceridemia being the most common feature. In addition, as suggested previously, altered lipid metabolism plays a pivotal role in fructose-induced cardiovascular dysfunction (Hsieh and Huang, 2001).

1.2.6 Fructose consumption and vascular dysfunction

Due to the fact that the vasculature is responsible for the transport of essential molecules to the tissues, any impairment in its function may result in metabolic disturbance (Baron and Clark, 1997, Rattigan et al., 1999). In relation to that, impaired vascular responses to endothelium-dependent vasodilators have been reported in small vessels from fructose-fed, insulin-resistant rats (Miller et al., 1999, Katakam et al., 1999, Erdos et al., 2002). However, it is also reported that high blood pressure in this model is brought by the impaired endothelial function caused by insulin resistance and hyperinsulinemia (Takagawa et al., 2001). In addition, increased sensitivity to vasoconstrictor agents was reported in the metabolic syndrome due to vascular dysfunction (Shinozaki et al., 2004). One possible mechanism which is responsible for vascular dysfunction is the impairment in the vascular smooth muscle K_{Ca} channels during insulin resistance (Dimitropoulou et al., 2002, Miller et al., 1999, Despres et al., 1996). Furthermore, it is suggested that the effect of insulin on endothelial nitric oxide synthase enzyme (eNOS) expression and nitric oxide (NO) production is impaired during insulin resistance resulting in impaired endothelium dependent relaxation (Verma et al., 1997a, Kamata and Yamashita, 1999) as illustrated in Figure 1.5. In addition, it has been shown that in type 2 diabetic or essential hypertensive patients with insulin resistance, the NO-

dependent vasodilatory response is impaired (Higashi et al., 1997). This is supported by the notion that the lipid lowering agents, statins were able to improve endothelial function in the metabolic syndrome through upregulation of nitric oxide synthase (NOS) expression, reduction of free radical formation and improving K_{Ca} function (Hernandez-Perera et al., 1998, Miller et al., 2004). From the above studies it becomes evident that endothelial dysfunction in the metabolic syndrome is associated with insulin resistance and may be responsible for higher blood pressure in this model.

1.2.7 Fructose consumption and renal sodium handling

Insulin resistance is characterized by a defective insulin-mediated vasodilation and this produces abnormal reabsorption of sodium from proximal tubules (Stenvinkel et al., 1995). Further to this, it has been suggested that this fructose-induced change in filtration and reabsorption of sodium is attributed to hyperinsulinemia in this model (Song et al., 2004b). In support of this notion, the infusion of insulin in animals was associated with impaired sodium excretion (Baum, 1987) and this was further shown in humans (ter Maaten et al., 1999, DeFronzo et al., 1975). Hence, sodium retention by renal tubules in hyperinsulinemia is therefore suggested as a possible mechanism by which hypertension is developed in this model (DeFronzo et al., 1975). Due to insulin resistance, the vasodilator effect of insulin is impaired and therefore becomes unable to offset the counterregulatory decrease in proximal tubular sodium reabsorption during hyperinsulinemia and thus predispose to sodium retention (Stenvinkel et al., 1995). Previous studies have shown the reduction in sodium excretion after the long-term consumption of fructose rich diet (Nandhini

and Anuradha, 2004, Onuma and Nakanishi, 2004, Abdulla et al., 2011c) but at the same time others have reported no change in sodium excretion (Navarro-Cid et al., 1995, Stepp et al., 2007). The lack of any change in sodium excretion after long-term fructose feeding suggests that sodium retention *per se* may not be the reason of hypertension in this model (Iyer and Katovich, 1996b). The urinary sodium excretion and the renal interstitial hydrostatic pressure have been reported to be regulated by NO system in the kidney (Majid et al., 2001, Kone and Baylis, 1997), therefore, any defect in that system may result in salt-sensitive hypertension (Ikenaga et al., 1993, Cowley et al., 1995, Barton et al., 2000). Furthermore, eNOS which is suggested to play a key role in sodium handling (Plato et al., 2000) has been reported to be inhibited in the kidney of fructose-fed and, salt-sensitive fructose-fed hypertensive rats (Kamata and Yamashita, 1999, Nishimoto et al., 2002). Therefore, the mechanism by which fructose results in sodium reabsorption as well as the association between insulin and sodium is not well understood and yet to be determined.

1.2.8 Fructose consumption and progression of renal disease

The metabolic syndrome is reported to be associated with microalbuminuria and hyperuricemia which are considered the early markers for renal disease (LeRoith et al., 2003). The data available regarding the relationship between fructose-feeding and the incidence of chronic kidney disease is varying. For example, it has been reported that fructose intake is associated with hyperuricemia (Choi and Curhan, 2008, Palanisamy et al., 2008, Choi et al., 2008) and albuminuria (Park and Meyer, 1992, Shoham et al., 2008, Palanisamy et al., 2008). Manitius et al., (1995) have shown that

fructose feeding for 2 weeks produced hyperplasia of mesangial cells in glomeruli and results in hyperfiltration. In addition, high fructose feeding has been reported to induce kidney hypertrophy, glomerular hypertension, cortical vasoconstriction and arteriolopathy (Sanchez-Lozada et al., 2007). Higher plasma creatinine and albuminuria have been reported by Palanisamy et al., (2008) following 60 days of fructose feeding in the rat. The authors also showed that it results in kidney morphological changes including fatty infiltration and thickening of glomeruli. It is suggested that hypertension in this model is an indirect cause of renal injury (Gersch et al., 2007). On the other hand, altered body homeostasis due to kidney damage may contribute to the development of hypertension. The renal damage associated with fructose feeding is suggested to be concentration dependent (Sanchez-Lozada et al., 2007). Accordingly, the development of altered renal haemodynamics and morphological changes depends on the occurrence of damaging features of the metabolic syndrome due to fructose feeding and is amount related. Furthermore, hyperinsulinemia due to fructose feeding plays an important role in the progression of renal disease. In support of that, treatment of fructose-fed rats with troglitazone as insulin sensitizer and to correct hyperinsulinemia has reduced the progression of renal disease (Yoshida et al., 2001). Roysommuti et al., (2002) have proposed that renal impairment characterized by impaired diuresis and natriuresis and higher glomerular filtration rate and filtration fraction after long term intake of diet high in carbohydrates is dependent on an intact RAS due to the fact that treatment with angiotensin converting enzyme (ACE) inhibitor captopril has corrected these impairments. On the other hand, it has been reported that the higher urinary NO excretion in fructose-fed rats may suggest a role of the renal NO system in the pathogenesis of the early renal changes induced by fructose intake (Cosenzi et al.,

2002b). In the mentioned study, fructose feeding for one month produced glomerular hypertrophy and increased expression of inducible nitric oxide synthase enzyme (iNOS) isoform in the kidney. The role of NO is evident by the ability of L-NAME, a nitric oxide synthase enzyme inhibitor to inhibit these renal changes (Cosenzi et al., 2002b). Although hyperinsulinemia is suggested to contribute to the pathogenesis of renal disease in this model but to date, the exact mechanisms responsible for the morphological and functional changes in the kidney of fructose-fed rat have not been identified.

1.3 Oxidative stress

1.3.1 What is oxidative stress?

Oxidative stress is an imbalance of the prooxidant antioxidant ratio. It is therefore the failure of the protective antioxidant mechanism to compensate the rise in free radical production. This results in cellular damage which ultimately leads to organ death. Oxidative stress plays a role in numerous clinical conditions such as diabetes, atherosclerosis, chronic inflammation and ischemia-reperfusion injury (LeRoith et al., 2003, Griendling and FitzGerald, 2003). As a result of oxidative stress, several defense mechanisms can be developed by the biological system. These mechanisms may either be preventive, repair, physical and antioxidant in nature.

1.3.2 Free radicals

Free radicals have been discovered in the biological systems more than 50 years ago (Commoner et al., 1954) and is defined as any atom or group of atoms that possesses one or more unpaired electron (Aitken et al., 2006). In addition, free radicals are either electrically charged or neutral and are unstable and highly reactive. Therefore, due to their high activity, free radicals can combine with another atom or atoms that have unpaired electrons causing their oxidation. High levels of free radicals produce harmful effect to cellular components such as proteins, membrane lipids and nucleic acids due to their ability to modify the structure of these biomolecules and ultimately result in cell death (Maritim et al., 2003).

1.3.3 Reactive oxygen species

Reactive oxygen species (ROS) are metabolites which are produced as a result of oxygen reduction. Examples of ROS are superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$), alkoxyl radical ($RO^{\bullet}$), hydrogen peroxide (H_2O_2) and organic hydroperoxides (ROOH). In addition, the term ROS may include strong oxidants such as peroxynitrite ($ONOO^-$) and the most biologically active free radical ($^{\bullet}NO$) (Taniyama and Griendling, 2003). Superoxide anion is one of the most important ROS in the vasculature (Taniyama and Griendling, 2003) and formed through a one electron reduction of O_2 (Wolin et al., 2002). It has a role in the regulation of cell proliferation, migration, and apoptosis through the formation of H_2O_2 (Rhee, 1999, Taniyama and Griendling, 2003). ROS play an important role in mediating the consequences of oxidative stress in the body in addition to promoting the biochemical pathways which are necessary for normal cellular function. In addition, ROS exert

multiple roles within the cardiovascular system by acting as signaling molecules at lower concentrations. Therefore, ROS could have a beneficial effect when they are in small quantities but a harmful effect when higher amount is generated (Aitken et al., 2006). The superoxide anion is considered as a primary ROS and is produced in the mitochondria by the one-electron reduction of O_2 (Valko et al., 2005). This process is reported to be mediated by either enzymatic (NADPH oxidase or xanthine oxidase) or nonenzymatic redox-reactive compounds (Droge, 2002) (Figure 1.5). Mitochondria are considered an important source of ROS (Andreyev et al., 2005, Raha and Robinson, 2000) and most importantly superoxide production. However, the formation of ROS contributes to mitochondrial damage in a range of pathologies (Droge, 2002). In addition, mitochondrial superoxide dismutase enzyme (MnSOD) (Weisiger and Fridovich, 1973) is reported to scavenge superoxide radical by converting it into H_2O_2 (Deby and Goutier, 1990, Fridovich, 1978). This indicates the biological significance of mitochondrial superoxide production (Murphy, 2009). The H_2O_2 can be then converted into the highly reactive hydroxyl radical ($^{\bullet}OH$) in the presence of reduced transition metals such as ferrous (Fe^{2+}) ion (Chance et al., 1979, Kehrer, 2000) through the Fenton reaction (Figure 1.5). However, the H_2O_2 may otherwise be converted into water by the enzymes catalase or glutathione peroxidase (Droge, 2002). The H_2O_2 is known to be a stable ROS and an important signaling molecule in vascular cells. It has been suggested that the oxidant imbalance during oxidative stress is characterized by increased $O_2^{-\bullet}$ and decreased NO availability (Zhang et al., 2003). Therefore, it is evident that different ROS coexist in the reactive environment each of which may be responsible for a given biological effect.