INTESTINAL HAEMODYNAMICS AND GLUCOSE UPTAKE FOLLOWING HEXOSES ADMINISTRATION IN DOGS

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ABSTRACT

An increase in blood glucose levels has been reported to induce a rise in Intestinal Glucose Uptake (IGU) through reduction in Intestinal Vascular Resistance (IVR) and increase in Intestinal Blood Flow (IBF). However, reports on other hexoses are few and inconclusive. The effects of fructose, galactose and glucose on intestinal haemodynamics and glucose uptake were therefore investigated. The roles of adrenergic receptors and Nitric Oxide (NO) were examined.

Experiments were carried out on sixty five fasted male anaesthetized Nigerian local dogs. The control (Group 1, n = 5) received normal saline, while groups 2-4 (n= 5) were infused with glucose (1.1mg/kg/min), fructose (1.1mg/kg/min) and galactose (1.1mg/kg/min), groups 5-7 (n=5), groups 8-10 (n= 5) and groups 11-13 (n= 5) were pretreated with prazosin (0.2mg/kg), propranolol (0.5mg/kg) and L-Nitro-Arginine-Methlyl-Ester (L-NAME) (35mg/kg) respectively followed by infusion with either glucose, fructose or galactose. Through a midline laparatomy, the upper jejunum was secured for IBF measurement by collection of effluent from the cannula placed in the jejunal vein for 60 seconds. Blood samples were obtained for determination of arterial and venous blood glucose. Arterial Blood Pressure (ABP) was measured throughout the experiment using a channel recorder. The IVR was calculated from ABP and IBF; IGU was derived from IBF and Arterio –Venous glucose difference. Blood glucose was determined using the glucose oxidase method. Data were analysed using ANOVA and Student's t-test at P= 0.05.

There were no significant changes in the mean ABP for glucose, fructose, and galactose relative to control (90.45±1.78mmHg). However, following L-NAME pretreatment, ABP for glucose (95.67±1.14mmHg), fructose (101.10±0.68mmHg) and galactose (111.17±1.57mmHg) were higher. The mean IBF in the glucose group (14.40±0.93ml/min) only was significantly

higher relative to the control (10.60±0.75ml/min). Arterio–Venous glucose differences were higher in glucose (24.20±2.13mg/dl), fructose (30.83±1.83mg/dl) and galactose (15.20±0.86mg/dl) relative to control (3.40±0.68mg/dl). IGU were higher in glucose (342.20-±40.77mg/dl), fructose (244.40±26.53mg/dl) and galactose (166.60±12.98mg/dl) relative to control (36.80±8.26mg/dl). Following L-NAME pretreatment, there was 50% reduction in the IBF; IVR was higher in glucose (29.87±1.67R.U), fructose (27.90±3.13R.U) and galactose (27.18±1.44R.U) relative to control (9.50±0.80R.U), and 200% reduction in IGU induced by the three sugars. The mean ABP were reduced in Prazosin (76.33±1.74mmHg) and propranolol (81.42±0.67mmHg). There were also reductions in IBF while there was no significant change in IVR following pretreatment with the adrenergic blockers. Propranolol also caused 500% (glucose), 450% (fructose) and 150% (galactose) reductions in IGU while prazosin had no effect on glucose and fructose-induced increases in IGU, but produced 160% reduction in IGU for galactose relative to control.

The hyperglycaemia and reduced vascular resistance induced by the three hexoses lead to increase in intestinal glucose uptake.Only glucose increased intestinal blood flow while fructose and galactose had no effect. These effects may be mediated in part by nitric oxide and β adrencoceptors.

Keywords: Hexoses administration, Glucose uptake, Intestinal Haemodynamics.

Number of words: 467.

DEDICATION

This piece of work is dedicated to the Almighty God for His love and mercies.

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CERTIFICATION

I certify that this work was carried out by MR. Toyin Mohammed SALMAN in the Department of Physiology, University of Ibadan.

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LIST OF ABBREVIATIONS

mg -- milligram

kg -- kilogram

min -- minute

ml -- milliliter

mmHg -- millimeter mercury

r.u -- resistance unit

i.v -- intravenous

GIT -- Gastrointestinal Tract

HGP -- Hepatic glucose production

GLUT2 - Glucose transporter 2

GK -- Glucokinase

HK -- Hexokinase

GS -- Glycogen synthase

GP -- Glycogen phosphorylase

PKA -- Protein Kinase A

GKS-3 -- glycogen synthase kinase –3

PP1 -- Protein Phosphatase -1

PTG -- Protein targeting to glycogen

MIDA -- Mass isotopomer distribution analysis

PEPCK -- phosphoenolpyruvate carboxykinase

PEP -- phosphoenolpyruvate

LIRKO -- liver specific disruption of the insulin receptor

MIRKO-- muscle-specific insulin receptor knock-out

HGP -- Hepatic Glucose Production

ER -- Endoplasmic Reticulum

HNF-4 α -- hepatocyte nuclear factor-4α

PGC-1α -- peroxisome proliferative activated receptor-γ co-activator-1α

cAMP -- Cyclic adenosine monophosphate

IL-6 -- Interleukin-6

AMPK -- AMP-activated protein kinase

TNF*α* -- tumour necrosis factor

siRNA -- small interfering Ribonucleic acid

ACC -- acetyl-CoA carboxylase

GLUT- 4 -- Glucose Transporter 4

UDPG -- uridine diphosphoglucose

G-6-P -- glucose-6-phosphate

G-1-P -- glucose-1-phosphate

GSD-Ia -- glycogen storage disease type Ia

GSD-Ib -- glycogen storage disease type Ib

AMPK -- AMP-activated protein kinase

ATP -- adenosine triphosphate

ADP -- adenosine diphosphate

3-MG -- 3-methylglucose

NOS -- Nitric oxide synthase

NO -- Nitric oxide

cGMP -- Cyclic Guanosine Monophosphate

RBC -- Red blood cells

CNS -- Central nervous system

GLP-1 -- glucagon-like peptide

NTS -- nucleus of the tractus solitarius

PBN -- parabrachial nucleus

DMNX -- dorsal motor nucleus of the vagus

GIP -- glucose-dependent insulinotropic polypeptide

SGLT3-- Sodium-dependent glucose transporter 3

LH -- Lateral Hypothalamic nuclei

PVN -- Paraventricular hypothalamic nuclei

PPA2 -- protein phosphatase A2

ChREBP-- carbohydrate response element binding protein

GI -- Glucose inhibited

GE -- Glucose excited

VMH -- ventromedial hypothalamus

AN -- arcuate nucleus

AP -- area postrema

2-DG -- 2-deoxyglucose

5-TG -- 5-thio-glucose

BLM -- basolateral medulla

GLUT2 -- glucose transporter type 2

Ca2+ -- Calcium

TRP -- transient response potential

AMPK -- AMP-activated protein kinase

T2DM -- type 2 diabetes mellitus

SGLTs -- sodium–glucose co-transporters

ATPase -- adenosine triphosphatase

FRG -- familial renal glucosuria

G6Pase -- Glucose 6-phosphatase

HK -- Hexokinase

F1,6Pase -- Fructose 1,6-bisphosphatase

PFK 1 -- phosphofructokinase 1

PC -- Pyruvate carboxylase

PEPCK -- Phosphoenolpyruvate carboxykinase

PK -- Pyruvate kinase (PK).

EGP -- endogenous glucose production

GLP -- glucagon-like peptide

Rd -- rate of disappearance

HGP -- hepatic glucose production

Ra -- rate of appearance

Vo_2 max -- maximum oxygen uptake

CHOox -- carbohydrate oxidation

NEFA -- non-esterified fatty acid

NHGU -- net hepatic glucose uptake

CFC -- capillary filtration coefficient

PO_2 -- oxygen tension

$[Ca^{2+}]i$ -- Ca^{2+} concentration

VOCs -- voltage-operated calcium channels

VSM -- vascular smooth muscle

SOD -- superoxide dismutase

ET_A -- endothelin-A

eNOS -- endothelial nitric oxide synthase.

ANG II -- angiotensin II

IR -- immunoreactivity

AG I -- angiotensin 1

ACE -- angiotensin converting enzyme

ET-1 -- endothelin-1

BFV -- blood flow velocity

CA -- cerebral arteries

SMA -- superior mesenteric arteries

L-NAME -- L-Nitro-N-Arginine-Methyl-Ester

EDHF -- endothelium-derived hyperpolarizing factor

NOS -- Nitric oxide Synthase

nNOS-IR -- nNOS immunoreactivity

SP -- Substance P

GC -- Guanylate cyclase

cGMP -- cyclic GMP

SNP -- sodium nitroprusside

SIN-1 -- 3-morpholinosydnonimine

ODQ -- 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one

Ach -- acetylcholine

L-NMMA -- N^G-Monomethyl-L-Arginine

EDRF -- endothelium-derived relaxing factor

EDNO -- endothelium-derived nitric oxide

LBF -- leg blood flow

TTX -- tetrodotoxin

CNS -- Central Nervous System

NPY -- Neuropeptide Y

PKC -- Protein kinase C

CHAPTER ONE

INTRODUCTION

The gastro-intestinal tract (GIT) is well established as a digestive and absorptive organ. After food intake, large molecules of carbohydrate, protein and fat are digested into smaller molecules such as glucose, amino acids and free fatty acids which are then absorbed in the small intestine. The digestive products cross the intestinal mucosa and enter the blood or lymph through which they are transported to body tissues for immediate use as metabolic fuel or for storage. The main dietary carbohydrates are polysaccharides, disaccharides and monosaccharides. The monosaccharides are readily absorbed while the polysaccharides and disaccharides are further broken down to monosaccharides such as glucose, fructose and galactose before being absorbed in the intestine. The fructose and galactose are later converted into glucose (Crane, 1968). Therefore, the monosaccharides will cause a steady increase in plasma glucose (Levine and Haft, 1970).

Maintenance of the normal blood glucose level in the body is of paramount importance and its failure can result in severe consequences for the body. The normal blood glucose level is maintained by a balance between glucose entering the blood stream and that leaving it. Glucose entering the circulation includes glucose absorbed from the intestine from ingested carbohydrate, glucose released from stored glycogen (glycogenolysis) and glucose newly synthesized from non-carbohydrate sources (gluconeogenesis).

Apart from the digestive and absorptive functions of the gastrointestinal tract, there are reports indicating that the gastrointestinal tract is a highly metabolic organ. For instance, Grayson and Kinear (1962) reported that the gastrointestinal tract contributes about 30% of the body's heat production by processes which are independent of digestion, absorption or bacterial

activity. Subsequent work by Durotoye and Grayson (1971) showed that oxidative processes alone could not account for this high level of heat production. When catecholamines were administered (Durotoye and Grayson, 1971), it was reported that the increase observed in glucose uptake and oxygen consumption were not compatible in quantity. In other words, the observed glucose uptake was far in excess of what could be accounted for by oxidative metabolism.

In a series of studies, Grayson and Oyebola (1983; 1985) also showed that when dogs were administered catecholamines or nicotine, glucose uptake and oxygen consumption by the intestine increased; but the increase in glucose uptake was far more than the increase in oxygen consumption. Also, the increase in glucose uptake and oxygen consumption did not occur at corresponding times. While a huge increase occurred in glucose uptake during the catecholamines infusion, oxygen consumption actually decreased. Since the results from the nicotine study are essentially similar to those of catecholamines, it was therefore postulated that the effects of nicotine on blood glucose are secondary to its action on adrenal medulla whereby catecholamines are released (Tsujimoto *et al.*, 1965).

Available evidence showed that the huge glucose taken up by the gastrointestinal tract is largely not utilized for oxidative metabolism and that glucose is not the main substrate of intestinal metabolism. Indeed, Windmueller and Spaeth (1978) showed that the substrates preferred for oxidative metabolism in the intestine are glutamine and ketone bodies while glucose contributes only about 7 %. Studies by other workers using perfused rat intestine (Hulsman, 1971) and dog intestine (Alteveer *et al.*, 1973) also suggested the importance of plasma esterified fatty acids as an energy source.

Current evidence suggests that the gastrointestinal tract is more involved in glucose homeostasis than in glucose metabolism. That is, the gastrointestinal tract takes up large quantity of glucose from the circulation during hyperglycemia induced by catecholamines (Grayson and Oyebola, 1983; Oyebola and Durosaiye, 1988; Alada and Oyebola, 1996); nicotine (Grayson and Oyebola, 1985); cow's urine concoction (Oyebola, 1982); glucagon (Alada and Oyebola, 1996); glucose (Alada and Oyebola, 1996); diabetes mellitus (Alada *et al.,* 2005) and that the gastrointestinal tract releases glucose into the circulation during hypoglycemia induced by insulin (Alada and Oyebola, 1996). Mithieux *et al.* (1996) had also provided strong evidence showing involvement of the gluconeogenic action of the intestine during insulin- and starvation-induced hypoglycemia. Alada and Oyebola (1996) had earlier postulated that the gastrointestinal tract may be playing a modulatory role in maintaining the normal blood glucose similar to the role of the liver.

The mechanism by which hyperglycemia causes a huge increase in glucose uptake by the intestine is still not clear. A number of questions remain unanswered. It was observed that there was no correlation between the percentage change in the blood glucose levels and the quantity of glucose that was taken up by the intestine during hyperglycemia (Alada *et al.,* 2005). The question is: What factor is responsible for the huge intestinal glucose uptake following an increase in blood glucose? Will the gastrointestinal tract for instance take up large quantity of glucose when the animal is infused with another hexose (apart from glucose) such as fructose or galactose? Infusion of glucose is known to increase plasma insulin level which is responsible for the vasodilatory effects of glucose. Will the infusion of other hexoses produce vasodilatory effects on intestinal blood flow similar to that of glucose? What are the contributions of the

haemodynamic changes in the effects of hexoses infusion on intestinal glucose uptake? What are the other possible mechanisms of increased glucose uptake by the intestine?

The present study was designed to investigate further the role of the gastrointestinal tract in glucose homeostasis. The study was designed to provide answers to the above questions. That is, to investigate how the gastrointestinal tract will handle infusion of glucose, fructose and galactose. The study also aimed at investigating the role of haemodynamics in the intestinal glucose handling during infusions of glucose, fructose or galactose. Attempts were also made in this study to elucidate the mechanisms of the observed responses.

Specifically, the study investigated:

(a) The effects of the infusion of glucose, fructose and galactose on mean arterial blood pressure, intestinal blood flow and vascular resistance in the dog.

(b) The effects of the infusion of glucose, fructose and galactose on blood glucose, arterio-venous glucose difference ((A-V) glucose) and intestinal glucose uptake in the dog.

(c) The role of adrenergic receptors in the observed responses.

(d) The role of nitric oxide in the observed responses.

CHAPTER TWO

LITERATURE REVIEW

2.1 ANATOMY OF THE DOG'S GASTROINTESTINAL TRACT RELEVANT TO THIS STUDY

The anatomy of the dog has been well documented (Miller*et al.*, 1964). The intestine can be classified into two parts: the small and the large intestine. The small intestine consists of two main parts, the relatively fixed and short proximal portion or duodenum, and the freely moveable, long distal portion consisting of the jejunum and ileum. The large intestine starts at the ano-rectal junction. A cross-section of the intestine shows that it consists of five layers. From the outside, these are the serosa layer, the longitudinal muscle layer, the circular muscle layer, the sub-mucosa layer and mucosa layer. The mucosa surface of the small intestine unlike the large intestine consists of folds with finger-like projections called villi. The small intestine in the dog has a length of about four metres while the large intestine is about 60-75cm in length (Grossman and Sisson, 1975).

There are no definite gross microscopic or developmental features marking the division between jejunum and ileum. This division was made by early investigators from the gross appearance of this portion of the bowel. According to Field and Harrison (1947), the term "jejunum" was used by galen to describe the middle portion of the small intestine because it is usually empty or appears emptier than the rest. The term "ileum" was first used by an anonymous author (Miller *et al*., 1964). It is applied to the relatively short and contracted portion of the small intestine in the domestic animals. In man, the distal three-fifth of the jejunuo-ileum regarded as the ileum and the proximal two-fifth as the jejunum. In the dog, unlike in man, the

ileum contains fewer aggregated lymph follicles than the more proximal part of the intestine (Titkemeyer and Callioun, 1955).

The serosa of the small intestine continues onto the mesentry, which contains the nerves, lymphatics and blood vessels supplying the intestine. According to Morton (1929), the proximal part of the small intestine has a much richer blood supply than the ileum, and it produces five to ten time more fluid.

2.1.1 Blood supply of the small Intestine

The small intestine receives its arterial blood supply from the superior mesenteric artery which arises from the aorta below the caeliac artery. It gives rise to the inferior pancreatico-duodenal arteries which supply the pancreas and duodenum; and then by means of some twelve to fifteen jejunal and ileal branches supply the jejunum and the ileum (Figure 2-1). These vessels form a system of vascular arcades, that communicate freely with each other and arising from them are short arteries of supply-vasa recta to the gut wall. This arrangement ensures good blood supply that depends on no single source and often capable of adequate functioning even in the presence of damage or kinking. A great deal of inter-and intra species variations exist in the arrangement of arterial supply and venous drainage (Rowell, 1974). Noer (1943) observed that in man there are three to four series of arcades, and the vasa recta do not communicate with each other; while in the dog, there are only primary and secondary arcades in close proximity to the gut wall and there are abundant vasa recta communications.

The veins of the small intestine drain into a series of arcades in close proximity to the arterial arcades. These arcades are drained by a number of collecting veins. Those draining the small intestine converge on the superior mesenteric vein. The splenic and the superior mesenteric veins join together to form the portal vein. The portal vein is a short vein, about 6cm long in dog

(Grossman and Sisson, 1975). It enters the porta hepatis in company with the hepatic artery and the bile duct. Within the portal hepatis, it divides into right and left branches which accompany the corresponding branches of the hepatic artery into the substance of the liver.

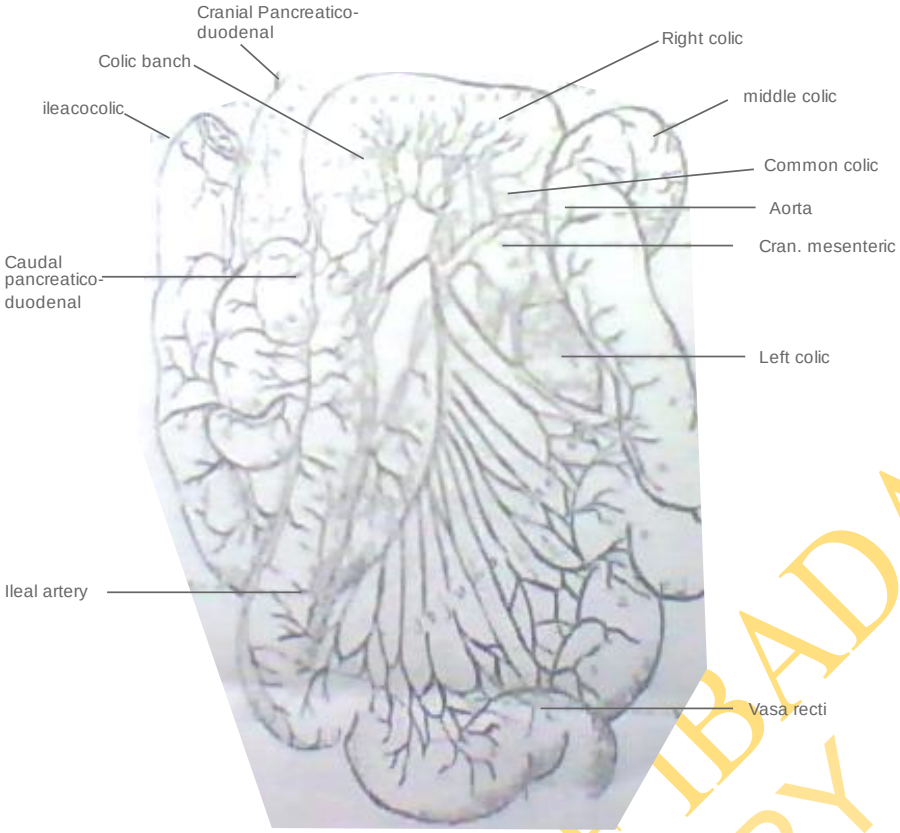


Figure 2-1: Branches of Superior mesenteric artery supplying the jejunum (Christensen and Evans, 1968)

2.2 Importance of Glucose Homeostasis

The maintenance of a stable level of glucose in the blood is one of the most finely regulated of all homeostatic mechanisms and one in which the liver, skeletal muscle, adipose tissue, kidneys and several other extrahepatic tissues along with interplay of several hormones play important role (Pickin *et al.*, 1988). The maintenance of blood glucose concentration usually between 80-90 mg/dl of blood is especially important to the brain, which normally uses glucose as the main source of energy and consumes 15-20% of the total glucose used each day (Kelley *et al.*, 1988; Seeley *et al.*, 1998; Meyer *et al.*, 2001). Glucose homeostatic mechanisms operate to maintain a constant net balance between the 'input' (various sources of plasma glucose) and 'output' (various sinks i.e. exit routes of glucose from plasma) (Owen *et al.*, 1969).

The maintenance of plasma glucose level within fairly narrow limit is of vital importance in the mammalian organism. If the plasma glucose rapidly falls to a low level (below 40-50 mg/ml) even for 5-10mins, the consequences can be dramatic and drastic. This is because the central nervous system (CNS) depends absolutely upon a continuing minute to minute supply of glucose, whereas most tissues can readily utilize free fatty acids or other blood-transported substrates when glucose is unavailable. Nerve tissue depends absolutely on glucose as the only energy substrate. Consequently, sustained hypoglycemia can lead to coma. If the hypoglycemia has been profound and prolonged, there will be inevitable brain damage that can be extensive and disabling and if uncorrected, this can lead to death (Andres *et al.*, 1956).

Abnormal elevation of plasma glucose level (hyperglycemia) is also ultimately life-threatening. If plasma glucose levels above 300 or 400 mg/dl are sustained for days, the patient will lose large amount of glucose and water in urine (glycosuria and polyuria) leading to progressive dehydration, decrease in blood volume (hypovolemia), hypertension, shock and

coma. Modest hyperglycemia over a period of years may account for the dysfunction of the central nervous system, blood vessels, kidneys and other tissues- the so-called late complications of diabetes mellitus. Prolonged hyperglycemia also can lead to death (Andres *et al*., 1956; West 1985).

These dramatic consequences of extreme departure from the normal illustrates the importance of glucose homeostasis. According to Owen *et al.* (1969), there are three sources of plasma glucose. The three sources are:

1) Intestinal absorption of dietary glucose and its precursors
2) Release of glucose from the liver.
3) Release of glucose from the kidney. Under ordinary circumstances, the kidney is a relatively minor third source. However, in prolonged starvation, it becomes significant. The amount of glucose in the circulation at a particular time will depend on the difference between absorption from the gastrointestinal tract and the release of glucose into the blood by the liver and to a lesser extent other extrahepatic tissues on one side and the amount of metabolic uptake of glucose by the liver and other tissues of the body (Otto *et al.*, 2001) as shown in figure 2.2. The body's homeostatic mechanism will thus increase glucose uptake during hyperglycemia due to increased input from various sources of glucose into the plasma glucose pool and decrease its uptake and release more glucose into the plasma pool during hypoglycemia.

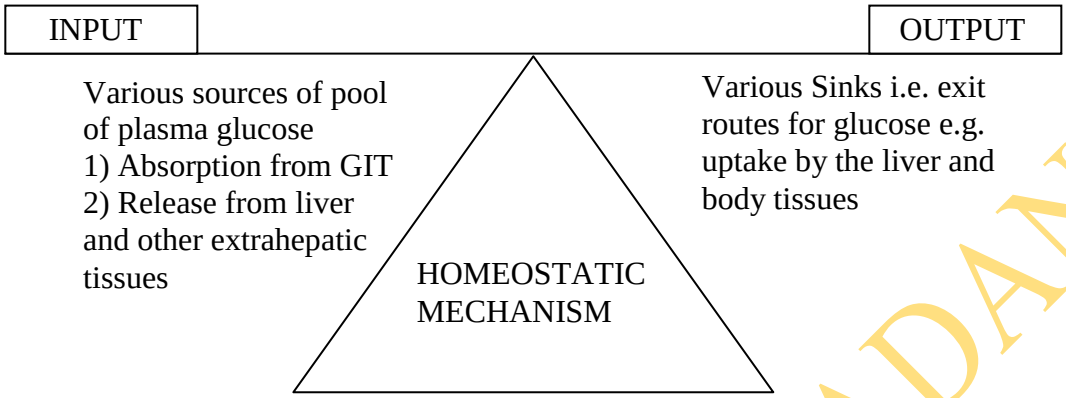


Figure 2-2: Homeostatic mechanism.

(Adapted from West, 1999)

Normal plasma glucose levels after an overnight fast range from 70-110 mg/dl. Following a meal, such as breakfast, glucose is rapidly elevated 30 to 50 percent above this level, but within two hours glucose level has been restored to normal, and there it remains at 80 mg/dl until the next meal and the pattern is repeated (Scheinberg *et al*., 1949; Nordlie *et al*., 1999).

Therefore, levels of blood glucose that are too high or too low are detrimental, causing diabetes or hypoglycemia. When glucose is not being ingested, it is the role of the liver to produce this glucose, either from its stores (glycogenolysis) or from three carbon precursors such as lactate through the process of gluconeogenesis. For instance, lactate produced in the muscle is transported to the liver where it is converted to glucose and returned to the muscle through the Cori cycle (Steinberg, 1985) as shown in figure 2-4.

2.1 Sources and sinks of plasma glucose

2.1.1 Sources of plasma glucose

The great majority of glucose in the plasma comes from two sources: intestinal absorption of dietary glucose and its precursors and the release of glucose from the liver (see figure 2-3).

Dietary sources of plasma glucose:

1-Glucose per se (a minor source)

2-Glucose-containing disaccharides

-Sucrose (fructosyl-glucose) (common name table sugar)

-Lactose (galactosyl-glucose) (milk sugar)

-Maltose (glucosyl-glucose)

3- Glucose-containing polysaccharides (a major source): Starch from plants and glycogen from animals tissues.

4-Sugars readily converted to glucose: Fructose and Galactose

5- Gluconeogenic amino acids

6- Glycerol moiety of triglycerides.

2.1.2 Sinks of plasma glucose

All body tissues can and do use plasma glucose, but some are obligatory users (cannot use alternatives such as free fatty acids when glucose is unavailable). The nervous system requires about 125-150g of glucose daily under most conditions. In prolonged starvation, study shows that the brain undergoes an interesting metabolic switch that allows it to utilize ketone bodies (β-hydroxybutyrate and acetoacetate) in place of over 50% of its usual glucose requirement (Owen *et al.*, 1967). Fatty acids are continually mobilized from the huge stores of adipose tissue triglycerides and a portion of them are continually converted to ketone bodies in the liver. This adaptation in metabolism of the brain ensures that it can survive without requiring drastic depletion of muscle protein to provide substrate for gluconeogenesis (West, 1999).

Red blood cells, the intestinal mucosa (inner epithelium) and the renal medulla (deepest region of the kidney) use glucose largely exclusively via anaerobic glycolysis. Most of the body tissues however, are facultative users of glucose. During fastings these tissues can and do switch to use free fatty acids (FFA) as their primary metabolic fuel (Zierler, 1999). The liver is both a

source and a sink for glucose and both uptake and release are occurring at all times. The net balance is under hormonal control (Zierler, 1999).

Normally, no glucose is lost to the urine via the kidney, so, the kidney is not normally a sink. Although glucose is freely filtered by the kidney, it is reabsorbed back into the blood stream unless the capacity for glucose reabsorption by the kidney is exceeded. If plasma levels of glucose are greatly elevated, then that amount of glucose that cannot be reabsorbed is lost in the urine (glucosuria) (Zierler, 1999).

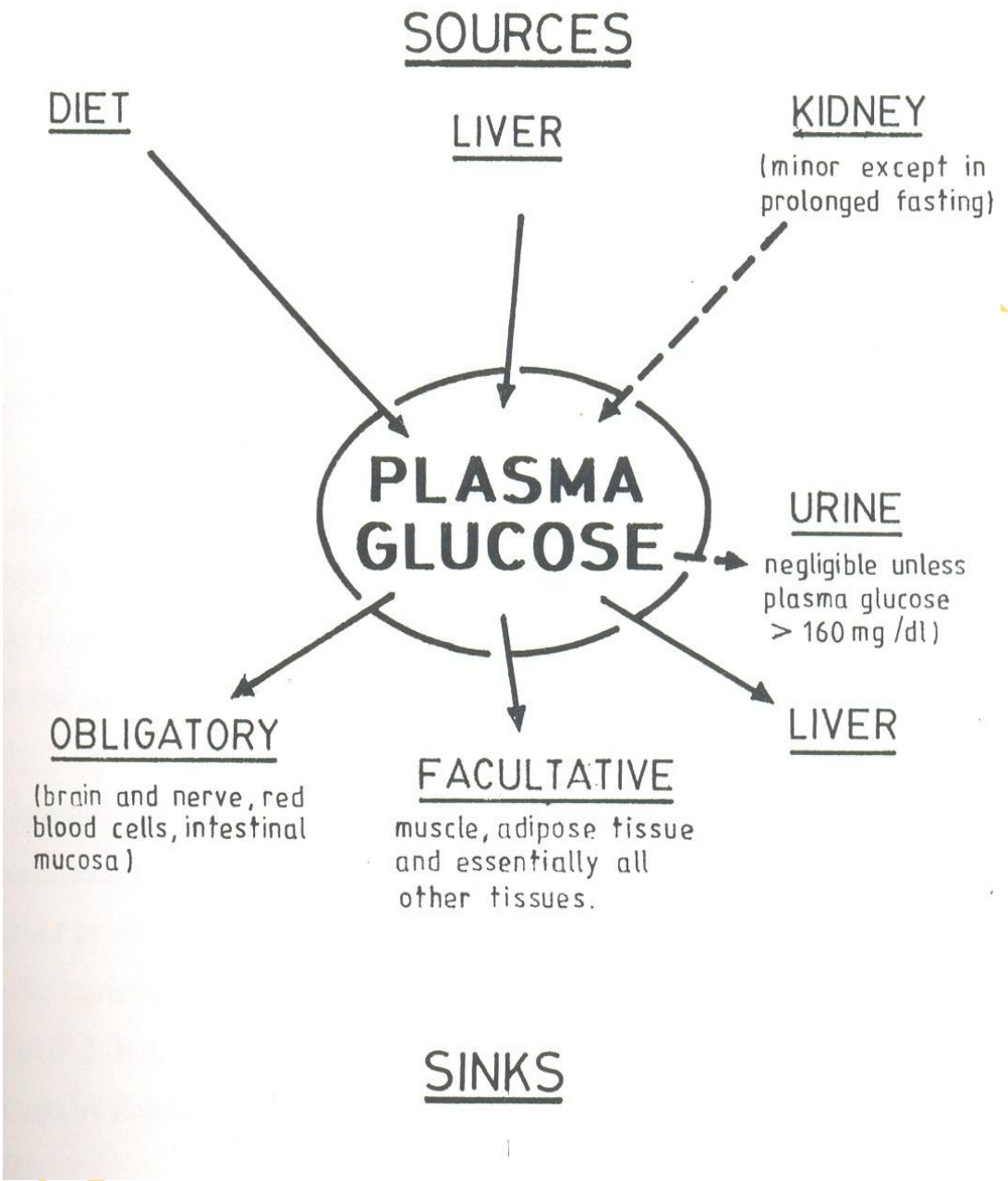


Figure 2-3: The sources and sinks of plasma glucose.

(Adapted from Steinberg, 1985)

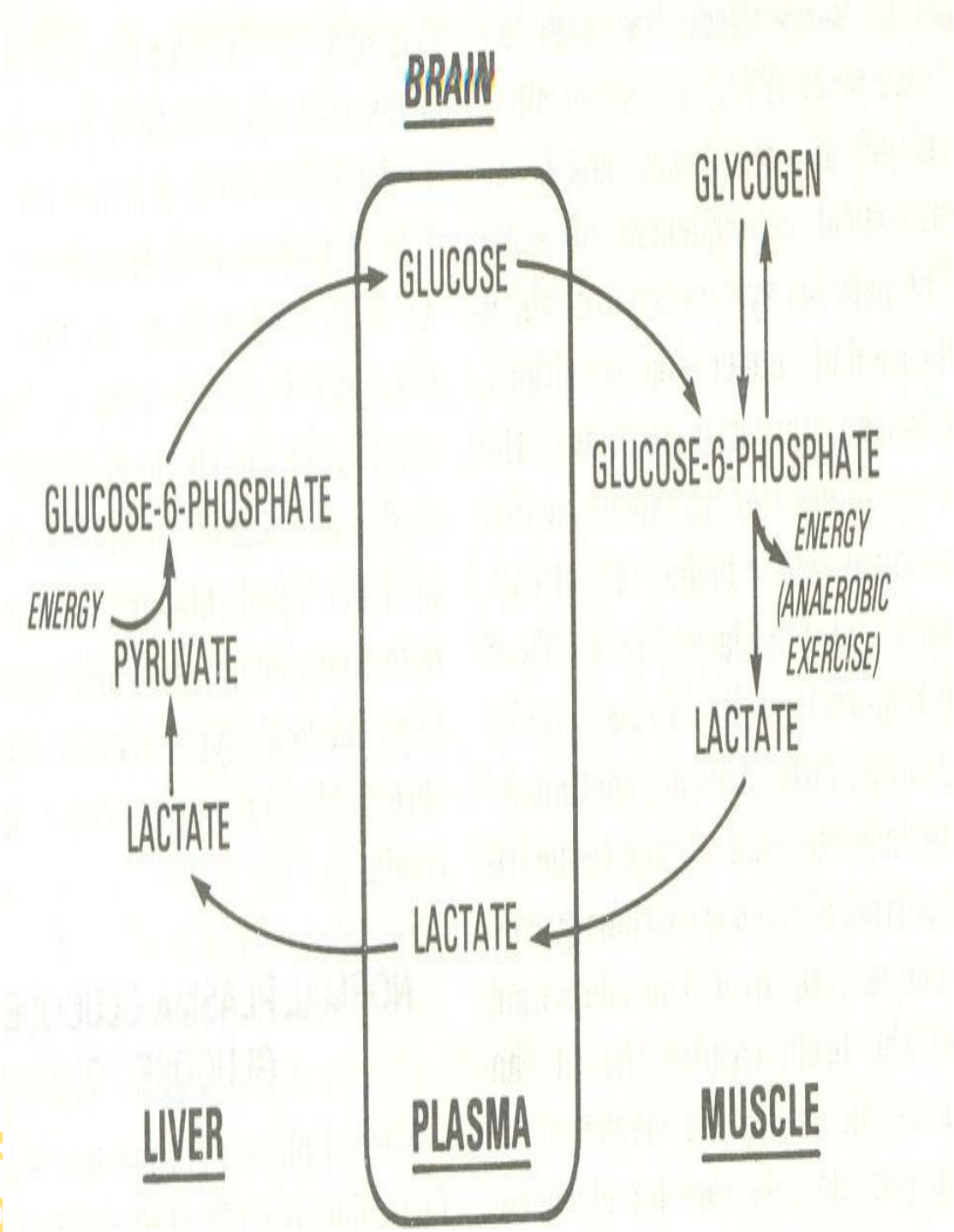


Figure 2-4: The Cori cycle (Steinberg, 1985)

2.3 Transport of Sugars across cell membranes

The transport of glucose in the various tissues of the body is achieved through different mechanisms. Active transport, i.e. an energy- dependent process that can transport against a concentration gradient, occurs in the intestinal epithelium and in the renal tubular epithelium. Either intestinal absorption of glucose or tubular reabsorption of glucose by this active transport mechanism is insulin-dependent.

In muscle, adipose tissue and other insulin-dependent tissues, glucose uptake occurs by a carrier-mediated transport mechanism. Glucose cannot be transported against a concentration gradient by this mechanism. It is a form of positive transport but more is involved than simple diffusion. This is evident from the following four characteristic properties of such a facilitated diffusion process:

(a)-Saturated kinetics: Transport increases with external glucose concentration but only to a certain maximum rate.

(b)-Stereospecificity: The unnatural isomer (L- glucose) is not transported.

(c)-Competition: Sugars of similar structure competitively inhibit glucose transport.

(d)-Countertransport-By taking advantage of the fact that a nonmetabolizable glucose analogue (3-0- methyl glucose) is transported by the same system, it can be shown (using an isolated rat diaphragm as an example of muscular tissue) that the carrier system is operating bidirectionally (Morgan and Johnson, 1988). On the inside of membrane, there is no comparable competition because the glucose is phosphorylated almost as soon as it enters the cell, and glucose-6-phosphate cannot bind to the carrier. Consequently, the outward movement of the analogue continues for a while at about the same rate that prevailed when the glucose was added. As the

concentration of the analogue on the inner side of membrane falls, its rate of transport outside falls until a new steady-state is established (West, 1999).

All of these properties can be accounted for if one postulates a set of carriers within the plasma membrane that shuttle from outer to inner surface and back, and are able to bind glucose reversibly at either surface. If the number of the carriers is finite, there will be a maximum rate of transport when all are occupied (saturation kinetics). If the configuration of the carrier is sharply defined, it will combine only with molecules of certain configurations (stereospecificity). If the configuration is not absolutely specific, some molecules of very closely related structure will also bind (competition). Even though the system operates symmetrically, differential metabolism of one or two competing ligands can lead to an apparent paradoxical transport against a gradient (countertransport) (Morgan *et al.*, 1964; West, 1999).

In muscle and adipose tissue, the rate-limiting step in the uptake of glucose is its transport across cell membrane by the carrier-mediated mechanism. The rate of glucose entry increases with the concentration of glucose presented to the cell (West, 1999). However, at very high levels the rate at which entering glucose is phosphorylated to form glucose-6-phosphate is so rapid that there is an almost unmeasurable low concentration of free glucose inside the cell. Insulin, the most important regulator of glucose transport in these tissues, can accelerate the inflow to the point that intracellular free glucose can be demonstrated (West 1999; Zierlar, 1999).

In contrast to muscle and adipose tissue membranes, liver membrane appear to be permeable to glucose, and the rate-limiting step for uptake is the rate at which the free glucose delivered into the cytoplasm can be phosphorylated in the liver. Two major types of kinases phosphorylate glucose: hexokinase, a relatively nonspecific enzyme saturated at very low

glucose concentration (k_m about 10^{-5} M); glucokinase, a specific enzyme with a k_m value near the physiological range of glucose concentration (k_m about 10^{-2}M). The latter is only partially saturated at normal glucose level so that flux can increase when glucose level increase and fall when glucose level fall (West, 1999; Zierlar, 1999). Glucokinase is not inhibited by glucose-6-phosphate: thus, the latter can build up and drive glycogen synthesis without inhibiting the continuing hepatic uptake of glucose when plasma level is high. The activity of glucokinase decreases in starvation and in diabetes mellitus (Weinhouse, 1976).

Insulin indirectly enhances membrane transport of glucose in the liver, this effect depends on induced glucokinase and the fact that glucose-6-phosphate is a feedback inhibitor of hexokinase. Reducing glucose-6-phosphate concentration accelerates glucose phosphorylation and thus, glucose transport. Insulin increases conversion to glycogen (by favouring activation of glycogen synthase) and by stimulating glycolysis. Insulin definitely stimulates hepatic glucose uptake and reduces hepatic glucose output, even though the hepatic plasma membrane is not insulin responsive (West, 1999; Zierlar, 1999).

In addition, the availability and activity of glucose transporters is crucial for the transport of sugars. For instance, the currently accepted model of intestinal hexose transport and absorption has entry across the brush-border membrane (BBM) mediated by the sodium-dependent glucose-galactose transporter (SGLT 1) and by the facilitated fructose transporter GLUT 5. The exit of all the three hexoses across the basolateral membrane (BLM) into the blood stream is mediated by another facilitated transporter, GLUT 2 (Cheeseman, 2002). SGLT 1 is highly stereospecific (Kimmich, 1981) and because of this, it transports galactose along with glucose since galactose has a similar stereochemistry with glucose and excludes fructose because of its different stereochemistry. GLUT 2 is a low affinity, high capacity facilitated hexose

transporter previously believed to be present only at the basolateral membrane. However, recent findings have shown that it is also present at the brush-border membrane and its expression at the brush-border membrane is highly regulated through the activation of protein kinase C β II when SGLT 1 transports glucose. GLUT 2 in the brush-border membrane would provide a high capacity, low affinity pathway for the entry of glucose, galactose and fructose into the epithelium i.e the so-called diffussional pathway. Thus, at the early stages of a meal, when the luminal concentrations of hexoses are high, entry would be rapid because of the involvement of GLUT 2 (Helliwell and Kellett, 2002).

A study of the kinetics of SGLT 1 and GLUT 2 has shown that SGLT 1 shows a saturation response while the low affinity, high capacity GLUT 2 was not saturated and gradually accounted for the major part of absorption as high glucose concentrations were reached. Detailed analysis revealed that the concentration dependence of GLUT 2 response to glucose is in fact cooperative because both activation of GLUT 2 and its protein level in the apical membrane increase with glucose concentration (Kellet and Helliwell, 2000). Moreover, GLUT 2 equilibrates glucose between plasma and enterocytes. Hence, when there is little glucose in the lumen before a meal, GLUT 2 is very low at the apical membrane and basolateral GLUT 2 operates in the opposite direction to supply glucose from the blood and maintain the energy requirements of the enterocytes.

2.4 Role of Various Tissues of the body in Glucose Homeostasis

2.4.1 The Liver

The liver is the principal organ that is involved in the regulation of plasma glucose. It is both a “source” and “sink” for plasma glucose (Owen *et al.*, 1989). Both uptake and release of glucose are going on all the times in the liver. The net balance is under multiple controls that

determine whether at any given time it represents a source (net input into plasma) or a sink (net uptake from plasma). The liver acts as a glucostat to regulate the plasma glucose level to a set point (Soskin *et al.,* 1943). Hence, in the absence of the liver, the plasma glucose level fluctuates tremendously with hyperglycemia following meals and profound hypoglycemia during the period between meals and during overnight fasting.

While it serves a variety of functions, the liver plays a unique role in controlling carbohydrate metabolism by maintaining glucose concentrations in a normal range over both short and long periods of times. Hepatocytes express dozens of enzymes that are alternatively turned on or off depending on whether blood glucose levels are either rising or falling out of the normal range. In the post-absorptive state (e.g overnight fast), hepatic glucose production (HGP) ensures a sufficient supply of glucose to the central nervous system and at the same time it regulates fasting plasma glucose concentrations (Defronzo *et al.*, 1992).

In the post-prandial period, the liver takes up a portion of ingested carbohydrates to restore glycogen stores. In addition, when glucose concentrations are elevated, the liver has the ability to synthesize lipids *de novo* through the lipogenic pathway. This net hepatic glucose uptake, which results from simultaneous suppression of glucose-producing pathways and stimulation of both hepatic glucose uptake and anabolic pathways of glucose disposal, restricts post-prandial increases in plasma glucose concentrations. In type 2 diabetes, alterations in hepatic glucose metabolism are observed, i.e. increased post-absorptive glucose production and impaired suppression of glucose production together with diminished splanchnic glucose uptake following carbohydrate ingestion (Defronzo *et al.,* 1992). In addition, the simultaneous overproduction of glucose and fatty acids in liver further stimulates the secretion of insulin by

the pancreatic β- cells, and exacerbates peripheral insulin resistance thereby establishing a vicious circle (McGarry, 1992).

After a meal, the secretion of insulin from alpha cells results in about 20-30% of the carbohydrate intake being stored in the form of glycogen in the liver and in skeletal muscle (Taylor *et al.,* 1993; Taylor *et al.,* 1996). Glycogen is considered the principal storage form of glucose and with up to 10% of its weight as glycogen, the liver has the highest specific content than any tissue. Therefore, defects in this process can be a major contributor to postprandial hyperglycemia, and glycogen contents of both liver and skeletal muscle are reduced in individuals with type 2 diabetes (Magnusson *et al.*, 1992; Shulman *et al.*, 1990).

Hepatic glycogen metabolism is controlled by the coordinated action of two enzymes, Glycogen Synthase (GS) and Glycogen Phosphorylase (GP), both of which are regulated by phosphorylation and allosteric modulators (Ferrer *et al.,* 2003). Although glucose and its metabolites can modulate their enzymatic activity and localization. GS and GP are principally regulated by phosphorylation. GS, which is the rate limiting enzyme of glycogen synthesis, is inactivated by phosphorylation on up to 9 regulatory residues by several kinases, including protein kinase A (PKA) and glycogen synthase kinase –3 (GKS-3) (Lawrence and Roach, 1997). Insulin regulates glycogen metabolism by promoting the dephosphorylation and the activation of GS through activation of the protein phosphatase-1 (PP1) and the inactivation of the upstream kinases. The identification of "targeting" subunits that would selectively allow the activation of PP1 only at specific sites has shed some light on the regulation of glycogen metabolism. Among them, protein targeting to glycogen (PTG) (Armstrong *et al.,* 1997; Moorhead *et al.,* 1995; Printen *et al.,* 1997) is a scaffolding protein that links PP1α to glycogen, and links it to enzymes involved in glycogen degradation or synthesis (Printen *et al.,* 1997). The recent inactivation of

PTG in mice has revealed that this protein is crucial for glycogen synthesis (Crosson *et al.*, 2003). While the homozygous mutation is lethal, heterozygous PTG knockout mice (PTG+/-) are viable but display a 40-50% decrease in steady-state glycogen concentrations and in glycogen synthase activity in fat, liver and muscle. In addition, PTG+/- mice develop a progressive glucose intolerance that is probably due to decreased glycogen content in liver but also in multiple sites (Crosson *et al.,* 2003). Similarly, overexpression of PTG in rat liver by adenoviral infection produces dramatic increases in steady-state glycogen accumulation and the glycogen synthase activity (O'Doherty *et al.,* 2000). Moreover, cells overexpressing PTG do not respond to glycogenolic stimuli and are thus store high levels of glycogen. All together these results demonstrate that PTG plays a crucial role in regulating glycogen synthesis *in vivo*.

As mentioned above, GS and GP are also regulated by allosteric modulators. It is now clear that glucose must be phosphorylated to allow the activation of GS (Ferrer *et al.,* 2003). Indeed, G6P, which is the main metabolite involved in the regulation of the enzyme, binds to GS, causing the allosteric activation of the enzyme through a change in conformation that converts it to a better substrate to synthase phosphatase, leading to the covalent activation of GS. The potency of the activation of GS seems to be determined by the origin of G6P since only G6P produced by hepatic GK is able to efficiently promote glycogen synthesis. Indeed, *in vitro* studies have demonstrated that adenovirus-mediated overexpression of GK, but not of HKI, has a potent effect on glycogen synthesis in primary cultured hepatocytes (Seoane *et al.*, 1997) and that only G6P produced by overexpressed GK is glycogenic because it effectively promotes the activation of glycogen synthase. It has also been confirmed that the ability of hepatocytes to efficiently synthesize G6P and glycogen is directly dependent on GK activity and not on HK. Using a model of liver-specific inactivation of GK (hGK-KO mice) (Postic *et al.*, 1999). It has

been shown that the efficiency of hGK-KO hepatocytes to synthesize G6P and glycogen was severely reduced in absence of GK, despite a marked increased in low *K*m HK activity (Dentin *et al.*, 2004).

In the postabsorptive state (after an overnight fast when absorption of nutrients from the intestine is completed), the liver releases glucose into the circulation to maintain normal blood glucose level. The net glucose release is the result of two simultaneous ongoing pathways that are tightly regulated. Indeed, liver produces glucose by breaking down glycogen (glycogenolysis) and by *de novo* synthesis of glucose (gluconeogenesis) from non-carbohydrate precursors such as lactate, amino acids and glycerol (Nordlie *et al.*, 1999; Pilkis *et al.*, 1992; Saltiel and Kahn, 2001) (see figure 2.3). In addition, hepatic gluconeogenesis from fructose (Anundi *et al.*, 1989; Patrick *et al.*, 1994) and galactose (Dirlewanger *et al.*, 2000; Gannon *et al.*, 2001) had been documented.The exact contribution of each of these two processes to glucose production remains however controversial.

Glycogenolysis occurs within 2-6 hours after a meal in humans. Even though, the energy content of the hepatic glycogen stores is small in comparison with the body's daily energy requirements, it is evident that liver glycogen is essential for blood glucose homeostasis in the postabsorptive state. Thus, direct determinations of liver glycogen concentration in biopsy material (Nilsson and Hultman, 1973) and ^{13}C-NMRS measurements of hepatic glycogen during the postabsorptive phase (12-24 hours) show that liver glycogen decreases linearly at rates corresponding to approximately 40% of the simultaneous whole body glucose turnover (Rothman *et al.*, 1991). During continued fasting, hepatic glycogenolysis decreases gradually, and the glycogen stores are almost completely exhausted after 48 hours (Nilsson and Hultman, 1973; Rothman *et al.*, 1991).

The rapid depletion of hepatic glycogen store during the postabsorptive state and early fasting period underscores the importance of gluconeogenesis from non-glucose precursors for the maintenance of blood glucose homeostasis. Our understanding of the quantitative contribution by gluconeogenesis to total glucose production has until recently been limited because of methodological problems (Wahren and Ekberg, 2007). Early estimates of hepatic glyconeogenesis were based on arterial-hepatic venous catheterization and balance measurements in healthy subjects. These indicated a relative contribution of gluconeogenesis to splanchic glucose production in the postabsorptive state of maximally 35% (Felig *et al.*, 1973; Wahren *et al.*, 1971; Wahren *et al.*, 1972). It should be recognized, however, that such estimates do not take into account splanchnic glucose utilization, nor extrahepatic splanchnic exchange or intraheptic precursor supply (Bjorkman *et al.*, 1990). The balance technique estimates seem low in comparison with determinations of gluconeogenesis based on the difference between ^{13}C-NMRS-measured rates of hepatic glycogen breakdown and glucose turnover rates estimated by tracer dilution methodology, which indicates a gluconeogenic contribution of 50% to 65% (Petersen *et al.*, 1996' Rothman *et al.*, 1991). Isotope tracing techniques using ^{14}C-lactate or ^{14}C-acetate have also been employed, but these are limited by inadequate labeling of the intracellular precursor pool or significant extra-hepatic metabolism of the tracer (Large *et al.*, 1995; Schmann *et al.*, 1991). Mass isotopomer distribution analysis (MIDA) using ^{13}C-glycerol has been employed for estimation of enrichment of the glucose precursor pool (Hellerstein and Neese, 1992), but it now appears that this method is limited by hepatic heterogeneity in the metabolism of glycerol (Landau *et al.*, 1995).

The above limitations in the determination of gluconeogenesis are avoided by the deuterated water technique (Landau *et al.*, 1995; Landan *et al.*, 1996). With this method, the

relative contribution of gluconegenesis to whole-body glucose production can be estimated from the ratio of ^{2}H enrichment at carbon 5 over that at carbon 2 of plasma glucose after the ingestion of $^{2}H_2O$. This approach is based on the observation that all glucose molecules exchange hydrogens between body water and those at carbon 5 of glucose during gluconeogenesis and additional hydrogens at carbon 2 during both gluconeogenesis and glycogenolysis. This technique indicates a contribution of gluconeogenesis, including any renal component, to glucose turnover ranging form 47% to 53% at 12-16 hours of fasting in healthy subjects (Chandramouli *et al.*, 1997; Landau *et al.*, 1996). Support for the validity of the deuterated water technique is obtained from the finding that after more than 42 hours of fasting, when the hepatic glycogen stores are almost completely depleted, gluconeogenesis as estimated by this method accounts for 93± 6% of glucose turnover (Chandramouli *et al.*, 1997). In view of these findings, it is now widely believed that glycogenolysis and gluconeogenesis each contribute appropriately 50% of glucose turnover in healthy subjects in the post absorptive state.

The rate of gluconeogenesis is controlled principally by the activities of unidirectional enzymes such as phosphoenolpyruvate carboxykinase (PEPCK), fructose-1, 6-bisphosphatase (FP2ase) and glucose-6-phosphatase (G6Pase). PEPCK catalyes one of the rate limiting steps of gluconeogenesis, the conversion of oxaloacetate to phosphoenolpyruvate (PEP), while G6Pase catalyzes the final step of gluconeogenesis, the production of free glucose from glucose-6-phosphate (G6P). The genes of these gluconeogenic enzymes are controlled at the transcriptional level by hormones, mainly insulin, glucagon and glucocorticoïds. Insulin inhibits gluconeogenesis by suppressing the expression of PEPCK and G6Pase, whereas glucagon and glucocorticoïds stimulate hepatic glucose production by inducing these genes (O'Brien and

Granner, 1996). In both type 1 and type 2 diabetes, excessive hepatic glucose production is a major contributor of both fasting and postprandial hyperglycemia (Taylor, 1999).

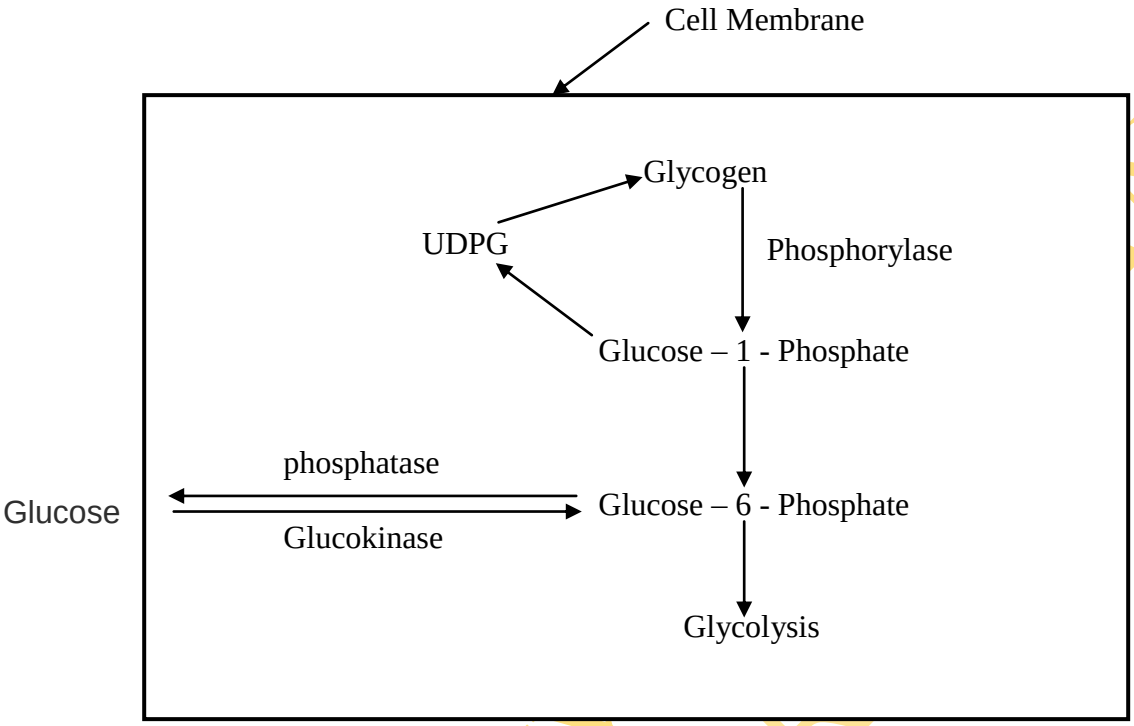


Figure 2-5: Chemical reactions of glycogenesis and glycogenolysis, showing also interconversions between blood glucose and liver glycogen (Adapted from Guyton and Hall, 2000).

2.4.2 Adipose Tissue

It is now well appreciated that, in addition to its functions related to energy storage and release, adipose tissue is also an endocrine organ, strongly involved in overall energy homeostasis and substrate partitioning. The most important hormones produced by adipose tissue are leptin and adiponectin. Leptin is a cytokine produced in proportion to the amount of adipose

tissue and which acts in specific brain hypothalamic nuclei to reduce food intake and in rodents to activate thermogenesis (Friedman, 2000). Leptin also has actions outside of the brain, one of which is to stimulate fatty acid oxidation in muscles and liver, at least in part through AMP-activated protein kinase (AMPK) activation (Minokoshi *et al.* 2002). Adiponectin belongs to the complement 1q family. It is one of the most abundant transcripts in adipocytes and its plasma concentration is high. It circulates and signals as a homomultimer. In contrast to leptin, its secretion and plasma concentration are inversely related to adiposity. Plasma adiponectin concentrations are decreased in obese and type 2 diabetic rodents, primates and humans (Tsao *et al.* 2002). Adiponectin is considered to be an insulin-sensitizing hormone since it activates muscle glucose utilization but also induces muscle and hepatic fatty acid oxidation (accumulation of fatty acids or fatty acyl-CoAs in insulin-sensitive cells is deleterious for insulin signalling) and decreases hepatic glucose production (Fruebis *et al.* 2001; Berg *et al.* 2002; Matsuzawa *et al.*, 2004). It has been shown, at least in the liver, that adiponectin effects require AMPK activation (Yamauchi*et al.* 2002). Cytokines such as interleukin-6 (IL-6) and tumour necrosis factor (TNFα) are produced by adipose tissue although probably not specifically by adipocytes but also by cells from the stroma–vascular fraction and can favour insulin resistance in insulin-sensitive tissues. AMPK is involved in other tissues in the maintenance of cellular as well as body energy homeostasis. When activated, it inhibits energy-consuming processesand activates energy-producing processes. Adipose tissue is a major component of energy homeostasis and a key player in the regulation of insulin sensitivity through fatty acid release and hormone secretion. AMPK exists in the cell as a heterotrimeric complex with a catalytic (α) and two regulatory subunits (β and γ) (Woods *et al.* 1996). Several isoforms have been identified for each subunit (α_1, α_2, β_1, β_2, γ_1, γ_2, γ_3), that can lead to the formation of 12 different complexes.

These combinations confer different properties to the AMPK complexes (Hardie & Carling, 1997) and show relative tissue specificity (Cheung *et al.* 2000). Muscle cells mainly express AMPK complexes containing the α_2 catalytic subunit and liver expresses both α_1 and α_2 isoforms (Stapleton *et al.* 1996; Woods *et al.* 1996). In adipose tissue, the α_1 catalytic subunit is the predominant isoform expressed and accounts for the major part ofAMPK activity (Lihn *et al.* 2004; Daval *et al.* 2005). Although the functional significance of these different complexes remains unclear, it can be emphasized that AMPK complexes containing the α1 isoform are less sensitive to AMP (Salt *et al.* 1998). At present there is no data concerning the respective expression of other AMPK subunits in adipocytes.

In adipose tissue, fasting and exercise activate AMPK (Park *et al.* 2002; Daval *et al.* 2005; Sponarova *et al.* 2005). Since both situations are concomitant with adrenergic stimulation, it could be anticipated that β-adrenergic agonists and their second messenger cAMP would stimulate AMPK activity. This is indeed the case (Haystead *et al.* 1990; Moule & Denton, 1998; Daval *et al.* 2005; Sponarova *et al.* 2005). It has been suggested that the effect of exercise on adipose tissue AMPK could also be secondary to the secretion of IL-6 by muscles (Kelly *et al.*2004). Indeed, IL-6 is able to activate AMPK in F442A adipocytes and a decreased AMPK phosphorylation is found after exercise in adipose tissue of IL-6 knock-out mice.

Leptin (Orci *et al.* 2004) and adiponectin (Wu*et al.* 2003; Sell *et al.* 2006) are able to activate AMPK in adipose tissue. Hypoglycaemic drugs such as biguanides are also inducing an increase of AMPK activity in adipocytes (Daval *et al.*2005; Huypens *et al.* 2005). More controversial results are found using thiazolidinediones, another class of hypoglycaemic agents which are ligands of the transcription factor PPARγ since Huypens *et al.* were unable to detect AMPK activation in 3T3-L1 adipocytes using 10 μmtroglitazone (Huypens *et al.* 2005) whereas

an increased AMPK activity was shown *in vivo* in adipose tissue of rats treated with pioglitazone (Saha *et al.* 2004) or rosiglitazone (Ye *et al.* 2004).

AMP and ATP concentrations in the cell are closely related due to the presence of the enzyme adenylate kinase. An increase in AMP is an exquisitely sensitive indicator of a decrease in the level of cellular energy charge. AMP activates AMPK by a complex mechanism involving allosteric effects and more importantly, the phosphorylation by upstream protein kinases of the threonine residue 172 within the activation loop of the α-catalytic subunit (Hardie and Carling, 1997). Two upstream kinases have been characterized. LKB1 is a kinase which is constitutively active and phosphorylates AMPK when AMP concentration rises in the cell and binds to the γ subunit, thus transforming AMPK in a suitable substrate for LKB1 (Hawley *et al.* 2003; Woods *et al.* 2003; Shaw*et al.*2004). The second kinase, calmodulin kinase kinase β, phosphorylates and activates AMPK in the presence of an increased calcium concentration, independently of an increase in AMP concentration (Hawley *et al.* 2005; Woods *et al.* 2005). In adipose tissue, several indirect arguments suggest that LKB1 is involved in AMPK activation. Treatment of adipocytes with AICAR, a drug which is transformed in the cell into ZMP, an analogue of AMP, activates AMPK in adipocytes (Sullivan *et al.* 1994; Corton *et al.* 1995; Salt *et al.* 2000; Lihn *et al.* 2004; Daval *et al.* 2005). In addition phenformin, a biguanide, induces AMPK activation and decreases ATP concentration (Daval *et al.* 2005). In transgenic mice expressing an uncoupling protein (UCP1) in white adipose tissue, the AMP/ATP ratio is increased and AMPK is activated (Matejkova *et al.* 2004). Finally, β-adrenergic lipolytic agents which induce AMPK stimulation are concomitant with a decrease in ATP concentration (Angel *et al.*, 1971; Issad *et al.*, 1995). A potential role of calmodulin kinase kinase β in AMPK activation has not been demonstrated in adipocytes (Daval *et al.*, 2006). Once activated, AMPK phosphorylates a number of proteins and

modulates the transcription of genes implicated in the regulation of energy metabolism to switch on catabolic pathways that produce ATP and switch off anabolic pathways that consume ATP (Daval *et al.*, 2006).

During hyperglycemia, insulin stimulates uptake of glucose into muscle and adipose tissue directly by increasing the translocation of GLUT-4 (Summer *et al.*, 2001). Among the multiple glucose transporter identified so far, only GLUT-4 has been demonstrated to be highly insulin-sensitive and exclusively expressed in insulin-sensitive tissues, such as the adipose tissue. Multiple studies have demonstrated that intracellular GLUT4 resides in two distinct, but related vesicular pools in adipocytes. Immunofluorescence analyses and subcellular fractionation techniques show that a significant fraction of GLUT-4 is localized in endosomal vesicles constitutively recycling to the plasma membrane, together with other recycling proteins, including GLUT1 and the transferrin receptor (Davis *et al.*, 1986; Clarke *et al.*, 1994). However, GLUT-4 can also be demonstrated in the 'GLUT-4 storage vesicle' compartment, which lacks endosomal markers, may contain more than 50% of the GLUT4 present in adipocytes, and is highly insulin-responsive (Rea and James 1997). Thus, in the basal state, GLUT4 slowly recycles between the plasma membrane and the endosomal compartment. Insulin induces a marked increase in GLUT4 exocytosis, mainly from the insulin-responsive vesicle pool, and a small reduction in the endocytotic rate, leading to a net increase of GLUT4 molecules at the cell surface and of glucose uptake rates (Clark *et al.*, 1994; Rea and James, 1997). `

AMPK activation stimulates glucose transport through increased GLUT4 translocation in muscles (Kahn *et al.* 2004). Only a few studies have addressed the potential role of AMPK in glucose uptake in adipose cells. Studies performed in 3T3-L1 adipocytes have reported that treatment of differentiated adipocytes with AICAR enhances basal glucose uptake by a

mechanism independent of insulin signalling (Salt *et al.* 2000; Sakoda *et al.* 2002). However, overexpression of a dominant negative form of AMPK in 3T3-L1 adipocytes treated with AICAR abolishes AMPK activation without affecting the increase in glucose uptake (Sakoda *et al.* 2002), raising the question of a direct involvement of AMPK in AICAR-stimulated glucose transport in this model. AThird study performed in primary rat adipocytes has shown that adiponectin activates AMPK and increases glucose uptake (Wu *et al.* 2003). In another study, the inhibitionof AMPK by pharmacological compounds abolishes the adiponectin-stimulated glucose transport and it occurs without affecting insulin-stimulated glucose uptake. This suggests a role of AMPK in glucose transport in adipocytes which could involve a mechanism independent of the insulin signalling pathway. However, whether AMPK induces the translocation of GLUT4 to the membranes of adipocytes remains unclear (Daval *et al.*, 2006).

Recently, it has also been reported that expression of a constitutively active Akt, using an adenoviral expression vector, promoted GLUT4 translocation to the plasma membrane, glucose uptake, and glycogen synthesis in both CHO cells and 3T3-L1 adipocytes. Conversely, inhibition of Akt either by adenoviral expression of a dominant-negative Akt or by the introduction of synthetic 21-mer small interfering RNA (siRNA) against Akt markedly reduced insulin stimulated GLUT4 translocation, glucose uptake, and glycogen synthesis. When 3T3-L1 adipocytes were treated with Akt isoform-specific siRNA, Akt2, and Akt1 to a lesser extent, appeared to play an essential role in insulin-stimulated GLUT4 translocation and glucose uptake, whereas Akt1 and Akt2 contributed equally to insulin-stimulated glycogen synthesis (Katome *et al.*, 2003). The latter findings demonstrated that Akt2 plays a major role in regulation of glucose transport responses in adipocytes and may partly reconcile the discordant results obtained with dominant-negative forms of Akt in earlier studies. In parallel to Akt, multiple PKC isoforms can

be activated by insulin in adipocytes and contribute to GLUT4 translocation and glucose uptake. Insulin was shown to activate PKC and, to a lesser extent, PKC-b in 3T3-L1 adipocytes (Bandyopadhyay *et al.*, 1997), and PKC-f/k in 3T3-L1 adipocytes and rat adipocytes (Kotani *et al.*, 1998, Standaert *et al.*, 1999); the latter effect appears to be mediated by the PI 3-kinase pathway (Standaert *et al.*, 1997). Overexpression of constitutively active atypical PKC isoforms in adipocytes increases GLUT4 translocation and glucose uptake, whereas the corresponding dominant-negative mutants inhibit insulin stimulation of glucose transport 50% (Kotani *et al.*, 1998, Bandyopadhyay *et al.*, 1999).

During hyperglycemia, insulin also stimulates glucose uptake indirectly by inhibiting free fatty acid release from the adipose tissues and reducing plasma free fatty acid levels. Plasma free fatty acid tends to inhibit glucose uptake (Randle effect) and thus a decrease in free fatty acid levels favours glucose uptake (Randle *et al.*, 1963). Therefore, during carbohydrate abundance and a rise in insulin, adipocyte takes in glucose mainly to facilitate the esterification and storage of fatty acid made elsewhere, and limits its output of fatty acid because of the antilipolytic effect of insulin (Rea and James, 1997).

In the adipocytes, activation of AMPK had also been reported to cause a decrease in lipogenesis, triglyceride synthesis, lipolysis and an increase in insulin sensitivity, fatty acid oxidation, glucose transport and uptake (Daval *et al.*, 2006).

One of the first proteins identified as a target of AMPK was acetyl-CoA carboxylase (ACC) which synthetizes malonyl-CoA from acetyl-CoA and is a key enzyme of the lipogenic pathway (Sim & Hardie, 1988). The phosphorylation and thus inhibition of ACC by AMPK has been shown in several studies *in vivo* and in intact cells.In adipocytes, a direct effect of AMPK activation on ACC phosphorylation and activity was shown in rodent adipocytes using

either AICAR (Sullivan *et al.* 1994) or expression of a constitutively active AMPK (Daval *et al.* 2005). This was concomitant with a decreased lipogenic rate (Sullivan *et al.* 1994). Conversely, overexpression of a dominant negative form of AMPK in adipocytes precludes the phosphorylation of ACC afterAICAR or isoproterenol (isoprenaline) treatment (Daval *et al.*, 2005). Exercise, which activates AMPK in adipose tissue is concomitant with a decreased ACC activity and malonyl-CoA concentration (Park *et al.* 2002). Exercise in rats also induces an increase in malonyl-CoA decarboxylase activity, thus further reducing malonyl-CoA concentrations, and a decrease in glycerol-acyl transferase activity, an enzyme involved in triglyceride synthesis. These effects are mimicked by AICAR treatment of the animals. As in the liver (Foretz *et al.* 1998; Leclerc *et al.* 1998), an activation of AMPK in adipocytes is concomitant with a decreased expression of lipogenic enzyme mRNA (Orci *et al.*,. 2004). Activation of AMPK in rodent adipocytes therefore leads to a decreased lipogenic flux and a decreased triglyceride synthesis (Daval *et al.*, 2006).

The other major function of adipose tissue is the breakdown of triglycerides through the lipolytic pathway that occurs during fasting to provide fatty acids and glycerol as fuels for peripheral tissues. In adipocytes, AMPK activation using AICAR has been shown to inhibit *β*-adrenergic-induced lipolysis (Sullivan*et al.* 1994; Corton *et al.* 1995). Recent work (Daval *et al.* 2005) has confirmed these studies in a more direct way, showing that overexpression of a constitutively active AMPK in rat adipocytes was indeed inhibiting isoproterenol-induced lipolysis, whereas overexpression of a dominant negative form of AMPK had a converse effect. Other activators of AMPK such as biguanides also had an inhibitory action on lipolysis (Daval *et al.* 2005). These results are at variance with the study of Yin *et al.* in 3T3-L1 adipocytes (Yin *et al.* 2003) since these authors have shown that overexpression of a dominant negative form of

AMPK inhibits isoproterenol-induced lipolysis suggesting, rather, a lipolytic action of AMPK activation. However, AMPK activity was not measured in these conditions and thus final conclusions from these experiments are difficult. Using the same cell line, it had also been demonstrated that AICAR and phenformin induce AMPK activity and strongly impair lipolysis (Daval *et al.* 2005). Interestingly, in mice lacking the predominant AMPK α_1 isoform, the size of adipocytes is reduced and basal and isoproterenol-induced lipolysis is higher than that of control adipocytes (Daval *et al.*2005). This argues in favour of an inhibitory role of AMPK activation on lipolysis. Mice with a general knock-out of the AMPK α_2 catalytic subunit have an increase in adipose mass due to adipocyte hypertrophy when fed a high fat diet and compared with high fat fed control mice (Villena *et al.,* 2004). Since the AMPK α_2 subunit represents only a very minor part of AMPK activity in adipose tissue, this adipocyte hypertrophy may be the consequence of the adaptation of adipose metabolism subsequent to the loss of AMPK α2 activity in other tissues such as muscle or liver. Thus, AMPK serves as a feedback mechanism limiting the cellular energy drain associated with lipolysis in adipocytes (Daval *et al.*, 2006).

However, during hypoglycemia induced by starvation or fasting, glucose uptake in the adipose tissue is drastically reduced. The response of the adipose tissue is metabolically, hormonally and neurally mediated. Hypoglycemia increases sympathetic stimulation and increases the release of adrenaline at sympathetic nerve endings (John and Gareth, 1991; Saladin, 1998; Ganong, 2001) and this in turn, activates hormone-sensitive lipase and promotes free fatty acid mobilization and decrease in glucose uptake.

2.4.3 Skeletal Muscle

The skeletal muscle consists of 40-50% of net body weight of a normal human (Shireh *et al.*, 2004) and stores about 300g of glycogen/70kg male, making it the largest overall reservoir of glycogen in the body. It is the major site of disposal of the excess postprandial plasma glucose (Katz, 1983; Hansen, 1986). Apart from this, the skeletal muscle accounts for about 20-30% of oxygen consumption during exercise (Rudermann *et al.*, 1990; Elia, 1992). Therefore, the importance of the skeletal muscle in glucose homeostasis is not in doubt.

There is elevation in blood glucose immediately after a meal but the elevation is only temporary and within two to three hours, plasma glucose is restored to the preprandial level (Wideman *et al.*, 2003). About 25-35% of ingested carbohydrate is immediately taken up by the liver (McMahon *et al.*, 1989; Mitrakou *et al.*, 1992; Yki-Jarvinen *et al.*, 1989; Buttler, 1991; Ferrannini *et al.*, 1985; Kelley *et al.*, 1994; Meyer *et al.*, 2001). Out of the remaining 65-75% of the ingested carbohydrate that enters the systemic circulation, the skeletal muscle takes up about 40% (Butler *et al.*, 1991; Firth *et al.*, 1986; Kelley *et al.*, 1994; McMahon *et al.*, 1989; Mitrakou *et al.*, 1992; Meyer *et al.*, 2001). The skeletal muscle is therefore the predominant site for peripheral glucose disposal (Butler, 1991).

The rise in blood glucose after a meal is immediately followed by a rise in insulin (Adkins, 2003). Insulin secretion is stimulated by several of the events associated with the glucose intake (Khan, 2002). It facilitates uptake of glucose into the skeletal muscle, thus promoting glycogenesis (Khan, 2002). Most of the glucose that is removed from the blood after a meal is converted into skeletal muscle glycogen (Shulman, 1990). Thus, glycogen synthesis in the skeletal muscle fibers is of particular relevance to blood glucose homeostasis. Insulin activates both glucose transport and glycogen synthase in the skeletal muscle to convert glucose

to glycogen (Lawrence, 1997). The increase in glucose transport results primarily from the translocation of the glucose transporters (GLUT-4) from the intracellular compartment to the sarcolemma and transverse-tubular membranes in response to insulin (Rodnick *et al.*, 1992). This allows more glucose to enter the muscle fiber, where it is converted to glucose-6-phosphate (G-6-P), glucose-1-phosphate (G-1-P) and uridine diphophoglucose (UDPG) in a sequence of reactions catalyzed by hexokinase, phosphoglucomutase and UDPG-pyrophosphorylase respectively (Edgerton, 2001). Glycogen synthase catalyzes the final step in which the glucosyl moiety from UDPG is added to pre-existing glycogen. Insulin activates glycogen synthase by dephosphorylating the enzyme (Lawrence, 1997). There is strong evidence that glucose transport is the principal rate-determining step for glucose metabolism under basal condition, as there appears to be very little free glucose in resting skeletal muscle fiber (Ziel, 1999).

The role of the skeletal muscle in blood glucose homeostasis when the blood glucose levels were raised by the administration of hyperglycemic agents such as adrenaline and caffeine have also been investigated. Caffeine administration was reported to cause an increase in glucose uptake by the canine hind limb in response to the increase in blood glucose caused by caffeine (Salahdeen and Alada, 2009). This observation is consistent with earlier observations in humans (Lee *et al.*, 2005; Greer *et al.*, 2001; Battram *et al.*, 2005) and animals (Pencek *et al.*, 2004). Previous studies have shown that glucose transport across skeletal muscle cell membrane is enhanced during hyperglycemia (Battram *et al.*, 2005; Zieler, 1999) irrespective of its causes. Some reports have also shown that caffeine-induced increases in skeletal muscle glucose uptake are through the release of calcium ions into the cytoplasm (Hardie and Sakamoto, 2006). Studies in which frog Sartorius muscle was incubated with caffeine resulted in several fold increase in glucose transport due to release of calcium ion into the myoplasm (Hardie and Sakamoto, 2006;

Rose and Richter, 2005). In rat epitrochlearis muscle, raising intracellular calcium ions by treatment with caffeine in-vitro also increased glucose transport (Hardie and Sakamoto, 2006). On the other hand, there are conflicting reports on the role of the skeletal muscle in glucose homeostasis during adrenaline hyperglycemia. For instance, several studies (Shikama *et al.*, 1978; Rizza *et al.*, 1980; Deibert *et al.*, 1980; Ekhelar *et al.*, 2002) have reported that catecholamines decreased glucose utilization by the skeletal muscles. However, Chiasson *et al*. (1981) in an *in-vivo* experiment reported that adrenaline exhibits a stimulatory effect on glucose uptake of the perfused rat hindlimb.

The breakdown of tissue glycogen into glucose is also critical for blood glucose homeostasis between meals (Massillion, 2001). Blood glucose homeostasis between meals is maintained by endogenous glucose production via glycogenolysis and gluconeogenesis. In the terminal stages of both pathways, glucose-6-phosphate is hydrolyzed to glucose and phosphate by the endoplasmic reticulum (ER)-associated glucose-6-phosphatase complex (Chou *et al.*, 2002; Chen *et al.*, 2003). This complex is composed of a glucose-6-phosphate transporter that transports glucose-6-phosphate from the cytoplasm into the lumen of the endoplasmic reticulum (ER) and a glucose-6-phosphatase catalytic subunit that hydrolyzes the glucose-6-phosphate to glucose and phosphate. Together they contribute, along with other factors, to the maintenance of blood glucose homeostasis. The glucose-6-phosphate transporter is encoded by a single copy gene (Hiraiwa *et al.*, 1999) that produces two alternatively spliced transcripts, glucose-6-phosphate transporter and variant glucose-6-phosphate transporter, differing by the absence or presence of a 66-bp exon-7 sequence (Hiraiwa *et al.*, 1999; Marcolongo *et al.*, 1998; Gerin *et al.*, 1999). Although the glucose-6-phosphate transporter transcript is expressed in many tissues, the variant glucose-6-phosphate transporter transcript is expressed only in the brain, heart, and

skeletal muscle (Lin *et al.*, 2000). Two distinct glucose-6-phosphatase genes, glucose-6-phosphatase-α (Lei *et al.*, 1993; Shelly *et al.*,. 1993) and glucose-6-phosphatase-β (Martin *et al.*, 2002; Shieh *et al.*, 2003), have been identified. The family prototype, glucose-6-phosphatase-α, is a 357-amino acid nine-transmembrane domain ER protein (Lin *et al.*, 2000; Shelly *et al.*, 1993; Pan *et al.*, 1998), which is expressed primarily in the liver, kidney, and intestine (Pan *et al.*, 1998). The primary function of glucose-6-phosphatase-α is to couple with the glucose-6-phosphate transporter to metabolize hepatic and renal Glucose-6-Phosphate to glucose.

The Glucose-6-phosphatase-α/Glucose-6-phosphate transporter complex is crucial for glucose homeostasis, and disruption of either component results in glycogen storage disease type Ia (GSD-Ia, Glucose-6-phosphatase-α deficiency) or type Ib (GSD-Ib, Glucose-6-phosphate transporter deficiency). Both GSD-Ia and -Ib patients manifest the symptoms of failed Glucose-6-Phosphate hydrolysis, characterized by a loss of blood glucose homeostasis and disorders of glycogen and lipid metabolism (Chou *et al.*, 2002; Chen *et al.*, 2003). Glucose-6-phosphatase-β is a 346-amino acid ubiquitously expressed phosphohydrolase with similar kinetic properties to Glucose-6-phosphatase-α (Shieh *et al.*, 2003). Like Glucose-6-phosphatase-α, Glucose-6-phosphatase-β is an integral membrane protein in the ER, containing nine transmembrane domains (Ghosh *et al.*, 2004). Moreover, the active-site structures of Glucose-6-phosphatase-α and –β are similar, and both form covalently bound phosphoryl-enzyme intermediates during catalysis (Ghosh *et al.*, 2002; 2004). Glucose-6-phosphatase-β also couples functionally with the Glucose-6-Phosphate transporter to form an active Glucose-6-Phosphatase complex that hydrolyzes Glucose-6-Phosphate to glucose (Shieh *et al.*, 2003). In addition to Glucose-6-Phosphate transporter, Glucose-6-phosphatase-β can couple functionally with variant Glucose-6-Phosphate transporter (Lin *et al.*, 2000). Although, it is assumed that Glucose-6-Phosphatase-α

can also couple to variant Glucose-6-Phosphate transporter, this remains to be demonstrated. Despite disruption of the Glucose-6-Phosphatase-α complex in GSD-I patients, several studies (Powell *et al.*, 1981; Tsalikian *et al.*, 1984; Collins *et al.*, 1990) indicate that GSD-Ia patients are still capable of producing glucose. In light of the discovery of Gulcose-6-Phosphatase-β, this implies that non-gluconeogenic tissues may be contributing to interprandial glucose homeostasis through the activity of a Gulcose-6-Phosphatase-β/Glucose-6-Phosphate transporter or a Gulcose-6-Phosphatase-β/variant Glucose-6-Phosphate transporter complex. This endogenous glucose production in GSD-Ia patients is mediated, at least in part, by the muscle Gulcose-6-Phosphatase-β complex (Shieh *et al.*, 2004).

Shieh *et al*. (2004) hypothesized that muscle glucose-6-phosphatase could catalyze the release of substantial amounts of glucose into the blood. This will have changed the long held dogma that only the liver and kidney can release glucose into the blood but this report had been challenged. Brunengraber *et al*. (2007) investigated glucose production by the gut, kidney, and leg of acutely hepatectomized dogs and of sham-operated dogs. They reasoned that allowing the deep hypoglycemia that sets in after hepatectomy would maximize the possibility of detecting glucose production by the gut and/or by muscle. Anesthetized dogs were fitted with carotid, jugular, portal vein, renal vein, and femoral vein catheters. They injected a small bolus of [6,6-$^{2}H_{2}$]glucose just after completing the hepatectomy (Daloze *et al.*, 1990) and at the end of the sham surgery in control dogs. At various times, blood was slowly sampled through the carotid, portal vein, renal vein, and femoral vein catheters. In hepatectomized dogs, glucose concentration decreased from 5.0 to 1.0 mM over 240 min, but remained stable at about 5 mM in shamoperated dogs. The average rates of glucose production for the three control and six hepatectomized dogs were 0.000 ± 0.006 and 0.007 ± 0.068 (SD) μmol · min^{-1} · kg^{-1},

respectively. Thus, even in hepatectomized dogs, where the deep hypoglycemia should have stimulated any gluconeogenic capacity of the intestine, there was no evidence of intestinal gluconeogenesis. Also, no glucose production was detected across the leg of hepatectomized dogs. This argues against the hypothesis that free glucose could be released from muscle glycogen via glucose-6-phosphate hydrolysis (Shieh *et al.*, 2004) or via glycogen debranching enzyme. Finally, glucose production by the kidneys accounted for the remaining glucose production after hepatectomy, i.e., about one-half of glucose production in the sham-operated dogs. However, the stimulation of renal gluconeogenesis by post hepatectomy hypoglycemia did not prevent the decrease of blood glucose concentration to 1 mM. This confirms the central role of the liver in the maintenance of blood glucose concentration (Previs *et al.*, 2009).

Moreover, when the body's store of carbohydrate decreases below normal, moderate quantities of glucose can be formed from amino acids, the glycerol portion of fat and lactate. About 60% of amino acids in the body protein can be converted to glucose. The remaining 40% have chemical configurations that make gluconeogenesis difficult or impossible (Guyton and Hall, 2006). Since neither glycerol nor lactate availability is adequate to meet the needs for gluconeogenesis, amino acids derived from proteolysis in the skeletal muscle, is the most important substrate. The presence of glucocorticoid and absence of insulin are the primary signals for enhanced protein degradation. This provides for the carbon chains of amino acids of skeletal muscle proteins to be delivered to the liver as gluconeogenic precursors. One complexity of glucocorticoid actions is that whereas it stimulates hepatic glycogen synthesis, it stimulates skeletal muscle glycogenolysis. The latter allows for more amino acids to be shipped to the liver (Basu, 2004).

Exercise and hypoxia are the major factors affecting glucose uptake by the skeletal muscle. Studies show that during a brief period (e.g. about 20min) of mild-to-moderate exercise, glucose uptake increases but blood glucose concentration tends to remain constant because endogenous glucose production matches the increased uptake (Havel *et al.,* 1967). During this period, insulin release is inhibited, and glucagon and catecholamine releases are increased, accounting for the increased hepatic glucose production (Zierler, 1999).

A prominent role has been identified for AMPK in the regulation of muscle metabolism during exercise. As its name suggests, AMPK is allosterically activated by 5'-AMP (Carling *et al.,* 1987), an effect antagonized by high concentrations of ATP (Corton *et al.,* 1995). Because of the reaction catalyzed by adenylate kinase (i.e the conversion of 2ADP to ATP + AMP), the AMP:ATP ratio varies approximately as the square of the ADP:ATP ratio (Hardie *et al.,* 2001), making the former ratio (essentially the parameter to which AMPK responds) a sensitive indicator of reduced cellular energy status. Consequently, any cellular or metabolic stress that either inhibits ATP synthesis [e.g., heat shock (Corton *et al.,* 1994), hypoxia (Marsin *et al.,* 2000), ischemia (Kudo et, 1995), or glucose deprivation (Salt *et al.,* 1998)] or that accelerates ATP consumption[e.g., contraction of skeletal muscle (Hutber *et al.,* 1997; Vavvas *et al.,* 1997; Winder, 1996)] causes AMPK activation. It had been known for many years that muscle glycogen phosphorylase and phosphofructokinase (the key enzymes regulating glycogen breakdown and glycolysis, respectively) can also be activated allosterically by a rise in the AMP:ATP ratio (Cori *et al.,* 1938; Passonneau and Lowry, 1962).The idea that this ratio was a key indicator of cellular energy status was therefore not new. However, the concept that a single protein kinase like AMPK could sense the same parameters and transmit the information to

potentially hundreds of downstream targets was an important new development (Hardie and Sakamoto, 2006).

The first indication that AMPK could be involved in regulation of muscle metabolism during exercise came from a study reported in 1996 (Winder and Hardie, 1996). Rats were run on a treadmill at 21 m/min up a 15% grade for periods up to 30 min. The deep red region of the quadriceps muscle was quickly removed and frozen for AMPK isolation and analyses. AMPK activity was found to increase two- to threefold within 5 min of the beginning of exercise and remained elevated for as long as the rat continued to run. In rats running on the treadmill, the increase in AMPK activity was found to be dependent on work rate (Ren *et al.,* 1994), and the activity remained elevated for several minutes following 5- and 30-min exercise bouts (Rasmussen *et al.,* 1996). When rat gastrocnemius muscle was stimulated in situ at a frequency of once per second, 10-ms duration, the estimated free AMP concentration increased, creatinine phosphate (CP) decreased, and AMPK activity increased (Hutber *et al.,* 1997). Interestingly, the decline in ACC activity (phosphorylation target for AMPK, a reporter for AMPK activity) correlated more closely with the decline in CP than with the rise in free AMP or with the increase in measurable AMPK activity. This illustrates the importance of the allosteric mechanisms. AMPK activity also increased in incubated epitrochlearis muscles in response to contraction and AICAR treatment (Hayashi *et al.,* 1998)

Vavvas *et al.* (1997) published the first information on the activation of specific isoforms of AMPK in response to muscle contraction. The gastrocnemius muscle was stimulated via the sciatic nerve (5 pulses/s, 100-ms trains, 50 Hz, 10-ms duration) to contract in situ for periods up to 5 min. The α_1- and α_2-isoforms were precipitated from muscle homogenates using specific antisera. The α_2-isoform was maximally activated (3- to 4-fold) within 30 s of the

beginning of stimulation, but no significant increase occurred in the α1-isoform (Vavvas *et al.*, 1997). In isolated epitrochlearis muscle, both the α_1- and α_2-isoforms were reported to be activated in response to a number of stimuli that decreased CP and glycogen, including contraction, hypoxia, dinitrophenol, rotenone, and sorbitol (hyperosmotic stress) (Fryer *et al.*, 2000; Hayashi *et al.*, 2000). Only the α_2-isoform was activated in epitrochlearis of rats running on the treadmill (Musi *et al.*, 2001).

Studies also indicate that the glycogen content of muscle may modulate the AMPK response to contraction (Derave *et al.*, 2000; Kawanaka *et al.*, 2000). Derave *et al.* (2000) subjected rats to 2 h of swimming, followed by feeding with a 100% fat diet overnight or with normal chow + 20% glucose in drinking water. The two protocols produced rats with low and high muscle glycogen, respectively. Hindlimbs of these rats were perfused with cell-free medium. After a 5-min washout period, one sciatic nerve was stimulated for 10 min with 100-ms trains (100 Hz) at 2-s intervals. In fast-twitch white fibers from the gastrocnemius, AMPK activity measured in postnuclear supernatants of stimulated muscles was increased 1.8-fold over resting muscles in the high-glycogen group compared with 4.6-fold over resting muscles in the low-glycogen group. In slow twitch soleus muscle, a contraction-stimulated activation of AMPK occurred in the muscles with low glycogen but not in those with high glycogen. The activity of ACC declined during contraction, however, indicating that the AMPK may have been activated allosterically. This would not have been detected in the final AMPK assay procedure. Studies using the incubated epitrochlearis muscle (fast twitch) have also demonstrated an attenuation of the AMPK response in muscles with high glycogen (Kawanaka *et al.*, 2000). It is unclear whether there is a direct effect of glycogen on AMPK activity or whether the attenuation is due to blunting of changes in ATP, AMP, and CP. ATP and CP responses to stimulation did not

appear to be significantly altered in the soleus regardless of glycogen content, although the time course of changes were not studied (Derave *et al.*, 2000).

Studies have clearly demonstrated AMPK to be activated in human muscle during exercise. Wojtaszewski *et al*. (2000) exercised (cycle ergometer) human subjects at 50% of maximal oxygen consumption for 90 min or at 55 min at 75% followed by 5 min at 90% of maximal oxygen consumption. They reported no change in AMPK activity in needle biopsies from the vastus lateralis in response to the lower intensity work bout but a three- to fourfold increase in activity of the α_2-isoform in response to the higher intensity work bout. The activity of the α_1-isoform was not changed in response to either work rate. The activity of the α_2- isoform had returned to baseline by 3 h postexercise. Fugii *et al*. (2000) earlier the same year reported no change in either isoform of AMPK in the vastus lateralis of human subjects in response to 20 min of work at 50% of maximal oxygen consumption, whereas activity of the α_2-isoform increased after 20 and 60 min of work at 70% of maximal oxygen consumption on the cycle ergometer. Muscle CP and glycogen were significantly decreased in response to subjects working at 70% but not at 50% of maximal oxygen consumption. A report also indicates that the activity of both α_1- and α_2-isoforms may increase with very high-intensity work. Chen *et al*. (2000) had subjects do a 30-s sprint (peak power 5 834 W) on a cycle ergometer and found the activity of both isoforms to be increased.

During the first hindlimb perfusion studies on the role of AMPK in regulation of fatty acid oxidation in muscle, it was noted that AICAR not only activated AMPK and increased palmitate oxidation but also it stimulated an increase in glucose uptake. It was hypothesized at the time that AMPK activated by muscle contraction stimulated both an increase in fatty acid oxidation and an increase in glucose uptake to meet the energy demands of working muscle

(Merrill *et al.*, 1997). The insulin-like effect of muscle contraction has been extensively studied (Goodyear, 1998; Goodyear, 2000; Hayashi *et al.*, 1997). The insulin-sensitive glucose transporter, GLUT-4, resides in two locations in the muscle (Goodyear, 1998; Goodyear, 2000; Hayashi *et al.*, 1997; Holloszy and hansen, 1996). In the basal state (low insulin), the GLUT-4 transporters reside in microvesicles beneath the sarcolemma and T-tubule membranes. In response to insulin or contraction, these transporters are translocated and inserted into membranes of the T tubules and sarcolemma, thus increasing the capacity for moving glucose into the muscle fiber. The signaling pathways for the two systems differ. The insulin pathway involves occupied receptor-catalyzed phosphorylation of insulin receptor substrate 1 followed by activation of phosphatidylinositol 3-kinase, whereas the contraction pathway does not involve either of these signaling proteins (Goodyear, 1998; Goodyear, 2000; Yeh *et al.*, 1995). The maximal effect of contraction on translocation of GLUT-4 is additive with the effects of high insulin. Although careful and extensive efforts have been expended by many laboratories in attempts to elucidate initial steps in the contraction-mediated GLUT-4 translocation, the signaling steps have been elusive. The possibility that AMPK activation may be involved has therefore generated considerable interest.

Using the incubated epitrochlearis, it was determined that AICAR would increase 3-methylglucose (3-MG) transport with characteristics similar to the contraction effect (Hayashi *et al.*, 1998). Wortmannin, an inhibitor of phosphatidylinositol 3-kinase, inhibited insulin-stimulation of 3-MG transport but not that caused by contraction or AICAR. Stimulatory effects of AICAR were additive with those of insulin but not with those of contraction. In perfused hindlimbs, AICAR was found to stimulate glucose uptake in the absence of insulin and to cause GLUT-4 translocation to the sarcolemmal fraction from the microvesicle fraction (Kurth-

Kraczek *et al.,* 1999). Infusion of AICAR into live rats resulted in increased labeled hexose uptake into all three muscle fiber types (Bergeron *et al.,* 1999). Other studies have demonstrated that several factors that decrease CP and glycogen in incubated epitrochlearis (contraction, hypoxia, dinitrophenol, rotenone, and sorbitol) also activate AMPK and stimulate glucose transport (Fryer *et al.,* 2000; Hayashi *et al.,* 2000). These data provide evidence that AMPK activation mediates the effect of contraction on stimulation of glucose transport.

Moreover, studies (Fryer *et al.,* 2000; Hayashi *et al.,* 2000) have demonstrated that both the contraction-induced increase in AMPK activity and the contraction stimulation of glucose uptake are decreased in muscles composed of type II fibers when glycogen content of the muscle is elevated. Kawanaka *et al*. (Kawanaka *et al.,* 2000) hypothesized that the smaller degree of AMPK activation was responsible for the decreased contraction stimulated translocation of GLUT-4 in glycogen supercompensated epitrochlearis muscles. Derave *et al*. (2000) reported a complete absence of AMPK activation in perfused supercompensated soleus muscles (composed of type I fibers) in response to electrical stimulation, but glucose transport was not diminished. On the basis of this apparent dissociation between the two processes in the soleus, the authors concluded that AMPK activation is not an obligatory signaling step in contraction activation of glucose transport. There was some evidence of allosteric activation of AMPK in the soleus, however, because ACC activity at 0.2 mM citrate was lower after stimulation. Nevertheless, it is entirely possible that redundant pathways are present for the insulin-like effect of contraction and that the AMPK-independent pathwaypredominates in type I fibers.

Part of the difficulty in identifying AMPK-dependent processes in the muscle has been due to the lack of specific inhibitors of AMPK. In isolated epitrochlearis, iodotubercidin, Ara-A, and 8-bromo-AMP inhibited effects of AICAR on 3-MG uptake and AMPK a2 activation but

had no or little effect on contraction-stimulated AMPK activity and 3-MG uptake (Musi *et al.*, 2001). It will be important to identify more specific inhibitors of AMPK to clearly link control of cellular processes to the AMPK signaling pathway.

Another approach to linking AMPK activation with contraction-stimulated glucose uptake is that of production of transgenic mice lacking functional forms of AMPK. This approach is complicated by the occurrence of multiple isoforms of the three subunits of AMPK.Mu and Birnbaum (Mu and Birnbaum, 2001) found that muscle from mice expressing a kinase-inactive α2-catalytic subunit of AMPK resulted in a depression of endogenous AMPK activity in both the resting and contracting muscle. Stimulation of hexose transport was completely blocked in AICAR-treated and hypoxic muscles but was only partially blocked in electrically stimulated contracting muscles. The authors concluded that a redundant AMPK-independent pathway must in part mediate the effect of contraction on glucose uptake in muscle. It is unclear whether residual endogenous AMPK could have been activated allosterically to mediate contraction-stimulated glucose transport in these transgenic mice. No mention was made of measurement of ACC activity as a reporter of possible allosteric activation.

Not all effects of AMPK on glucose transport may be due to effects on translocation of GLUT-4. Abbud *et al*. (2000) studied effects of AICAR on glucose transport in C2C12 myoblasts, which rely on GLUT-1 to transport glucose. In these cells, a two- to threefold increase in glucose transport was observed in response to incubation with AICAR. Thus enhancement of the activity of other isoforms of glucose transporters may also help explain the effect of AICAR on glucose transport in muscle.

Some attention has been given to identifying the specific target protein(s) for mediating the effect of AMPK on GLUT-4 translocation and glucose transport.

Previous studies have demonstrated a possible role of nitric oxide (NO) in contraction stimulation of glucose transport (Balon, 1996; Balon, 1998). Fryer *et al*. (2000) reported nitric oxide synthase (NOS) activity to be increased along with glucose transport in H-2K myotubes in response to activation of AMPK with AICAR. NOS inhibitors blocked the increase in glucose transport after AMPK activation. Both the neuronal NOS and endothelial NOS isoforms can be phosphorylated by AMPK in vitro. NO activates guanylate cyclase, which then catalyzes synthesis of cGMP. Treatment of these cells with an inhibitor of guanylate cyclase prevents the increase in glucose transport brought by AICAR. In soleus and EDL muscle strips, the stimulatory effect of AICAR on glucose transport was also blocked by the NOS inhibitor, *N*G-nitro-L-arginine methyl ester. These results provide evidence that an obligatory target of AMPK in inducing an increase in glucose transport is NOS. It has also been suggested that skeletal muscle nNOS is the major isoform involved in AMPK activation of GLUT4 expression in skeletal muscle (Lira *et al.*, 2007). Specifically, the experiments of Lira *et al.* (2007) demonstrated that GLUT4 expression in L6 myotubes, both at mRNA and protein levels, is positively regulated by NO and cGMP. In addition, the results show that NOS activity is necessary for AICAR-induced activation of AMPK and expression of GLUT4. These findings are consistent with a role for NO in the regulation of AMPK kinase activity, or perhaps inhibition of AMPK phosphatase activity (Lira *et al.*, 2007).

Two distinct pathways regulate glucose utilization in skeletal muscle: insulin and contractile activity. Insulin or insulin-like factors increase eNOS activity in endothelialcells and iNOS expression in rat and human myoblasts (Jaffrey *et al.*, 1998) through a phosphatidylinositol (PI) 3-kinase-dependent pathway. Contractile activity of skeletal muscle also increases NOS activity and the expression of eNOS and nNOS (Balon and Nadler, 1997;

Sessa *et al.*, 1994). Classic pharmacological studies in intact skeletal muscles (Balon and Nadler, 1994; Balon and Nadler, 1997) and Baron (Baron, 1994; Baron, 1996) in both rats and human subjects have established that NOS activity increases glucose transport. Specifically, infusion of *N*G-monomethyl-L-arginine (LNMMA) systemically into rats or humans or its addition to isolated muscle fiber bundles (rat EDL) after contractile activity, decreases total 2-deoxyglucose transport. Current evidence indicates, however, that insulin-mediated NO effects are mainly microvascular, not myocyte, in origin; that is, insulin-mediated GLUT-4 transport of glucose in isolated skeletal muscle is not dependent on NO.

Insulin increases muscle blood flow in rats and humans (Baron, 1996; Baron *et al.*, 1996; Roy *et al.*, 1998; Scherrer *et al.*, 1994; Steinberg *et al.*, 1994). This effect is probably mediated through the PI 3-kinase/AKT kinase pathway, an established mechanism of eNOS activation by receptor tyrosine kinases (Dimmeller *et al.*, 1999; Fulton *et al.*, 1999). The increase in muscle perfusion (Baron *et al.*, 1996) is thought to increase the delivery of insulin's major substrate, glucose, to the muscle cell. Indeed, Baron and co-workers (Baron *et al.*, 1995; 1996) suggest that 30% of insulin's effect on glucose uptake can be accounted for by increases in muscle perfusion. NOS inhibition by L-NAME in the standard hyperinsulinemic-euglycemic clamp model reduces whole body insulin action and glucose disposal by 15–30% in the conscious rat (Rodriguez-Crespo *et al.*, 1998), in keeping with previous studies in isolated limbs of human subjects (Steinberg *et al.*, 1994). Although Butler *et al*. (Butler *et al.*, 1998) reported that L-NMMA infusion increases rather than decreases whole body glucose transport in 16 healthy male subjects, the NOS inhibitor also increased rather than decreased calf blood flow and hardly affected blood pressure; that is, L-NMMA inexplicably produced diametrically opposite hemodynamic responses from those seen in numerous previous studies, including those in which

it caused insulin resistance (Baron *et al.*, 1995). Fryburg (1996) and Scherrer *et al*. Schmidt *et al* (1996) have also challenged the strength of the relationship between insulin-mediated increases in blood flow and glucose transport, but good reasons have been forwarded to negate their results (Roy *et al.*, 1998). Thus there is good evidence that insulin-mediated vasodilation is partly NO dependent and that such increases in perfusion contribute to increased skeletal muscle glucose transport (i.e., independent of insulin-mediated increases in the GLUT-4 transporter). EDRF thus amplifies insulin's action to stimulate glucose uptake (Stamler and Meissner, 2001).

More recently, Salahdeen and Alada (2009) reported the role of adrenergic receptors in caffeine- induced increase in glucose uptake by the canine hindlimb at rest and during contraction. There was a decrease in hindlimb glucose extraction and hindlimb glucose uptake produced by caffeine following pretreatment of the dogs at rest with either prazosin or propranolol; suggesting the involvement of both alpha and beta adrenergic receptors in the effect of caffeine on hindlimb glucose uptake at rest. Failures of the two blockers to alter these variables during hindlimb contraction also shows the non-involvement of both alpha and beta adrenoceptors in the response to caffeine during hindlimb contraction. It is however instructive to note that the significant reduction in blood flow to the hindlimb in response to caffeine administration at rest was completely abolished during hindlimb contraction. In other words, caffeine had no significant effect on the blood flow to the hindlimb during contraction. Again, failure of the adrenergic receptor blockers to alter the effect of caffeine on the hindlimb blood flow during contraction shows that the adrenergic receptors are not involved in the effect of caffeine on blood flow during contraction of the hindlimb (Salahdeen and Alada, 2009).

2.4.4 Erythrocytes

The mature human erythrocyte meets its energy requirement primarily from the metabolism of carbohydrates, chiefly glucose, although other monosaccharides can be utilized (Bishop, 1964). Normally 6-8 µmoles of glucose per g of hemoglobin per hr are broken down to lactate via the Embden-Meyerhof pathway (Tsuboi, 1965). In addition, a small fraction of the glucose-6-phosphate formed is metabolized via the hexosephosphate pathway yielding reduced nicotinamide adenine dinucleotide phosphate (Murphy, 1960).

The erythrocytes represent one of the most important tissues after the brain in terms of glucose consumption (Jacuez, 1984). The red blood cells (RBC) could take up glucose and store it as glycogen. Thus, it has been demonstrated that the glucose-^{14}C is both incorporated into and released from glycogen, that redistribution of the glycosyl units within the glycogen molecule occurs, and that the outer branches are shortened after periods of incubation in the absence of added glucose (Shimon *et al.*, 1968). All of the enzymes concerned with glycogen synthesis and breakdown are also present in the normal erythrocytes (Shimon, 1968). It is therefore possible that these cells could be involved in blood glucose homeostasis. Information on a possible role of RBC in the postprandial state is scanty. The few available studies indicated that fetal and neonatal erythrocytes of all mammalian species are highly permeable to glucose. Glucose concentration, in red blood cell water are about the same as those in plasma water. The report of previous studies showed that in all mammals except for porpoise rhesus monkey and human, red blood cell glucose content decreases at about the same time as fetal hemoglobin decreases (Andreen-Svedberg, 1933). It was also observed that the ratio of red cell water glucose to plasma water glucose varies widely among mammals, from near zero (in swine) to near unity (in primates and porpoise) with many species at about 0.5 (Goodwin, 1956). Thus, whole blood

glucose concentration underestimates plasma water glucose concentration, which is the glucose concentration that is driving the force for movement into interstitial water (Zierler, 1999).

In adult humans, studies have indicated that glucose concentration in plasma water is nearly the same as in red blood cell water over a wide range of glucose concentration, until plasma glucose concentration becomes extremely high when erythrocyte glucose transporters are fully saturated (Mckay, 1932). Jaguez (1984) pointed out that glucose enters human red blood cells at a rate that exceeds its red blood cell utilization by a thousand-fold and thus erythrocytes glucose serves as buffer to damp the amplitude of variations in plasma water glucose concentration.

In rats, reports showed that red blood cells take up glucose when there is hyperglycemia and stored most of it as glycogen and during hypoglycemia under the influence of a β-adrenergic agonist, red blood cell glycogen is dissimilated to glucose and moves into plasma (Guarner and Alvarez-Buylla, 1989). Therefore, red blood cells can be described as circulating hepatocytes (Zierlar, 1999).

2.4.5 Brain

The central nervous system (CNS) has been identified as a key regulator of whole body homeostasis. In fact, from the respiratory system to the circulatory system, thermoregulation to energy expenditure, the CNS plays a fundamental role in our body's homeostatic controls. Within the entire CNS, the hypothalamus in particular is generally accepted to mediate the day-to-day regulation of a number of factors including body temperature (Gordon, 1986); blood pressure (Guyenet, 2006); thirst (Anderson, 1978) and hunger (Schwartz *et al.*, 2000), and is a fundamental structure for the integration of the nervous and endocrine systems. The record of

CNS control of peripheral glucose homeostasis began with the finding by Claude Bernard that punctures in the floor of the fourth ventricle resulted in glucosuria (Bernard, 1855). It was not until over a century after Bernard's initial observation that the boom in the field occurred. Over the past decade, it has been shown that the CNS senses 1) hormones (Coppari *et al.*, 2005; Gelling *et al.*, 2006; Inoue *et al.*, 2006; Klevit *et al.*, 2006; Knauf *et al.*, 2005; Obici *et al.*, 2002), namely insulin, leptin, and most recently identified, glucagon-like peptide (GLP)-1, and 2) nutrients (Lam, 2005; Obici *et al.*, 2002; Parton *et al.*, 2007), namely fatty acids and glucose, to regulate glucose homeostasis.

Glucose is an important regulatory signal that controls the secretion of hormones by various endocrine cells and activates neurons in the peripheral and central nervous systems. Because the brain derives its metabolic energy almost exclusively from glucose and thus requires that glycemic levels do not fall markedly below ~5 mM, critical glucose-sensing systems are located in the central nervous system and control glucosehomeostasis, feeding behavior, and energy storage and expenditure (Marty *et al.*, 2007).

Glucose triggers many neuronal and endocrine responses from the time it appears in the oral cavity to its rise in the systemic circulation. In the mouth, glucose stimulates nervous reflexes, in part initiated by activation of taste receptors and of their afferent fibers (Ahren, 2000), which project to the brain stem and are in relation to the nucleus of the tractus solitarius (NTS), the reticular formation, the parabrachial nucleus (PBN), and the dorsal motor nucleus of the vagus (DMNX) (Berthoud and Mogenson, 1977; Berthoud and Powly, 1990). Activation of this reflex is responsible for the cephalic phase of insulin secretion, which plays an essential role in glucose tolerance (Ahren, 2000).

In the intestine, glucose stimulates the secretion of the gluco-incretin hormones, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide-1 (GLP-1) by, respectively, the intestinal endocrine K- and L-cells (Deacon, 2005) and activates autonomic and enteric neurons (Mei, 1978; Thorens and Larson, 2004). Activation of neurons may be through glucose binding to the SGLT3 isoform of the Na+/glucose cotransporters, which are involved in signal transduction rather than glucose transport and are expressed in cholinergic neurons in the submucosal and myenteric plexuses (Deiz-Sampedro *et al.*, 2003); some enteric neurons express the ATP-sensitive K+ channel (KATP channel) subunits Kir 6.2 and SUR1 (Liu and Kirchgessner, 199), suggesting that they sense glucose by a mechanism similar to that of the pancreatic beta-cells . Appearance of glucose in the portal vein stimulates sensors, which activate vagal afferents that project to the NTS and lateral (LH) and paraventricular (PVN) hypothalamic nuclei (Adachi *et al.*, 1984; Shimizu *et al.*, 1983). Glucose sensing by the hepatoportal sensor leads to several adaptive responses, such as stimulation of glucose storage in liver and a subset of peripheral tissues, including the soleus, the heart, and brown adipose tissue; inhibition of counterregulation; termination of food intake; and stimulation of first phase insulin secretion (Preitner *et al.*, 2004; Thorens, 2004).

In hepatocytes, glucose uptake triggers several responses that increase the glucose storage capacity of the liver as well as the conversion of glucose into fat. First, glucose is an allosteric inhibitor of glycogen phosphorylase and glucose-6-phosphate, an activatorof glycogen synthase (Carabaza *et al.*, 1992). Thus increased glucose uptake and phosphorylation augment glycogen storage. Second, the glucose metabolite xylulose-5-phosphate, generated through the pentose-phosphate shunt, is an activator of the protein phosphatase A2 (PP2A), which dephosphorylates the cytoplasmically located transcription factor ChREBP (carbohydrate response element

binding protein) and allows its translocation into the nucleus to activate the expression of genes involved in glycolysis (L-pyruvatekinase) and fatty acid synthesis [AcylCoA synthase (ACC) and fatty acid synthase] (Dentin *et al.*, 2006; Kabashima *et al.*, 2003).Elevation in systemic glycemia stimulates insulin secretion by pancreatic beta-cells, which induces glucose utilization in liver and glucose uptake in fat and muscle and suppresses glucagon secretion. In con-trast, a fall in glycemia below ~5 mM stimulatesglucagon secretory activity by various mechanisms (Marty *et al.*, 2007).

In the central nervous system, glucose regulates the activity of glucose-sensitive neurons present in the brain stem and the hypothalamus. These neurons have a critical role in regulating glucose and energy homeostasis through secretion of endocrine pancreas hormones, regulation of liver glucose production, feeding behavior, and energy expenditure.

2.4.5.1 Sites and mechanisms of glucose sensing in the Brain

(a) Sites of gluco-detection: The earliest demonstration that the brain is involved in glycemic control was provided by Claude Bernard, who showed, in the dog, that lesioning the hypothalamus induced hyperglycemia. In 1953, Jean Mayer proposed that cells located in the hypothalamus could be specialized to monitor plasma glucose variations and postulated that these cells translate variations in glucose concentrations in electrical or chemical signals that control feeding behavior (Mayer, 1953). In the early 1960s, two groups identified, by electrophysiological analysis of hypothalamic slices, neurons able to modulate their firing activity in response to changes in extracellular glucose levels (Anand *et al.*, 1964; Oomura and Yoshimatsu, 1984). These are glucose-excited (GE; previously called glucoseresponsive) neurons, which increase their firing rate with elevation in extracellular

glucose concentrations, or glucose-inhibited (GI; previously called glucosesensitive) neurons, which are activated by a decrease in extracellular glucose concentration or by cellular glucoprivation (Routh, 2002; Yang and Low, 2004). Both types of neurons are widely distributed in the brain but highly represented in hypothalamic nuclei and the brain stem, regions involved in the control of energy homeostasis and food intake. At the hypothalamic level, GE neurons are found mostly in the ventromedial hypothalamus (VMH), the arcuate nucleus (AN), and the PVN (Dunn-Meynell *et al.*, 1998; ; Wang *et al.*, 2004), and GI neurons are mostly located in the LH, median AN, and PVN. Both types of neurons are also found in the brain stem, in particular in the NTS, the area postrema (AP), and the DMNX (Adachi *et al.*, 1984; Dallaporta *et al.*, 1999; Mizuno and Oomura, 1984; Yettefti *et al.*, 1997). Recently, the presence in the AN of GE and GI neurons responsive to glucose over either a low (0–5 mM) or a high glucose concentration range (5–20 mM) have been described; the latter are referred to as HGE (high GE) or HGI (high GI) neurons, respectively (Floramonti *et al.*, 2004; Penicaud *et al.*, 2006).

Evidence for the presence of glucose-regulated neurons has also been obtained by intravenous or intracerebroventricular injections of the glucose antimetabolites 2-deoxyglucose (2-DG) or 5-thio-glucose (5-TG). The glucoprivic signal generated by these compounds induces metabolic or behavioral responses, and the activated neurons can be identified by electrophysiological recordings or detection c-fos-like immunoreactivity. Responsive neurons have been found in the AN, VMH, PVN, LH, PBN, NTS, AP, DMNX, and the region of the basolateral medulla (BLM) containing the A1/C1 noradrenergic and adrenergic neurons (Briski and Sylvester, 2001; Dallaporta *et al.*, 1999; Ritter and Dinh, 1994).

(b) Mechanisms of gluco-detection.

There is considerable evidence that the mechanisms of glucose sensing by cells in the central nervous system may rely on different mechanisms, and one may be similar to that of the pancreatic beta-cells (Schuit *et al.,* 2001; Yang *et al.,* 1999). Glucose signaling in these cells requires glucose uptake by the low-affinity glucose transporter type 2 (GLUT2), glucose phosphorylation by glucokinase, and the consequent metabolism of glucose to increase the intracellular ATP-to-ADP ratio. This leads to the closure of KATP channels, membrane depolarization, and the entry of Ca2+, which triggers insulin secretion. In this pathway, the rate-controlling step is the phosphorylation of |glucose by glucokinase, and glucose uptake is a permissive step (Matschinsky, 1996). The KATP channel also plays a fundamental role since it links changes in glucose metabolism to plasma membrane electrical activity (Ashcroft and Gribble, 1999). Thus many studies have evaluated the role of GLUT2, glucokinase, and the KATP channel subunit SUR1, SUR2, and Kir6.2 in central glucose sensing. Because GE neurons increase their firing activity when extracellular glucose rises, they may share similarity to beta-cells. The presence of GLUT2 in hypothalamic nuclei where glucose-sensitive neurons are present has indeed been reported (Arluison *et al.,* 2004 Li *et al.,* 2003; Navarro *et al.,* 1996) as well as the presence of glucokinase (Dunn-Meynell *et al.,* 2002; Wang *et al.,* 2004; Yang *et al.,* 2004), which is a critical regulator of VMH glucose sensing (49). In GE neurons, the increase in extracellular glucose leads to an augmentation of the ATP-to-ADP ratio and the closure of the KATP channels (Ashford *et al.,* 1990; Miki *et al.,* 2001; Van *et al.,* 2007), which leads to plasma membrane depolarization and Ca2+ entry through voltage-gated channels, thereby increasing neuronal activity and neurotransmitter secretion (Amoroso *et al.,* 1990; Moriyama *et al.,* 2004).

There is, however, also indication that neuronal gluco-detection could be independent of KATP channels (Floramonti *et al.*, 2004), glucokinase (Song and Routh, 2005) or GLUT2 (Kang *et al.*, 2004). For instance, the activation by glucose of HGE neurons seems to depend on the glucose-regulated activity of a TRP (transient response potential) channel (Floramonti *et al.*, 2004).

In GI neurons, the mechanism linking a decrease in glucose concentrations to increased firing activity is less clear, but suppression of firing activity may becontrolled by the increase in the ATP-to-ADP ratio, which leads to an increase in Na-K-ATPase activity (Oomura *et al.*, 1974; Silver and Erecinska, 1998) and/or an opening of ATP-regulated chloride channels (Routh, 2002; Song and Routh, 2005), which hyperpolarize the plasma membrane. For GI orexin neurons, it has been shown that tandem-pore K+ channels could mediate their inhibition by glucose (Burdakov *et al.*, 2006).

2.4.5.2 Central glucose sensing and counterregulation.

A fall in blood glucose level below ~5 mM induces a rapid counterregulatory response to restore normoglycemia. This involves the activation of glucagonsecretion from pancreatic alpha cells and of catecholamines from adrenal glands (Cryer, 2004Mitrakou *et al.*, 1991; Taborsky *et al.*, 1998), as well as the activation by the autonomic nervous system of hepatic glucose production (Perseghin *et al.*, 1997). The sites of hypoglycemia detection are multiple and can be activated either by hypoglycemia or by peripheral or central glucoprivation. Classically, experimental counterregulation is activated by insulin-induced hypoglycemia or by injection of 2-DG or 5-thioglucose (5-TG). In both conditions, afferent neurons located in the abdominal regions relay the information to the brain stem and the hypothalamus, which can also be directly activated by hypoglycemia or the glucoprivic signal. Some of the glycemia-sensitive abdominal afferent neurons are located in the hepatoportal vein area as shown by the possibility to suppress

hypoglycemia- induced glucagon secretion by portal vein glucose infusion (Hevener *et al.*, 2000). The role of central glucose sensing in the control of counterregulation can be similarly evidenced by intracarotid glucose infusion, whichblocks hypoglycemia-induced secretion of counterregulatory hormones and endogenous glucose production (Biggers *et al.*, 1989; Frizzell *et al.*, 1993), or by intracerebroventricular injection of 2-DG, which stimulates glucagon and catecholamine secretion and endogenous glucose production toinduce hyperglycemia . The involvement of hypothalamic nuclei, in particularthe VMH, has been assessed in lesion studies and by pharmacological or genetic interference with glucose detection systems (Frizzell *et al.*, 1993). For instance, glucagonsecretion can be induced by direct injection of 2-DG in the VMH (Borg *et al.*, 1995), or, in contrast, hypoglycemia-induced glucagon secretion can be suppressed by direct VMHinjection of glucose (Borg *et al.*, 1997). Interestingly, the corticotrophin-releasing factors CRF and urocortin I, which are agonists of CRF receptor 1 and 2, respectively, either increase or suppress, respectively, hypoglycemia-induced counterregulation by directly modulating the firing rate of the glucose-sensitive VMH neurons (Cryer, 2006; McCrimmon *et al.*, 2006).

There is also strong evidence that brain stem nuclei play an important role in the control of glucagon secretion. For instance, when the cerebral aqueduct, which allows circulation of cerebrospinal fluid between the third and fourth ventricle, is obstructed, 5-TG induces a glucoregulatory response only when injected in the fourth but not in the third ventricle. In addition, whereas 5-TG injections in different nuclei of the hypothalamus fail to induce a glucoregulatory response, its injections into the NTS and the basolateral medullary regions containing the A1/C1 catecholaminergic neurons, which project to different sites of the hypothalamus, induce a strong response (Fraley and Ritter, 2003; Ritter *et al.*, 2000; 2001). Also,

in decerebrated rats, the hyperglycemic response to an intraperitoneal injection of 2-DG is preserved (Ritter *et al.*, 2000), and c-fos immunostaining revealed that the activated neurons are present in the NTS, the DMNX, and the catecholaminergic neurons of the basolateral medulla (Ritter *et al.*, 1998). Thus glucose-sensing units involved in the physiological control of counterregulation arepresent at multiple locations in the hepatoportal vein region, the brain stem, and hypothalamus. These sites are synaptically connected and form a network for monitoring blood glucose levels. This information is integrated at the central level to control the counterregulatory response by activating afferent autonomic nerves that stimulate glucagon secretion and the secretion of epinephrine by the adrenals, and also block insulin secretion.

2.4.6 Kidney

The human kidney is involved in the regulation of glucose homeostasis and in abnormalities found in diabetes mellitus via three different mechanisms: (i) release of glucose into the circulation via gluconeogenesis; (ii) uptake of glucose from the circulation to satisfy its energy needs; and (iii) reabsorption into the circulation of glucose from glomerular filtrate to conserve glucose carbon.

The study of Meyer *et al.* (2002) had shown that about 10% of an ingested glucose load is taken up by the kidney. Renal uptake of glucose has been previously shown to increase during hyperglycemia (Cerosimo *et al.*, 1997; Dzurik and Chorvathova, 1972) and to be stimulated by insulin (Cerosimo *et al.*, 1994; Meyer *et al.*, 1998). Increases in renal glucose fractional extraction and renal blood flow both contributed to the increased postprandial renal glucose uptake (Meyer *et al.*, 2002). These changes mayhave resulted, at least in part, from postprandial hyperinsulinemia, because insulin has been shown to increase renal blood flow and glucose

fractional extraction in normal postabsorptive humans (Cerosimo *et al.*, 1999; Meyer *et al.*, 1998). Had these changes in blood flow and fractional extraction not occurred, the observed renal glucose uptake would have been reduced by approximately 50%. Thus the mass action effects of hyperglycemia and changes in blood flow and fractional extraction contributed similarly to the postprandial renal glucose uptake.The magnitude of the postprandial renal glucose uptake may seem surprising because the kidney, in contrast to liver and muscle, does not normally store appreciable glycogen (Biava *et al.*, 1966). However, all of the glucose taken up by the kidney could be accounted for by its being released as lactate and its being oxidized, as a substitute for FFA. Lipid is normally the major oxidative fuel of the kidney (Wirthensohn and Guder, 1986). However, postprandial renal FFA net uptake was reduced by 65% (i.e., by 48 µmol/ min). In other words, there was substitution of glucose for FFA (Meyer *et al.*, 2002). These considerations provide further, albeit indirect, evidence in favor of a renal glucose-fatty acid cycle, as has been recently postulated (Meyer *et al.*, 1997) and analogous to the situation for skeletal muscle postulated by Taylor *et al*. (1993). These investigators observed no net glycogen accumulation in skeletal muscle for nearly 2 h after ingestion of a meal containing 290 g of carbohydrate. Because muscle glucose uptake would have increased substantially during this period, as demonstrated in previous studies (Butler *et al.*, 1997; Firth *et al.*, 1986; Jackson *et al.*, 1986; Mitrakou *et al.*, 1992; Meyer *et al.*, 2002), it was postulated that the glucose taken up was oxidized in place of FFA.

Not only did the kidney play a role in the disposal of the ingested glucose, it also influenced postprandial glucose homeostasis by increasing its release of glucose. Thus, in contrast to liver, whose postprandial release of glucose was markedly decreased, there was a more than twofold increase in renal glucose release, so that it on average actually exceeded that

of the liver during the 4.5-h postprandial period. This finding wassurprising, because hyperglycemia and hyperinsulinemia would have been expected to suppress renal glucose release (Cerosimo *et al.*, 1997; Meyer *et al.*, 1998). Several factors could explain the reciprocal changes in hepatic and renal glucose release. First of all, the liver is exposed to higher (i.e., portal) insulin concentrationsthan is the kidney. Second, suppression of hepatic glycogenolysis, which was postulated to have largely accounted for the suppression of hepatic glucose release, is known to be more sensitive to insulin than is hepatic gluconeogenesis (Chiasson *et al.*, 1976). Third, suppression of glucagon secretion may have played a role, because glucagon supports hepatic glucose release (Chiasson *et al.*, 1983) but has no effect on renal glucose release (Stumvoll *et al.*, 1998). Fourth, glucose ingestion increases sympathetic nervous system activity (Welle *et al.*, 1980), which may have preferentially augmented renal glucose release. In support of this view is the observation that infusion of epinephrine causes a sustained increase in renal glucose release while increasing hepatic glucose release transiently and to a lesser extent (Stumvoll *et al.*, 1995). From a teleological point of view, this increased postprandial release of glucose by the kidney would permit greater suppression of hepatic glycogenolysis so that there could be more efficient glycogen replenishment (Meyer *et al.*, 2002).

These differences in regulation and reciprocal change in renal and hepatic glucose release have led to the concept of hepatorenal glucose reciprocity (Meyer *et al.*, 1999). This concept refers to the situations in which a physiological or pathological decrease in glucose release by kidney or liver is associated with a compensatory increase in glucose release by liver or kidney so as to prevent hypoglycaemia or to optimize homeostasis. Examples of this include the anhepatic phase after liver transplantation, prolonged fasting, acidosis, meal ingestion and insulin overdoses in diabetes mellitus (Meyer *et al.*, 1999; Joseph *et al.*, 2000; Gerich, 2002).

The metabolic fate of glucose is different in different regions of the kidney. Because of its low oxygen tension, and low levels of oxidative enzymes, the renal medulla is an obligate user of glucose for its energy requirement and does so anaerobically. Consequently, lactate is the main metabolic end product of glucose taken up in the renal medulla, not carbon dioxide (CO_2) and water. In contrast, the renal cortex has little glucose phosphorylating capacity but a high level of oxidative enzymes. Consequently, this part of the kidney does not take up and use very much glucose, with oxidation of FFAs acting as the main source of energy. A major energy-requiring processin the kidney is the reabsorption of glucose from glomerular filtrate in the proximal convoluted tubule (Brenner, 2004).

In diabetics, in addition to increased glucose production, renal glucose uptake is increased in both the post-absorptive and postprandial states in patientswith type 2 diabetes mellitus (T2DM) (Triscari *et al.,* 1979; Meyer *et al.*, 2004). Meyer *et al*. (1998) showed that, in the post-absorptive state, renal glucose uptake is significantly greater in patients with T2DM than in normal individuals (353 ± 48 vs. 103 ± 10 µmol ⁄min), actually exceeding increased glucose production to result in a net glucose uptake of 92 µmol ⁄min. This contrasts with a net output of 21 µmol ⁄min in non-diabetic individuals (Meyer *et al.,* 1998). In the postprandial state, uptake of glucose by tissues is increased in patients with T2DM and its distribution and fate are altered (Meyer *et al*., 2004). Glucose uptake by the kidneys is raised by more than twofold [21.0 ± 3.5 vs. 9.8 ± 2.3 g in diabetic vs. non-diabetic individuals during a 4.5-h period following ingestion of 75 g of glucose (Meyer *et al.,* 2004), whereas glucose uptake in muscle is not significantly altered (Mayer *et al.,* 2004). Moreover, less glucose is oxidized (Meyer *et al.,* 2004).

Moreover, in the postabsorptive state (after 14- to 16-h overnight fast), glucose is released into the circulation at a rate of approximately 10 µmol⁄(kg min) (Gerich, 2000; Landau

et al., 1996; Stumvoll *et al.*, 1997). Approximately 50% of this is the result of the breakdown of glycogen (glycogenolysis) stored in the liver and the other half is because of the production of new glucose molecules from precursors such as lactate, glycerol, alanine and other amino acids (gluconeogenesis) by liver and kidneys (Gerich, 2000; Landau *et al.*, 1996; Stumvoll *et al.*, 1997). The kidney is unable to release glucose through glycogenolysis because it contains very little glycogen and those renal cells that are able to synthesize glycogen lack the enzyme glucose-6-phosphatase and therefore cannot release glucose (Stumvoll *et al.*, 1997). In humans, only the liver and kidney contain significant amounts of the enzyme glucose-6-phosphatase and therefore are the only organs that are able to perform gluconeogenesis. Research over the last 15–20 years has established that the human liver and kidneys provide about equal amounts of glucose via gluconeogenesis in the postabsorptive state. Consequently, after an overnight fast, 75–80% of glucose released into the circulation derives from the liver and the remaining 20–25% derives from the kidneys.As the duration of fasting increases, glycogen stores in the liver become further depleted until, after 48 h, virtually all the glucose released into the circulation is derived from gluconeogenesis (Consoli *et al.*, 1987; Landau *et al.*, 1996). Consequently, as the length of fast increases, the proportion of overall glucose release accounted for by renal gluconeogenesis increases (Davidson and Peters, 1997).

It is important to note that kidney and liver differ in their use of gluconeogenic precursors and the effect of hormones on their release of glucose. Lactate is the predominant gluconeogenic precursor in both organs, but otherwise the kidney preferentially uses glutamine (Meyer *et al.*, 2002), whereas the liver preferentially uses alanine (Stumvoll *et al.*, 1998).

The kidney can be considered as two separate organs because glucose utilization occurs predominantly in the renal medulla, whereas glucose release is confined to the renal cortex

(Schoolwerth *et al.,* 1988; Wirthensohn and Guder, 1896; Guder, 1984). This functional partition is a result of differences in the distribution of various enzymes along the nephron. For example, cells in the renal medulla have appreciable glucose-phosphorylating and glycolytic enzyme activity, and, like the brain, they are obligate users of glucose (Cahill, 1970). These cells, however, lack glucose-6-phosphatase and other gluconeogenic enzymes. Thus, although they can take up, phosphorylate, glycolyse, and accumulate glycogen, they cannot release free glucose into the circulation (Schoolwerth *et al.,* 1988; Wirthensohn and Guder, 1896; Guder, 1984). On the other hand, cells in the renal cortex possess gluconeogenic enzymes (including glucose-6-phosphatase), and thus they can make and release glucose into the circulation. But these cells have little phosphorylating capacity and, under normal conditions, they cannot synthesize appreciable concentrations of glycogen (Schoolwerth *et al*., 1988; Wirthensohn and Guder, 1896; Guder, 1984). Therefore, the release of glucose by the normal kidney is mainly, if not exclusively, a result of renal cortical gluconeogenesis, whereas glucose uptake and utilization occur in other parts of the kidney.

Consistent with numerous studies in diabetic animal models (Teng, 1954; Landau, 1960; Flinn *et al.,* 1961; Kamm and Cahill, 1969; Chang and Sneider, 1970), patients with type 11 diabetes mellitus (T2DM) have an increased release of glucose into the circulation by the kidney in the fasting state (Meyer *et al.,* 1998). Although the liver is commonly viewed as being largely responsible for increased release of glucose into the circulation in T2DM, the absolute increase in renal glucose release is comparable in magnitude (2.60 and 2.21 mmol /kg/ min) for liver and kidneys, respectively) (Meyer *et al.,* 1998). In fact, the relative increase in renal gluconeogenesis is substantially greater than the increase in hepatic gluconeogenesis (300 vs. 30%). Similar to the

liver, the increased glucose release by the kidney in the fasting state is solely, if not exclusively, a result of gluconeogenesis (Meyer *et al.*, 1998).

In addition to releasing glucose into the circulation by synthesizing new glucose molecules via gluconeogenesis and its utilization of glucose, the kidney can also influence glucose homeostasis by returning glucose to the circulation via the reabsorption of glucose from glomerular filtrate. Normally, approximately 180 litres of plasma are filtered by the kidneys each day. As the average plasma glucose concentration throughout a24-h period is approximately 5.5 mmol ⁄ l (100 mg⁄ dl), approximately 180 g of glucose is filtered by the kidneys each day. In healthy individuals, virtually all of this is reabsorbed into the circulation and the urine is essentially free from glucose (Silvermann and Tumer, 1992). To put this into perspective, in a given day, the kidneys produce 15–55 g glucose via gluconeogenesis and metabolize 25–35 g glucose. Therefore, in terms of glucose economy, it is clear that renal reabsorption is the primary mechanism by which the kidney influences glucose homeostasis. Alterations in renal tubular glucose reabsorption may therefore be expected to have a considerable impact on glucose homeostasis.

Reabsorption of glucose from glomerular filtrate occurs by means of sodium–glucose co-transporters (SGLTs) in the proximal convoluted tubule. There are six members of this family (Wright *et al.*, 2007). In animal models, approximately 90% of glucose is reabsorbed by SGLT2, a high-capacity low-affinity glucose transporter (Km, approximately10 mmol⁄ l; Vmax , approximately 10 nmol ⁄ (min mg) protein (Brown, 2000). SGLT2 is thought to be located exclusively on the luminal surface of the epithelial cells lining the S1 and S2 segments of the proximal tubule (Lee and Han, 2007; Chen *et al.*, 2008). Transport of sodium and glucose by SGLT2 occurs in a 1:1 ratio (Brown, 2000; Wright, 2001). The remaining 10% of glucose

reabsorption is mediated by SGLT1, a high-affinity, low-capacity glucose/galactose transporter (Km 0.2 mmol/l; Vmax, approximately 10 nmol/(min mg) protein; sodium: glucose coupling ratio = 2:1) located on the luminal surface of epithelial cells lining the S3 segment of the proximal tubule (Brown, 2000; Wright, 2001). SGLT1 is also extensively expressed in the small intestine and in other tissues (Wright, 2001). Glucose reabsorbed from the proximal tubules by SGLTs is then released into the circulation through the action of facilitative glucose transporters (GLUTs) at the basolateral membrane of the epithelial cells lining the proximal tubules (GLUT2 in the S1/2 segments and GLUT1 in the S3 segment) (Hediger and Rhoads, 1994). SGLT-mediated glucose transport is an active process, moving glucose against a concentration gradient, utilizing energy derived from the sodium electrochemical potential gradient across the brush border membrane and maintained by the transport of intracellular sodium into the blood via sodium:potassium adenosine triphosphatase (ATPase) pumps at the basolateral membrane (Wright *et al.*, 2007). In contrast, GLUTs facilitate passive transport (equilibration) of glucose across membranes and do not require an energy source (Wright *et al.*, 2007).

Glucose is freely filtered in the glomerulus, so that, as plasma glucose levels increase, the amount of glucose in the glomerular filtrate increases linearly. Reabsorption of filtered glucose also increases linearly until the maximal reabsorptive capacity is exceeded (Silvermann and Tumer, 1992). This is often referred to as the renal threshold and equates to a filtration rate of 260–350 mg/ min per 1.73 ^{m2} (Zelikovic, 2004), which occurs at plasma glucose concentrations of 11.0 mmol/l in healthy adults (Moe *et al.*, 2008). Above this plasma glucose concentration, the percentage of filtered glucose that is reabsorbed decreases and the percentage of the filtered load of glucose that is excreted in the urine increases, resulting in glucosuria (Silvermann and Tumer, 1992).

The renal threshold for glucose is decreased in individuals with a rare condition known as familial renal glucosuria (FRG), caused by a range of mutations to the SLC5A2 gene, which encodes SGLT2. Depending on the nature of the mutations, these individuals have varying degrees of glucosuria, but in the most severe form (so-called 'Type 0' disease) they can lose > 100 g glucose per day to the urine (Santer *et al.*, 2003). Interestingly, the large majority of patients exhibit no symptoms and their condition is only identified incidentally. Typically, they do not become hypoglycaemic or dehydrated and have no electrolyte imbalance or increased risk of urinary tract infections (Santer *et al.*, 2003). Even the most severe form of the condition appears to carry a favourable prognosis (Scholl-Burgi *et al.*, 2004) (although it should be noted that only small numbers of patients have been described in the literature). In contrast, patientswith SGLT1 gene mutations have low levels of glucosuria but suffer from glucose–galactose malabsorption in the gut, which can be associated with life-threatening severe diarrhoea and dehydration unless a glucose- and galactose-free diet is carefully followed (Turk *et al.*, 1991).

It is well recognized that glucosuria in diabetic patients does not occur at plasma glucose levels that would normally produce glucosuria in non-diabetic individuals (Mogensen, 1971). This is the result of increased glucose reabsorbtion from glomerular filtrate in people with diabetes mellitus. The transport maximum (Tm) for glucose is increased and glucosuria only begins to occur at higher than normal plasma glucose levels. In one study, the Tm increased from approximately 350 mg ⁄ min in normal individuals to approximately 420 mg⁄min in those with diabetes mellitus (Mogensen, 1971). Studies of renal cells isolated from the urine of people with diabetes as well as cells from several animal models have demonstrated enhanced expression of SGLT2 transporters (Rahmoune *et al.*, 2005; Vestri *et al.*, 2001). Hyperglycaemia, albumin and

angiotensin II have all been reported to up-regulate expression of SGLT2 in T2DM (Rahmoune *et al.*, 2005). The role of altered renal glucose reabsorption in the pathogenesis of diabetic nephropathy is unclear (Gerich, 2010).

2.4.7 Gastro-Intestinal Tract

In glucose homeostasis, the role of the gastro-intestinal tract (GIT) with regards to digestion and absorption of carbohydrates is well known. The GIT is capable of taking up large quantities of glucose (Durotoye and Grayson, 1971; Grayson and Oyebola, 1983; Grayson and Oyebola, 1985, Oyebola and Durosaiye, 1988). However, the glucose uptake was found to be far in excess of what could be accounted for by oxidative metabolism (Durotoye and Grayson, 1971; Grayson and Oyebola, 1983).

Studies in normal dogs and have shown that the intestine takes up substantial amount of glucose at rest and whenever the blood glucose level was increased (Oyebola, 1982; Grayson and Oyebola, 1985; Alada and Oyebola, 1996; Alada and Oyebola, 1997; Alada *et al*., 2001). Thus, when hyperglycemia was induced by adrenaline (Grayson and Oyebola, 1983), glucagon (Alada and Oyebola, 1996), nicotine (Grayson and Oyebola, 1985), glucose infusion (Alada and Oyebola, 1996), cow's urine concoction (Oyebola, 1982) or induction of diabetes (Alada *et al.*, 2005), the GIT increased its glucose uptake by as much as 400-700% so as to bring down the blood glucose. On the other hand, when hypoglycemia was induced by insulin injection (Alada and Oyebola, 1996), the gastrointestinal tract pushes out glucose into the circulation. Also, since there was increased uptake in all cases of hyperglycemia, it was concluded that the canine gut increased its glucose uptake whenever there was hyperglycemia irrespective of the cause (Alada and Oyebola, 1996).

The mechanism of the increased glucose uptake in response to hyperglycemia induced by adrenaline, glucagon or glucose and the negative glucose uptake in response to insulin-induced hypoglycemia had also been studied. It has been reported that prazosin significantly reduced the increase in glucose uptake caused by adrenaline injection whereas it had no effect on the increased glucose uptake induced by glucagon or glucose infusion and the negative glucose uptake induced by insulin (Alada and Oyebola, 1997).

Moreover, it was observed that a combination of prazosin and propranolol completely abolished the effects of adrenaline on blood glucose and intestinal glucose uptake (Alade and Oyebola, 1997). This is consistent with earlier reports in man (Antonis *et al.,* 1967) and Cat (Al-Jibonri *et al.,* 1980). The mechanism of adrenaline-induced hyperglycemia and increased glucose uptake by the gut thus seems to involve alpha and beta adrenoceptors.

Furthermore, propranolol alone also significantly reduced adrenaline-induced hyperglycemia and adrenaline-induced increase in glucose uptake by the intestine. Propranolol also caused a significant decrease in glucagon-induced increase in glucose uptake by the intestine. The alpha and beta receptors are therefore almost equipotent in their effects on adrenaline-induced hyperglycemia and glucose uptake by the intestine (Alada and Oyebola, 1997). The reduction by propranolol of the increase in glucose extraction and intestinal glucose uptake produced by glucagon suggests the involvement of beta adrenoceptors in the effects of glucagon on glucose uptake by the bowel. Failure of alpha blockers to do so shows the non-involvement of the alpha adrenoceptors in the response to glucagon. In addition, prazosin had no effect on glucose-induced hyperglycemia and increased glucose uptake while propranolol significantly reduced same. These findings suggest that the increase in the intestinal glucose uptake in response to high blood glucose levels, irrespective of its cause is mediated partly by

beta receptors. Some other receptors are most probably involved since β-blockade alone did not abolish increased uptake. Alpha-receptors, unlike in the adrenaline response, are not involved in the increased uptake induced by glucagon and glucose infusion. Also, while prazosin had no effect on insulin-induced hypoglycemia and negative glucose uptake in the intestine, propanolol reduced significantly but did not abolish the insulin-induced negative glucose uptake. These findings suggest that the beta adrenoceptors mediated in part the increased negative glucose uptake induced by insulin while again, the alpha receptors are not involved (Alada and Oyebola, 1997).

Whether such reciprocity exists or not can only be proven by simultaneous measurement of hepatic and gut glucose uptakes in the post absorptive state. This possibility deserves further investigation. However, the enormous quantity of glucose taken up by the gut in diabetic hyperglycemia suggested that the post-absorptive gut in the diabetic state may well be involved in reducing the degree of diabetic hyperglycemia by mopping up a lot of glucose from the circulation. The huge glucose uptake observed in the diabetic state was mainly due to increased glucose extraction without an increase in blood flow. This contrasts with the effects of adrenaline, glucagon and glucose infusion (Alada and Oyebola, 1996) where significant increases in gut blood flow contributed substantially to the increased glucose uptake.

Furthermore, the response of the diabetic gut to insulin with respect to glucose uptake had also been studied. Insulin caused a reduction in glucose uptake in a dose-dependent manner in diabetic dogs. The decrease in glucose uptake was mainly due to reduction in glucose extraction because insulin had no effect on blood flow at all the doses studied. Insulin also caused negative glucose uptake in normal dogs at low doses (2.5 iu/kg and 5.0 iu/kg). The reduced glucose uptake and negative glucose uptake produced by insulin at these low doses in

normal dogs suggest that these effects are more probably not due to the blood-glucose lowering effect of insulin. Other mechanism(s) not related to the glycemic are probably involved and this deserves further study (Alada *et al.*, 2005).

It is noteworthy however, that higher dose of insulin (8.0 iu/kg) in normal dogs caused just a transient negative glucose uptake and that for most of the post-injection period which lasted 90 mins, there was in fact significant increase in glucose uptake. This was in spite of the fact that this dose caused significant reduction in adrenal and venous glucose levels. This finding shows that it is not the degree of hyperglycemia alone (and therefore the amount of glucose transport to the gut tissue in a given time) that determines that glucose uptake in the gut. Other factors that are yet to be identified are involved. However, it is reasonable to suggest that the reduced blood glucose level produced by insulin triggered the secretion of counter-regulatory hormones, especially adrenaline, glucagon and growth hormone. The secretion of the counter-regulatory hormones in response to lowering of blood glucose is well-documented (Griffin and Ojeda, 1988, Defronzo *et al.*, 1986, Wasserman and Vranic, 1986). This may well be a similar phenomenon to the post-hypoglycemic hyperglycemia or the Somogyi effect which has long been known to occur in humans given insulin therapy (Bolli *et al.*, 1984).

However, inspite of these numerous studies, there are still many questions yet unanswered: (1) Is the response (i.e huge increases in intestinal glucose uptake) specific for only hyperglycemic agents and glucose? (2) How will the postabsorptive intestine respond when it is challenged with other sugars such as fructose and galactose? (3) Will haemodynamics have any role in the responses to other sugars like it did in the case of glucose? (4) What will be the role of adrenoceptors in the responses to other sugars? (5) Does nitric oxide have any role in the responses of the postabsorptive intestine to these sugars?

All these questions constitute a clear need for further studies on the effects of sugars on glucose uptake by the intestine in the postabsorptive state. Moreover, the observation that there was no correlation between the percentage changes in blood glucose and intestinal glucose uptake suggests that other factors other than glucose may be contributing to the huge increases in intestinal glucose uptake observed when glucose and hyperglycemic agents were administered. Thus, studying the handling of glucose by the postabsorptive intestine after administration of other sugars (e.g fructose and galactose) such that the blood levels of these sugars are increased instead of that of glucose will provide an insight into other factors that may be contributing to the huge increases in glucose uptake by the postabsorptive gut.

Traditionally, the only two organs believed to be capable of gluconeogenesis have been the liver and the kidney, with the latter being of importance only at times of metabolic acidosis and during prolonged starvation (when hepatic gluconeogenesis has decreased considerably). The possibility that organs other than liver and kidney could contribute to whole-body glucose production arose from the identification of some gluconeogenic enzymes in tissues not known to be gluconeogenic. This led to a number of reports, most of which concentrated on the capacity of the small intestine to produce glucose. The possibility that the intestine could contribute to glucose production, even under some conditions, is intriguing because it would affect our long-held view that glucose production is restricted to liver and kidney (Watford, 2005). Also, it has been hypothesized (Troy *et al.*, 2008) that intestinal gluconeogenesis may be involved in the rapid improvement of type II diabetes in obese humans who underwent bariatric surgery (Nugent *et al.*, 2008; Porie, 2008). Thus, intestinal gluconeogenesis, if it were unequivocally proven to occur, may be relevant to public health. The presence of intestinal gluconeogenesis would have profound consequences on how we view glucose homeostasis, but the evidence is far from

uniequivocal and there are alternative explanations for the presence of gluconeogenic enzymes in the small intestine (Previs *et al.*, 2009).

Studies on intestinal gluconeogenesis that have been conducted so far fall into three categories, i.e., enzymatic activities, mRNA levels, and flux rates. The interpretations of these studies have been debated without a clear consensus. In 2005, Watford reviewed the field and expressed concerns about the validity of some reports, but left the door open to further evaluation of the concept of intestinal gluconeogenesis (Watford, 2005). Although the identification of activities of gluconeogenic enzymes and the measurement of mRNA levels are suggestive of the existence of gluconeogenesis, the proof of the concept requires the unequivocal measurement of a gluconeogenic flux in the intestine.

The major substrates for gluconeogenesis are lactate and pyruvate, glycerol, and most of the common amino acids (lysine and leucine being exceptions).For a cell to carry out gluconeogenesis, it must express some or all of four key enzymes to bypass the irreversible steps of glycolysis. These enzymes include:

(1) Glucose 6-phosphatase (G6Pase) to bypass hexokinase (HK),

(2) Fructose 1,6-bisphosphatase (F1,6Pase) to bypass phosphofructokinase 1 (PFK 1)

(3) Pyruvate carboxylase (PC) together with phosphoenolpyruvate carboxykinase (PEPCK) to bypass pyruvate kinase (PK).

There is no doubt that some of these enzymes are present in non-hepatic/non-renal tissues, but since one or more is usually lacking, such tissues have not been considered to be capable of gluconeogenesis. Examples include the high levels of F-1,6-Pase in some skeletal muscles, which are believed to play a role, through substrate cycling, in the regulation of glycolysis (Challis *et al.*, 1981), and the presence of PEPCK in adipose tissue, where it functions

in glyceroneogenesis (Hanson and Reshef, 2003). The mucosa of the small intestine appears to be an exception in that it clearly expresses all the enzymes at relatively high levels during the neonatal period in the rat, rabbit, and mouse (Hahn and Smale, 1982; 1983; Westbury and Hahn, 1984; Hahn and Wel-Ning, 1986; Hahn *et al.*, 1991; Watford and Tatro, 1989; Asins *et al.*, 1996). This has led to proposals that this organ could be a site of gluconeogenesis in the newborn, but since expression of key gluconeogenic enzymes decreases to very low levels after weaning, intestinal gluconeogenesis has not been considered to be of physiological significance in the adult.

Recently, however, Mithieux *et al*. (2001; 2004) have argued that there is sufficient expression of all gluconeogenic enzymes in the rat during starvation or streptozotocin diabetes for this organ to be a significant site of gluconeogenic enzymes in the rat during starvation or streptozotocin diabetes for this organ to be a significant site of gluconeogenesis, and using tracer methodology they claim that intestinal glucose release may represent between 19% and 35% of total body glucose production under such conditions (Croset *et al.*, 2001; Mithieux, 2001; Mithieux *et al.*, 2004a). Azzout-Marniche *et al*. (2007) have argued that the main glucose-6-phosphatase isoform expressed in the small intestine (G6PC3) is present in many non-gluconeogenic organs and is different from the G6PC1 form expressed in gluconeogenic organs.

The production of glucose by additional non-gluconeogenic organs has been hypothesized by Shieh *et al*. (Shieh *et al.*, 2003). They reported on a glucose-6-phosphate hydrolase (glucose-6-Pase-β) that can couple with the glucose-6-P transporter to hydrolyze glucose-6-phosphate to glucose. They later showed that astrocytes, which are the main brain cells that accumulate glycogen, express glucose-6-Pase-β and the glucose-6-P transporter (Ghosh *et al.*, 2005). These activities can couple to form an active glucose-6-Pase complex. Shieh *et al*.

(2003) hypothesized that in hypoglycemia, astrocytes may provide glucose directly to neurons and possibly to plasma. Although this is an attractive hypothesis, they acknowledged that stimulation of glycogenolysis in astrocytes in vitro leads to the release of lactate, not glucose (Dringen *et al.*, 1993; Pellerin, 2003; Wiesinger *et al.*, 1997). Also, Shieh *et al*. (2004) hypothesized that muscle glucose-6-phosphatase could catalyze the release of substantial amounts of glucose into the blood.

The data from Mithieux's group were met with much skepticism in the field. As was pointed out by Watford in a recent review (Watford, 2005), computations by Mithieux *et al*. (1996) of intestinal glucose release were based on very small differences in glucosespecific activity in mesenteric vein plasma versus arterial plasma. For example, in diabetic rats, the average specific activities of venous versus arterial glucose differed by 1.4%, while the coefficients of variation of the arterial- and venous-specific activities were 21.9% and 11%, respectively [13,646 ± 946 versus 13,464 ± 470 dpm/μmol (SE, n = 10)]. It is not clear how the authors concluded that the two average specific activities were significantly different at the $P < 0.01$ level.

Martin *et al*. (2007) tested the capacity of the rat small intestine to synthesize glucose from glutamine, the main fuel of intestinal metabolism (Windmueller and Spaeth, 1980). They incubated segments of small intestine from 72-hr-fasted rats (Wistar and Sprague-Dawley) with l-[3-^{13}C]glutamine, and followed the ^{13}C-labeling of metabolites by NMR. The 72-hr fasting corresponds to the maximal induction of gluconeogenic enzymes in the rat small intestine (Mithieux *et al.*, 2004). The viability of the intestinal segments for 30 min was verified by the constancy of ATP concentration, the linearity of [3- ^{3}C] glutamine uptake, and the linearity of the accumulation of metabolites (glutamate, alanine, lactate, pyruvate, aspartate). The ^{13}C-

labeling of metabolites indicated cycling of the label of [3-^{13}C] glutamine in the citric acid cycle (evidenced by the labeling of multiple carbons of glutamine and glutamate) and its transfer to gluconeogenic intermediates and amino acids (lactate, alanine, ornithine, citrulline, proline, aspartate). The authors reported finding a very small amount of glucose in the incubation medium, equivalent to 0.4% of the [3-^{13}C]glutamine uptake, but unlabeled. Because a small amount of unlabeled urea was also found, it is likely that the traces of unlabeled glucose and urea were present in the extracellular fluid of the intestinal segments taken from the rats. Martin *et al*. (2007) concluded that the small intestine of the 72-hr-fasted rat does not synthesize glucose from glutamine, its main substrate. In subsequent studies from the same group, Baverel *et al*. (2007) reported incubations of intestinal cells from adult fed and 48-hr-fasted diabetic ZDF rats with [3-^{13}C] glutamine. The data were similar to those of their previous study on intestinal segments, i.e., labeling of a number of metabolites, but no formation of glucose. In a similar study, Burrin *et al*. (2005) incubated epithelial cells of pig intestine with lactate or glutamine. They could not detect the formation of glucose using a sensitive enzymatic assay.

Burrin *et al*. (2005) also infused [$^{13}C_6$] glucose to 28-day-old piglets fasted for 36 hr. After 4 hr, they measured the concentration and molar percent enrichment (MPE) of glucose in the carotid artery and portal vein. Although, the uptake of glucose by the gastrointestinal tract was significantly different from zero, the mean difference in MPE of glucose (measured by gas chromatography–mass spectrometry) across the gut was not significantly different from zero. They concluded that there is “no significantgluconeogenesis by the gastrointestinal tissues in the fasted piglet.”

Brunengraber *et al*. (2007) also investigated glucose production by the gut, kidney, and leg of acutely hepatectomized dogs and of sham-operated dogs. They reasoned that allowing the

deep hypoglycemia that sets in after hepatectomy would maximize the possibility of detecting glucose production by the gut and/or by muscle. Anesthetized dogs were fitted with carotid, jugular, portal vein, renal vein, and femoral vein catheters. They injected a small bolus of [6,6-$^{2}H_{2}$]glucose just after completing the hepatectomy (Daloze *et al.*, 1990) and at the end of the sham surgery in control dogs. At various times, blood was slowly sampled through the carotid, portal vein, renal vein, and femoral vein catheters. In hepatectomized dogs, glucose concentration decreased from 5.0 to 1.0 mM over 240 min, but remained stable at about 5 mM in shamoperated dogs. The average rates of glucose production for the three control and six hepatectomized dogs were 0.000 ± 0.006 and 0.007 ± 0.068 (SD) μmol · min^{-1} · kg^{-1}, respectively. Thus, even in hepatectomized dogs, where the deep hypoglycemia should have stimulated any gluconeogenic capacity of the intestine, there was no evidence of intestinal gluconeogenesis. Also, no glucose production was detected across the leg of hepatectomized dogs. This study argues against the hypothesis that free glucose could be released from muscle glycogen via glucose-6-phosphate hydrolysis (Shieh *et al.*, 2004) or via glycogen debranching enzyme. Finally, glucose production by the kidneys accounted for the remaining glucose production after hepatectomy, i.e., about one-half of glucose production in the sham-operated dogs. However, the stimulation of renal gluconeogenesis by post hepatectomy hypoglycemia did not prevent the decrease of blood glucose concentration to 1 mM. This confirms the central role of the liver in the maintenance of blood glucose concentration (Previs *et al.*, 2009).

Based on these substantive counter arguments against the findings of Mithieux *et al.* (2004; 2008; 2006a; 2006b), it is therefore concluded that, at the present time, there is no credible evidence that the small intestine is a source of glucose (Prervis *et al.*, 2009).

2.5 Effects of different sugars on glucose homeostasis

2.5.1 Glucose

Previous studies in animals (Burcelin *et al.,* 2000; Gardemann *et al.,* 1986; Hevener *et al.,* 1997; Ishida *et al.,* 1983) and humans (Defronzo *et al.,* 1978; Smith *et al.,* 2002) have provided compelling evidence for the existence of a portal glucose "sensor" that regulates both hepatic and peripheral glucose uptake. Enteric absorption of glucose causes concentrations to be higher in the portal vein than in the systemic circulation (Adkins-Marshall *et al.,* 1990; Ishida *et al.,* 1983). The resultant portal-to-hepatic venous glucose gradient has been reported to enhance hepatic glucose uptake and inhibit extrahepatic glucose disposal (Adkins-Marshall *et al.,* 1990; Adkins *et al.,* 1987; Galassetti *et al.,* 1998; Ishida *et al.,* 1983; Pagliassotti *et al.,* 1991). Both hyperglycemia and hyperinsulinemia are required for the portal glucose "signal" to be detected (Adkins-Marshall *et al.,* 1990; Galassetti *et al.,* 1998; Pagliassotti *et al.,* 1991). ACh activates and vagotomy or hepatic denervation abolishes the portal glucose signal, implying a neural origin (Adkins-Marshall *et al.,* 1992; Stumpel *et al.,* 1998). In addition, infusion of glucose into the portal vein dampens the counterregulatory response to systemic hypoglycemia (Hevener *et al.,* 2000). Taken together, these data suggest that activation of the portal glucose sensor minimizes changes in portal and systemic glucose concentration.

The mechanism by which the portal vein senses glucose is an area of active investigation. Burcelin *et al.* (2001; 2000a; 2000b), in an elegant series of experiments, have strongly implicated GLUT2 transporters in this process. They have shown that infusion of glucose in the portal vein of mice at a rate that approximated basal endogenous glucose production (EGP) markedly increased peripheral glucose clearance (Burclin *et al.,* 2000b). This effect did not occur

in mice in whom the GLUT2 transporter was knocked out and could be abolished by concurrent infusion of either somatostatin or the glucagon-like peptide (GLP)-1 antagonist exendin (Burcelin *et al.,* 2001). In addition to providing interesting mechanistic insights, these studies were both surprising and provocative since intraportal glucose infusion caused a paradoxical fall in the plasma glucose concentration to hypoglycemic levels (Burcelin *et al.,* 2001).

The effects of glucose infusion on intestinal glucose uptake had also been reported in dogs. Glucose infusion caused an immediate rise in both arterial and venous glucose levels reaching peak values at the end of the 90 minutes infusion period. The increase in blood glucose was followed by corresponding increases in both glucose extraction and intestinal glucose uptake. The increase in intestinal glucose uptake was partially mediated by beta adrenergic receptors as propranolol significantly reduced the glucose-induced increase in intestinal glucose uptake (Alada and Oyebola, 1996; 1997).

It has also been demonstrated previously that peripheral and splanchnic glucose uptake were the same in nondiabetic humans when glucose was infused intraduodenally or intravenously at a rate of 4 $mg.kg^{-1}$ min^{-1} (Vella *et al.,* 2002). However, in those experiments, glucose was clamped at approximately 150 mg/dl and insulin at approximately 400 pmol/l to stimulate hepatic and peripheral glucose uptake. Somatostatin had to be given to inhibit endogenous insulin secretion so as to ensure comparable portal insulin concentrations on both occasions. Somatostatin also has been given for the same reason in the animal experiments that have shown that intraportal glucose infusion decreases rather than increases extrahepatic glucose uptake (Adkins-Marshall *et al.,* 1990; Cardin *et al.,* 1999; Galassetti *et al.,* 1998; Pagliassotti *et al.,* 1991). Therefore, it is possible that the use of somatostatin in these experiments either dampened or ablated the response of the putative portal glucose sensor. Alternatively, the

response to intraportal glucose delivery in dogs and humans may differ from that in mice because in healthy nondiabetic humans, intraduodenal glucose infusion at rates bracketing normal EGP does not cause hypoglycemia. Rather, glucose and insulin concentrations increase slightly. The higher glucose and insulin concentrations appropriately suppress EGP and stimulate glucose disappearance, thereby minimizing the rise in glucose concentration (Farhad *et al.*, 2003).

During exercise, blood glucose is an important fuel for contracting muscle. Studies in several species have demonstrated that glucose uptake [rate of dissapearance (Rd)] by muscle increases during physical exercise (Coggan, 1991). Therefore, an increase in hepatic glucose production (HGP) is required to meet this demand for blood glucose and to avoid hypoglycemia. Indeed, studies in both humans (Jenkins *et al.*, 1985) and dogs (Berger *et al.*, 1994) during moderate-intensity exercise have demonstrated that the dynamics of HGP are similar to those of the increase in glucose Rd, such that plasma glucose concentrations are maintained within a narrow range. Furthermore, glucose infusion in an amount corresponding to the exercise-induced increase in HGP has been demonstrated to largely abolish endogenous glucose production [rate of appearance (Ra)] in these species during moderate exercise (Jenkins *et al.*, 1985; Berger *et al.*, 1994). These findings suggest that metabolic feedback mechanisms associated with an imbalance between glucose supply and demand stimulate the exercise-induced increase in HGP. During more strenuous exercise [>70% of maximum oxygen uptake (Vo_2max)], however, HGP greatly exceeds glucose Rd, and plasma glucose concentrations increase, suggesting that other mechanisms contribute to the mobilization of liver glucose in these circumstances. In support of this hypothesis, some (Manzon *et al.*, 1998; Wiersma *et al.*, 1993), but not all (Howlett *et al.*, 1998), studies have demonstrated that infusion of glucose only partially attenuates HGP during

heavy exercise. During exercise at these higher workloads, neural feed-forward mechanisms, rather than metabolic feedback, may control the increase in HGP.

In horses, unlike humans and dogs, plasma glucose concentrations increase (2–4 mM) even during moderate-intensity exercise (35–50% Vo_2max) (Farris *et al.*, 1985; Rose and Hodgson, 1994). This finding indicates a mismatch between glucose Ra and Rd in the horse during moderate exercise and suggests that neural feed-forward mechanisms may, in part, control liver glucose mobilization. However, because there are no reports of whole body glucose turnover in the horse during sustained exertion, no information exists concerning glucoregulatory mechanisms in this species. We hypothesized that, if feed-forward mechanisms control the glucose Ra response to exercise independent of glucose feedback, an intravenous glucose infusion would not suppress HGP under such circumstances.

In humans, it is well established that provision of exogenous glucose (intravenous infusion or glucose ingestion) influences metabolism during exercise (Ahlborg n Felig, 1977; Coggan and Coyle, 1991). Specifically, an increase in blood glucose availability augments glucose Rd by muscle and, during prolonged mild-to-moderate-intensity exercise (i.e., 30–70% Vo_2max), attenuates the decrease in carbohydrate oxidation (CHOox) and enhances endurance performance. However, most (Coyle *et al.*, 1986; 1991; Hargreaves and Briggs, 1988), but not all (Tsintzas *et al.*, 1995; 1996), studies in humans have reported that net muscle glycogen utilization is not altered by carbohydrate ingestion. In horses during moderate-intensity exercise, intravenous glucose infusion (approximately 2 g/min) prolonged running performance by 14% relative to the control treatment (Farris *et al.*, 1995).

Thus, it was hypothesized that, if feed-forward mechanisms control the glucose rate of appearance (Ra) response to exercise independent of glucose feedback in dogs, an intravenous

glucose infusion would not suppress HGP under such circumstances. Raymond *et al*. (2000) carried out a study to confirm this hypothesis. They observed that an infusion of glucose at a rate that approximated the average endogenous Ra measured in Con trials only partially suppressed hepatic glucose output during exercise. They observed that the increase in glucose availability as a result of glucose infusion (3 g/min) resulted in two-fold increases in whole body glucose rate of disappearance (Rd).

Since exogenous glucose only partially suppressed HGP, the results of the present study differ from those of previous studies in humans and animals. In humans and dogs during moderate-intensity exercise, an intravenous glucose infusion that matches the endogenous Ra response completely inhibits the exercise-associated increment in HGP (Berger *et al.,* 1994; Howlett *et al.,* 1998; Jenkins *et al.,* 1985). Although the reasons for this species variation cannot be determined from the data, it is possible that greater sympathoadrenal activation in the horse during low-intensity exercise plays a role in the feed-forward regulation of hepatic glucose output. After 100 min of moderate-intensity (2-fold increase in heart rate) exercise in dogs, plasma norepinephrine and Epinephrine concentrations were approxiamtely two-fold higher than at rest (Berger *et al.,* 1994). Similarly, in trained human athletes exercising at approximately 50% of Vo_2max, plasma norepinephrine and Epinephrine were only two- to- threefold higher compared with preexercise norepinephrine and Epinephrine values (Deuster *et al.,* 1989). A more striking increase in plasma catecholamines was observed in the horse and this likely reflects a greater degree of sympathoadrenal activation compared with responses measured in dogs and humans at similar relative exercise intensities. Increases in circulating catecholamines, and possibly direct sympathetic neural activation, can enhance hepatic glucose mobilization during exercise, although species differences exist (Wasserman, 1995).

Given the substantial endogenous Ra response in the group of horses given glucose, total glucose supply (the sum of exogenous glucose and HGP) was approximately two-fold higher compared with that in the Control. Furthermore, by a similar magnitude, whole body glucose Rd was higher in glucose group than Control throughout exercise. A number of factors regulate glucose uptake by muscle, including glucose delivery to muscle, membrane glucose transport, and intracellular metabolism. Glucose delivery is a function of muscle blood flow and prevailing glucose concentrations. In exercising dogs, tracer-determined whole body and leg Rd are augmented after an increase in blood glucose availability (Zinker *et al.*, 1993). Similarly, carbohydrate ingestion or intravenous glucose infusion that elevates plasma glucose concentrations has been demonstrated to increase leg (Ahlborg and Felig, 1976) and whole body (Coyle *et al.*, 1991; Howlett *et al.*, 1998; McConell *et al.*, 1994) glucose disposal in humans during moderate-intensity exercise. Therefore, part of the increment in glucose uptake in the glucose group can be attributed to an increase in blood glucose concentration. However, the ratio of the rate of disappearance of glucose to the blood glucose concentration (Rd/glucose concentration) was significantly higher in glucose group than in the control between 30 and 90 min of exercise, suggesting that other factors may have contributed to the increase in glucose Rd. Studies in humans (Wasserman *et al.*, 1991) and dogs (Zinker *et al.*, 1993) have demonstrated that muscle contractions and insulin have synergistic effects on glucose Rd by muscle during exercise. In addition, a suppression of circulating non-esterified fatty acid (NEFA) availability observed in the horses had earlier been shown to increase whole body and limb glucose Rd significantly in dogs during exercise (Bracy *et al.*, 1995).

2.5.2 Fructose

In humans, approximately one-half of the fructose given by prolonged intravenous infusion is taken up by the splanchnic tissues, with the liver accounting for >75% of this removal (Mayes, 1993; Van den Berghe, 1996). Hepatic fractional extraction of fructose (first pass extraction) was 50% over a wide range of sinusoidal fructose concentrations (60–300 μmol/l). On the other hand, overall net hepatic fructose uptake could account for 77, 70, and 77% of the fructose infused, indicating that almost three-fourths of the infused hexose was eventually cleared by the liver. These results confirm that the liver is the major site of fructose clearance from blood and indicate that this is so even when blood fructose levels are <500 μmol/l.

Since the presence of GLUT2 in the liver allows a rapid equilibration of the intracellular glucose level with the extracellular glucose level (Niewoehner and Nuttall, 1988), net hepatic glucose flux represents a balance between glucokinase flux and glucose-6-phosphatase flux. Wals and Katz (1994) showed that low concentrations of fructose (50–200 μmol/l) increased both glucose phosphorylation and recycling between glucose and glucose-6-phosphatase proportionally in isolated rat hepatocytes, indicating that fructose did not inhibit glucose-6-phosphatase flux . Niewoehner *et al*. (1987) reported that the administration of a wide range of fructose loads (0.28–2.8 mmol/kg) to intact animals did not affect glucose-6-phosphatase activity, despite a marked increase in the intracellular content of fructose-1-phosphate in the liver. In hepatocyte studies, on the other hand, glucokinase was activated by only 50–200 nmol/l of intracellular fructose-1-phosphate, which was associated with 100–200 μmol/l of extracellular fructose (Van Schaftingen *et al.*, 1994; Agius and Peak, 1993).

Furthermore, small amounts of fructose have been reported to activate glucokinase in catalytic manner. Van Shaftingen *et al*. (1994) demonstrated that glucokinase activity is acutely

regulated by its interaction with a regulatory protein. The regulatory protein binds to glucokinase and allosterically inhibits it by decreasing the apparent affinity of the enzyme for glucose. The regulatory protein with fructose 6-phosphate bound is in a conformation capable of interacting with, and inhibiting, glucokinase. Fructose-1-phosphate competes with fructose 6-phosphate for binding to the regulatory protein. The regulatory protein with fructose-1-phosphate bound is in a conformation that is not capable of interacting with glucokinase, thus glucokinase is not inhibited. It has been shown that intraportal infusion of small amount of fructose at 1.7, 3.3, or 6.7 µmol.kg^{-1}.min^{-1}, which raised the portal blood fructose concentration from <6 (basal) to 113, 209, and 426 µmol/l, respectively, increased NHGU from 15 to 41, 54, and 69 µmol.kg^{-1}.min^{-1}, respectively, during a hyperglycemic/hyperinsulinemic clamp in 42-h fasted dogs (Shiota *et al.,* 1998). The glucose produced from fructose that entered the liver was stored as glycogen (69%), released as lactate (17%), or oxidized (8%). Almost all (90%) of the stored glycogen was deposited via the direct pathway. The inclusion of small amounts of fructose with the intraduodenal glucose load augmented NHGU, increased hepatic glycogen synthesis via the direct pathway, and augmented hepatic glycolysis. As a result, postprandial hyperglycemia and insulin release were reduced (Shiota *et al.,* 2002). It has also been shown that fructose administration stimulates hepatic glycogen synthesis in the presence of hyperinsulinemia in healthy subjects (Peterson *et al.,* 2001). These studies demonstrated that small amounts of fructose markedly stimulate hepatic glucose uptake with a resulting increase in its storage as glycogen and of its catabolism by glycolysis. Because insulin stimulates glycogen synthesis by activating glycogen synthase (Bollen *et al.,* 19986, Ferrer *et al.,* 2003) and glycolysis by increasing fructose 2,6-phosphate levels (Assimacopoulos-Jeannet and Jeanrenaud, 1990; Hue and Rider, 1987), it is possible that the effect of insulin to stimulate glucose 6-phosphate disposal

exerts a permissive effect on the stimulation of glucokinase flux by fructose. It has been reported, on the other hand, that in individuals with type 2 diabetes the ability of hyperglycemia per se to suppress hepatic glucose production was nearly normalized by the addition of a catalytic amount of fructose (Hawkins *et al.*, 2002) and that fructose decreases the glucose and insulin responses to an oral glucose tolerance test (Moore *et al.*, 2001). It is likely that small amounts of fructose could stimulate not only hepatic glucose phosphorylation but also glycogen synthesis and glycolysis in the liver independently from the action of insulin.

To evaluate whether a catalytic amount of fructose can lessen postprandial hyperglycemia, even in the absence of an increase in plasma insulin, Masakazu *et al*. (2005) examined the effects of the inclusion of small amounts of fructose with an intraportal glucose load on the resulting increments in plasma glucose under euinsulinemic conditions in conscious dogs. They observed that even in the absence of an increase in plasma insulin, small amounts of fructose can significantly lessen the hyperglycemia resulting from intraportal glucose infusion by increasing the ability of the liver to take up glucose and store it as glycogen.

In a previous study in 42-h-fasted conscious dogs (Masakazu *et al.*, 1998), an increase in NHGU resulting from an intraportal infusion of a small amount of fructose was accompanied by increases in hepatic glucose 6-phosphate content, glycogen synthesis by the direct pathway, and glycolytic flux in the presence of the rise in plasma insulin levels, indicating that small amounts of fructose stimulate glucokinase flux in this condition. Also, in the study of Masakazu *et al*. (2005), in which plasma insulin levels were maintained at basal, fructose-stimulated increase in NHGU was also accompanied by increases in hepatic glucose 6-phosphate content, glycogen synthesis by the direct pathway, and glycolytic flux, suggesting that small amounts of fructose are able to stimulate glucokinase flux even in the absence of a rise in plasma insulin. It has been

demonstrated that fructose activates glucokinase activity in the liver via increasing intracellular concentration of fructose 1-phosphate (Agius and Peak, 1993; Ferrer *et al.,* 2003; Van Schaftingen *et al.,* 1994). The conversion (phosphorylation) of fructose to fructose 1-phosphate is catalyzed by fructokinase, an enzyme not regulated by insulin (Mayes, 1993). Indeed, the fractional extraction (50%) of fructose by the liver in the absence of the rise in plasma insulin observed by Masakazu *et al*. (2005) is very similar with that (46%) in a previous study (Eggleston and Woods, 1970) in which small amounts of fructose were infused intraportally in the presence of hyperglycemia and hyperinsulinemia. Van Shaftingen *et al*. (1994) and Agius and Peak (1993) showed that the addition of very low concentrations of fructose rapidly increases fructose-1-phosphate content in cultured hepatocytes and induces the release of glucokinase from its regulatory protein even in the absence of insulin. Furthermore, it has been shown that in the absence of insulin, fructose at low extracellular concentrations (50–200 _mol/l) stimulated glucose phosphorylation as measured by the formation of $^{3}H_2O$ from [2-$^{3}H_3$]glucose and the glycolytic flux as measured by the release of $^{3}H_2O$ from [3-^{3}H]glucose (Fillat *et al.*,1993; Van Schaftingen *et al.,* 1994). Therefore, the production of fructose-1-phosphate from fructose and activation of GK by fructose 1-phosphate do not depend on an increase in plasma insulin levels (Masakazu *et al.*, 2005).

The stimulation of net hepatic glucose uptake (NHGU) by small amounts of fructose was accompanied by increased glycogen content in the liver even in the absence of the rise in plasma insulin. Net glycogen deposition depends on the activities of glycogen synthase and phosphorylase. The activities of these enzymes are regulated not only via phosphorylation/dephosphorylation but also allosterically by some metabolic intermediates (Bollen *et al.,* 1998; Ercan-Fang *et al.,* 2002; Ferrer *et al.,* 2003). The effects of fructose

administration on phosphorylase activity are controversial. Bollen *et al*. (1986) observed an activation of phosphorylase and an inhibition of phosphorylase phosphatase by fructose 1-phosphate in liver extracts. Kaufmann and Froesch (1973) showed an inhibition of phosphorylase by fructose 1-phosphate. Ercan-Fang *et al*. (2002) reported that phosphorylase was directly inhibited by high concentration of fructose 1-phosphate but not by physiological levels of the sugar. There is no evidence that glycogen synthase activity is regulated directly by changing fructose 1-phosphate content (concentration). On the other hand, the inclusion of small amounts of fructose increased the intracellular content of glucose 6-phosphate, which has been reported to be a potent inhibitor of phosphorylase activity (Aiston *et al.*, 2003) and activator of glycogen synthase (Villar-Palasi and Guinovart, 1997). There is in vitro evidence that glucose 6-phosphate can stimulate dephosphorylation of phosphorylase-a by stimulating phosphorylase phosphatase (Cadefau *et al.*, 1997) and inhibit phosphorylation of phosphorylase-b by inhibiting phosphorylase kinase (Tu and Graves, 1973) by a substrate-mediated mechanism. Aiston *et al*. (2003) changed glucose 6-phosphate content in cultured hepatocytes by using three different approaches, involving incubation with substrates, overexpression of glucokinase, and inhibition of glucokinase with 5-thioglucose, and demonstrated that phosphorylase-a activity was decreased by increasing glucose 6-phosphate content.

An activation of hepatic glycogen synthase has been repeatedly observed with fructose administration in in vivo and in vitro studies (Van den Berghe, 1986). Niewoehner *et al*. (Niewoehner *et al.*, 1987) suggested that activation of glycogen synthase by the administration of relatively small fructose loads to intact animals is secondary to increased glucose 6-phosphate, a potent activator for glycogen synthase (Villar-Palasi and Guinovart, 1997). Glucose 6-phosphate inhibits phosporylase-a which inhibits glycogen synthase phosphatase allosterically (Agius and

Peak, 1993) as mentioned before. It is possible, therefore, that fructose activates glycogen synthase via an increase in the intracellular content of glucose 6-phosphate, which in turn results from the activation of glucokinase and an increase hepatic glucose uptake. Therefore, a small amount of fructose was able to stimulate glycogen synthesis via activation of glycogen synthase and inhibition of glycogen phosphorylase by increasing glucose 6-phosphate resulting from the activation of glucokinase even in the absence of the rise in plasma insulin.

Net hepatic lactate production induced by the intraportal glucose load was increased by intraportal infusion of small amount of fructose even when plasma insulin levels remained basal. Although, glucose per se is known to stimulate glycolysis via increasing intracellular concentration of fructose-2,6-bisphosphate by dephosphorylating fructose-6-phosphate,2-kinase:fructose-2,6-bisphosphatase (Nishimura *et al.*, 1994). Plasma glucose levels during the intraportal glucose load were lower in the presence of fructose infusion, indicating that increased glycolytic flux in the presence of fructose might not be mediated by the rise in plasma glucose. The increased intracellular content of glucose 6-phosphate in all likelihood increased glycolytic flux by its mass action. In addition, it is known that pyruvate kinase and phosphofructokinase are key regulatory sites in glycolysis. Low-fructose loads when given to intact animals (Ogawa *et al.*, 1993) or isolated hepatocytes (Fillat *et al.*, 1993) increased the intracellular content of fructose 2,6-bisphosphate, a potent activator of phosphofructokinase (Hue and Rider, 1987). This effect might be secondary to increased glucose 6-phosphate content because fructose-induced activation of glycolysis was not observed in rat hepatocytes incubated in the presence of mannoheptulase which inhibited glucokinase (Fillat *et al.*, 1993). In perfused liver, pyruvate kinase has been reported to be activated only by very high fructose loads (1–5 mmol/l) (Eggleston and Woods, 1970). Even in the absence of the rise of plasma insulin levels, therefore,

the inclusion of fructose could increase glycolytic flux via an increase in the intracellular glucose 6-phosphate content secondary to the activation of glucokinase, perhaps with an associated increase in the activity of phosphofructokinase even when plasma insulin remains at the basal.

Insulin stimulates glucose 6-phosphate disposal to glycogen by activating glycogen synthase and inactivating glycogen phosphorylase in the liver. It has been reported that insulin stimulates glycolysis in the liver by increasing the level of fructose-2,6-bisphosphate and by modifying the activity ratio of pyruvate kinase (Assimacopoulos-Jeannet F and Jeanrenaud, 1990; Hue and Rider, 1987). These actions of insulin accelerate the rate of glucose 6-phosphate disposal. Furthermore, insulin has been reported to induce glucokinase translocation in cultured rat hepatocytes (Agius and Peak, 1993) and in vivo in normal rats (Chu *et al.*, 1994), although its mechanism remains unknown. However, it remains to study whether the rise in insulin interacts with fructose to stimulate NHGU additively or synergistically.

Increases in plasma insulin and/or glucose concentrations, glucose delivery to the liver, and the arterial-portal glucose gradient increase the magnitude of NHGU (Cherrington, 1999). To evaluate the effects of fructose on NHGU and intracellular glucose metabolism, plasma insulin and/or glucose concentrations, therefore, these parameters have to be matched between the fructose and control groups. In the study of Masakazu *et al*. (2005), the fructose group had lower sinusoidal glucose levels and glucose delivery to the liver during the test period as a result of higher NHGU with an equivalent rate of intraportal glucose infusion compared with control group. Plasma insulin levels were also lower in the fructose group than the control group. The difference in plasma insulin levels resulted from the rise in the hormone in the control group despite continuous infusion of somatostatin to inhibit endogenous secretion of the hormone. Markedly increased plasma glucose levels might overcome the inhibitory effect of somatostatin.

Therefore, our evaluation based on the differences in NHGU and intracellular glucose metabolism between the fructose and control groups may underestimate the effects of fructose on NHGU and intracellular glucose metabolism (Masakazu *et al*., 2005)

The glycemic index of fructose is very low compared with glucose (19 and 100, respectively) (Foster-Powell and Miller, 1995). This property initially elicited a great interest for the use of fructose as a potential beneficial sweetener in patients with diabetes mellitus. One further characteristic of fructose, which suggested that it was well suited for diabetic patients, is that fructose does not require insulin either for its transport into hepatic cells or for the initial steps of its hepatic metabolism. When administered to diabetic patients, fructose indeed produced minor increases in plasma glucose and insulin concentrations compared with glucose (Crapo *et al.,* 1980; 1981). The plasma insulin response to fructose was however markedly enhanced in diabetic patients compared with nondiabetic subjects. The stimulation of carbohydrate oxidation and of gluconeogenesis after fructose ingestion appeared globally similar in healthy nondiabetic subjects and in diabetic patients (Paquot *et al.,* 1996; Simonson *et al.,* 1988). As in healthy subjects, the enhanced gluconeogenesis induced by fructose appeared to be compensated by an autoregulatory process, involving minor inhibition of glycogenolysis, so that overall glucose output and glycemia did not change to any great extent (Paquot *et al.,* 1996). Of interest, glucose-induced thermogenesis is frequently blunted in insulin-resistant patients, while fructose-induced thermogenesis remains comparable to that observed in controls (Simonson *et al.,* 1988). This is likely explained by the fact that, in insulin-resistant subjects, intracellular glucose metabolism is decreased, leading to lower glucose-induced thermogenesis, while hepatic fructose metabolism is not impaired (Luc and Kim-Anne, 2009).

Physical exercise requires a continuous supply of energy to the working muscle, and muscle contraction increases muscle glucose oxidation by several folds (Halloszy, 1992). Glucose oxidized by muscle during exercise originates either from blood glucose through exercise-induced translocation of GLUT4 (Winder *et al.*, 2006), or from muscle glycogen. Muscle fatigue is a complex phenomenon, still incompletely understood, in which a decrease in glycemia and/or exhaustion of muscle glycogen store can play a major role (Allen *et al.*, 2008). The development of sport drinks and supplements, aimed at preventing a drop in glycemia during exercise and sparing muscle glycogen oxidation, has therefore been the focus of intense research. In this context, fructose has attracted considerable attention.

Fructose can indeed be metabolized during exercise. When infused intravenously during an exercise of moderate intensity, it was shown that approximately 80% of the dose of fructose administered was metabolized in splanchnic tissues to be released as glucose, pyruvate, and lactate which were subsequently metabolized in working muscle. The remaining 20% were metabolized directly in working and resting skeletal muscle (Ahlborg and Bjorkman, 1990). Due to intravenous rather than oral administration, fructose concentration was however very high (up to 6 mM), and it is unlikely that such direct muscle fructose metabolism occurs with the low plasma fructose concentrations elicited by oral fructose. When oxidation of oral glucose or fructose drinks were compared during an exercise of moderate intensity, it was reported that fructose oxidation was comparable to that of glucose (Adopo *et al.*, 1994), or slightly lower (Jandrain *et al.*, 1993), and that fructose conversion into glucose accounted for about half of the total glucose production (Jandrain *et al.*, 1993). Thus, even though fructose ingestion per se does not increase plasma glucose concentration, it may nonetheless contribute to maintain glycemia by sustaining glucose production during exercise (Jandrain *et al.*, 1993).

Sport drinks aim to prevent a drop of glycemia and to provide exogenous glucose to the working muscles. When oral glucose was administered, exogenous glucose metabolism was however limited to a maximum of approximately 1.0 –1.1 g/min, most likely due to saturation of intestinal glucose transport when higher doses are administered (Jeukendrup, 2004). When a mixture of glucose and fructose was administered, total carbohydrate oxidation could however be further enhanced by approximately 40%. This may be explained by the different transport systems used for intestinal absorption of glucose and fructose and by their different metabolism, i.e., essentially hepatic for fructose versus primarily within the skeletal muscle for glucose during exercise. It was also reported that moderate doses of fructose reduced the perception of fatigue and stress during exercise (Rowlands *et al.*, 2008) and improved exercise performance during a cycling exercise (Currell and Jeukendrup, 2008).

Regarding the effects of fructose on muscle glycogen synthesis, few contradictory studies were performed. One study showed that fructose was more efficient than glucose to prevent the decrease in muscle glycogen (assessed from a postexercise muscle biopsy) (Levine *et al.*, 1983), but another study, using similar techniques, observed no difference between fructose and glucose drinks (Koivisto *et al.*, 1985). One study compared muscle glycogen recovery after exercise with glucose and fructose feeding. In this study, muscle glycogen repletion, evaluated with 13C-NMR spectroscopy, was considerably more efficient with glucose than with fructose (Van den Bergh *et al.*, 1996).

On the basis of these studies, the use of fructose as a supplement in sports drinks may possibly have modest advantages, which however remain to be better documented by larger studies in which performance or endurance are the primary outcome. One concern with the use of fructose during exercise is that it may be incompletely absorbed from the gut and get

fermented by intestinal bacteria (Mitsui *et al.*, 2001), which may limit the amount that can be administered without adverse gastrointestinal symptoms.

2.5.3 Galactose

Many mammalian tissues are capable of metabolizing galactose and several metabolic pathways have been described (Segal, 1972). The entry of galactose into many cells may be modulated by insulin due to the stereoconfiguration of the first three carbon atoms which are similar to glucose (Krahl, 1961). In addition, earlier experiments have demonstrated a marked influence of one sugar on the rate of metabolism of the other. Thus, in adipocytes it was found that the uptake of galactose was enhanced not only by insulin but also by the addition of glucose (Naito and Okada, 1972). In other experiments on the liver, it was noted that glucose administration shifted the oxidation-reduction potential of the liver to a more reduced state as determined by an organ microfluorometer. This shift in oxidationreduction state could be blocked by a timely administration of a suitable amount of galactose (Naito *et al.*, 1970).

Oral administration of galactose had also been reported to elevate (Maclagan, 1940; Bakx *et al.*, 1946, Bird and Hartmann, 1994), depress (Pierce, 1935; Wagner, 1943), or not alter the blood glucose levels of healthy adults (Cori, 1928; Peters, 1946) and to increase that of subjects with diabetes (Koehler *et al.*, 1935; Bollman *et al.*, 1934). In addition, Harding and Grant (1932) had reported that following the administration of galactose in normal subjects, there was a temporary increase in blood glucose which reached a peak by 15 minutes and returned to the basal value by 1 hour due to increased utilization of glucose as evidenced by increased respiratory quotient and lactate production. However, these changes were not observed in diabetics (McCullag *et al.*, 1931; Roe *et al.*, 1935). The temporary increase in blood glucose

following the administration of galactose may also be prevented by accelerating glucose utilization with insulin (Maclagan, 1940).

In several species of neonatal animals, it has also been reported that galactose is incorporated into hepatic glycogen more rapidly than glucose and the activity of galactokinase in the liver is greater than that of either hexokinase or glucokinase (Kliegman & Sparks, 1985). Furthermore, Katz *et al.* (1986) reported that, after the administration of an oral dose of glucose to rats, much of the absorbed glucose passed through the liver and was metabolized by the peripheral tissues. These findings suggest that, while most of the absorbed galactose is taken up by the liver and is available to replenish hepatic glycogen, most of the glucose passes through the liver without being metabolized and is available to correct hypoglycaemia and serve as an obligatory energy supply to tissues such as the brain. In this context the galactose moiety of lactose may be important for metabolic homeostasis of young mammals. Indeed, newborn piglets have very little insulation and low fat reserves (10-20 g/kg body weight; Mellor and Cockburn, 1986) and, therefore, the galactose moiety of lactose would appear to facilitate optimally the replenishment of hepatic glycogen for postprandial glucose homeostasis.

Furthermore, in order to study the effects of galactose on glucose metabolism, isolated adipocytes were prepared using either the glucose- or the galactose-containing buffer and were both incubated in a glucose-containing buffer. It was observed that adipocytes prepared in galactose-containing buffer increased significantly glucose as well as galactose metabolism by the cells (Chikayuki and Kodo, 1974).An increase in oxygen consumption was also observed in these adipocytes. The increase in the incorpration of ^{14}C into CO_2 or lipids from [U-^{14}Cglucose or [1-^{14}C]galactose could not be due to an extracellular dilution effect, because the specific activity of the labeled compound in the incubation medium was carefully designed to be the

same in each group of experiments. On the other hand, the difference in the incorporation might have been due to intracellular dilution of the labeled sugar. The cells treated with galactose before incubation might have accumulated less glycogen and the intermediary metabolites of glucose than the cells treated with glucose. Therefore, the isotopically labeled glucose would be less diluted in the cells isolated in galactose-containing buffer and thus the incorporation of [U-^{14}C]glucose would have apparently increased in these cells. However, the increase in the incorporation of [U-^{14}C]glucose by the addition of galactose to the cells incubated in the glucose-containing buffer can not be explainable properly from the above considerations. Furthermore, if this were the case, the increase in oxygen consumption observed in the adipocytes prepared in galactose seems to be unexplainable on this basis (Chikayuki and Kodo, 1974).

The incorporation of [I-^{14}C]galactose into CO_2 and lipids was significantly higher in the adipocytes prepared in galactose. If the results obtained were due to intracellular isotope dilution effects, the incorporation of [I-^{14}C]galactose in the cells prepared in the galactose would be expected to be lower. However, the opposite effect was observed (Chikayuki and Kodo, 1974).

These results suggest that the observed increase in the incorporation of [U-^{14}C]glucose into CO_2 and lipids was due to a real increase in glucose uptake and metabolism by adipocytes. The significant increase in oxygen consumption by cells prepared using galactose suggests a real increase in glucose metabolism. On the other hand, while the incorporation of ^{14}C from [U-^{14}C]-glucose into CO_2 in the cells prepared in galactose was 5.3 times greater than that in the cells prepared in glucose, the oxygen consumption of the former cells was 1.2 times greater than that of the latter. This result suggests a preferential utilization of glucose in cells treated with galactose (Chikayuki and Kodo, 1974).

2.6 Intestinal Metabolism

The metabolism of food substrates present within the intestinal cells has received some attention over the years. Grayson and Kinner (1962) have shown that the gastro-intestinal tract (GIT) is metabolically very active. Grayson and Kinnear (1962) used heat production as the main indicator of gastro- intestinal tract metabolic activity. They showed that the gastro-intestinal tract contributes about 30% of the body's total heat production by processes which are independent of digestion, absorption or bacterial activity.

The cells of the alimentary tract are unique in that they have two sources of metabolic substrates. Like all mammalian cells, they receive nutrients from tissue fluid via the blood and also receive a considerable supply of nutrients from the gut lumen from the normal food intake, the proteinaceous secretions of the alimentary tract and from the exfoliated, degenerating mucosal cells. Studies by Windmueller and Spaeth (1974) have shown that mucosal cells of the small intestine take up among other nutrients large quantity of glutamine from arterial blood. Hydrolysis of glutamine to glutamate is followed by a rapid metabolism to CO_2, organic acids, and several other amino acids including citrulline, ornithine, proline and alanine. About one-third of the respired CO_2 produced by the intestine in fasted rats is derive from circulating glutamine; an indication of the quantitative importance of this substrate in gut metabolism (Windmueller and Spaeth, 1974). Although, many amino acids taken up from the blood may be converted to glucose, glutamate appears quantitatively to be the most important as a metabolic fuel in the intestine (Felig, 1973).

The second source of metabolic substrates for intestinal mucosal cells in addition to the blood, is the intestinal lumen. Luminal glucose, amino acids and other nutrients are directly utilized by the mucosal cells of the intestine (Smyth, 1963, Spencer and Zamcheck, 1961). The

relative importance of the two sources of intestinal metabolic substances is not clear, nor is it known whether substrates taken up by the cells from the lumen and from the blood enter a common metabolic pool (Parsons, 1975, Clark and Sherratt, 1967). Earlier studies in several animal species have shown for instance that glutamate administered intraluminally is poorly recovered in portal blood and that the glutamate gives rise to increased portal concentrations of alanine (Mathews and Wiseman, 1953; Neame and Wiseman, 1957; 1958). Windmueller and Spaeth (1975) have also shown that only 30% of the glutamate carbon from the absorbed glutamate was incorporated into alanine.

Earlier studies by Dickens and Wei-Malharbe (1941) showed a high rate of anaerobic glycolysis by intestinal mucosa in vitro. This was confirmed by wolff and Bergman (1972). Thus, glucose is generally regarded as the major source of energy and is usually the only substrate added during incubation of intestine in-vitro. Hohenleitner and senior, (1969), in a series of in-vitro studies on dogs showed that under hyperglycemic or normoglycemic conditions, the intestinal preparations removed perfusate glucose at a high rate and release large amount of lactate. Since the high lactate production was invariably associated with considerable glucose removal from the perfusate, it was therefore suggested that most of the lactate was derived from the metabolism of the perfused glucose.

The metabolism of circulating substances was further studied by windmueller and Spaeth (1978) in-vivo in rat jejunal segments having intact arterial supply. They identified ketone bodies and glutamine as the major respiratory fuels in post-absoptive small intestine, while glucose contributes only 7%. The low rate of glucose utilization observed by Windmueller and Spaeth (1978) may well be explained by the fact that ketone bodies suppress glucose uptake in several tissues (Robinson and Williamson, 1979).

Most of the studies on the intestine were based on measurement of oxygen consumption and carbon dioxide production (alteveer et al., 1973). Studies by Durotoye and Grayson (1971), Grayson and Oyebola (1983; 1985) showed that the resting glucose uptake in the upper jejunum was more than could be accounted for on the basis of oxidation. When the dog used was administered with catecholamines or nicotine, glucose and oxygen uptake rose, but the increase in glucose uptake was far more than that of oxygen uptake. Furthermore, Grayson and Oyebola (1983; 1985) observed that the increase in glucose uptake and oxygen consumption did not match in timing either.

The mucosal portion of the small intestine has been identified as the site largely responsible for the metabolism of plasma substrates (Windmueller and Spaeth, 1974). The mucosa is however composed of several cell types and these cells have different substrate preferences and metabolic activities.

2.7 Mesenteric Circulation

In a 70.kg resting adult human male in good health, the majorinflow vessel of the mesenteric circulation, thesuperior mesenteric artery, delivers about 12% of thecardiac output and is therefore unsurpassed in sizeamong all branches of the entire aorta. This vessel suppliesthe entire small intestine, the proximal half of thecolon, and part of the pancreas.After blood has been distributed by the superiormesenteric artery to the small intestine, it accumulatesin the mesenteric veins en route to the great portal vein,which transports the blood to the liver. Since the arterial supply of the liver is provided by the hepatic artery, a branch of another splanchnic vessel (celiac artery), the mesenteric circulation is in parallel with the hepatic artery and in series with the portal vein. Within the walls of the small intestine the mesenteric

circulation is also organized in a manner featuring both in-series and in-parallel relationships. Thus the circulation of the mucosa is in an in-series relationship with the submucosal circulation from which the mucosal vessels arise and is also in an in-parallel relationship with the microcirculation of the muscular layer of the wall. These kinds of relationships permit internal redistribution of blood flow within the organ without altering total blood flow.

The mesenteric circulation has three major functional vascular elements (Folkow, 1967). The first category of vessels consists of the microscopic arteries and the arterioles. These vessels have relatively thick walls composed mostly of vascular smooth muscle and are reactive to various stimuli, especially the catecholamines (Zweifach, 1961). The largest portion of the resistance to blood flow in the entire mesenteric circulation from its origin at the aorta to the portal vein occurs as the blood flows through the microscopic arteries and arterioles; hence they are termed the "resistance vessels" (Folkow, 1967). For a drug to increase or decrease blood flow markedly in the mesenteric circulation, the agent must act on the walls of the resistant vessels.

From the arteriole, the blood flows into the capillaries located adjacent to the parenchymal cells. Nearly all transport of materials between the blood and the cells takes place at the level of the capillary component of the circulation. Thus 0_2, nutrients, and fluids move from the blood into the cells, whereas CO_2, heat, metabolites, and fluids move in the opposite direction. Hence the capillary units have been termed the "exchange vessels" (Folkow, 1967). The degree of exchange will depend on the population density of perfused capillaries because only a fraction of all capillaries is open to the flow of blood at any moment in time (Folkow *et al.,* 1963). The structure regulating blood flow through the individual capillary is a smooth muscle thickening around the origin of the microvessel. This thickening has been termed the "precapillary sphincter." When this sphincter contracts, it closes off the capillary to the flow of

blood and thereby reduces exchange between blood in that capillary and adjacent cells. When the sphincter relaxes from the contracted state, the capillary is again perfused with blood, thereby accelerating the exchange. The open capillaries at any moment in time are also referred to as the "nutrient circulation.

The third functionally important component of the mesenteric microcirculation is the microscopic veins that drain the blood from the capillaries. The thin walls of these low-pressure vessels also contain smooth muscle and are responsive to extrinsic stimuli.When their walls contract, blood is expressed centrally from the veins. Since 80% of the total blood in the mesenteric circulation is stored in these microscopic veins, contraction of their walls propels previously stored blood back to the heart, as occurs at the outset of exercise. Because of this storage function the venules have been referred to as "capacitance vessels" (Folkow, 1963).

2.8- Measurement of Gastrointestinal Blood flow

2.8.1 Direct method

Most of the direct methods for the study of blood flow in an organ have been critically reviewed (Folkow, 1952; Alada and Oyebola, 1992). This technique requires arterial cannulation with an extra corporal circuit with or without a pump. However, the physiological nature of these procedures and the striking effects of the preparation on vascular tone make it suitable.

2.8.1.1 Venous outflow

This is likely the oldest method for the measurement of blood flow in an organ and requires collection of the venous effluent. The basic assumption of the technique is that venous drainage reflects arterial inflow. Its major virtues are ease of measurement, reproducibility, and

accuracy. It has been employed effectively to confirm blood flow measured using other techniques (Moody, 1967; Alada, 1992). The obvious disadvantage of this procedure is the trauma to the experimental animal; it can only be used in acute preparations. The technique yields no information about distribution of blood flow.

2.8.2- *In-vivo* fluorescence microscopy

This technique involves direct microscopic observation of vessels in the living animal. The tissue is transilluminated with a high-intensity light source and the vasculature is visualized with a microscope. Fluoresce in isothiocyanate is injected intravenously. The fluorescent agent emits light when excited by filtered light from the microscope system. The emitted light is visualized either by the microscope or on a TV monitor. Vessel diameter may be measured using an image splitting technique via a microscope recording system. This technique has been applied to investigations of the morphology and flow patterns in the gastric microcirculation, including vascular responses to stimuli and changes in microvascular permeability (Guth, *et al.*, 1980). A major limitation of this technique is that it only provides information for a limited portion of tissue, from which one must extrapolate findings to the whole organ. Thus, an additional technique is required to measure whole organ blood flow.

2.8.3- Photometric velocity method

Photometric velocity measurements detect the nearly identical patterns of changes in light intensity caused by a red cell (or group of red cells) as it passes two measuring points along a blood vessel. The time of delay is the transit time of the blood over the known distance between two light detectors (Bohlen, 1981); the distance divided by the transit time is the velocity of the moving red blood cells. This technique may be applied to observing neural and local control of

blood flow and vessel dimensions (Bohlen, *et al.*, 1978) and has the advantage of allowing direct visualization of the enteric vasculature.

2.8.4-Morphological studies:

The premise underlying these approaches is that the vascular architecture reflects its function. Much morphologic information has been obtained from injection procedures using India ink, gelatin, graphite, radio opaque materials, starch granules, various colours of latex preparations, and various plastic agents. A recent adaptation of this method involves injection of a silicone elastomer into which a pigment has been milled (Microfil). Once the injection is complete and surrounding tissues have been cleared, specimens are scanned using a dissecting microscope. This technique has been applied to studies of the small intestine (Reynolds *et al.*, 1972). There are a number of disadvantages with this technique. First, injection of the substance alters blood flow and may distort the geometry of the vessels even when injection pressure is kept within physiological limits. Second, this technique only allows one estimation and is limited by the resolution and magnification of the dissecting microscope. To overcome the latter difficulty, scanning electron microscopic examinations of casts of the microcirculation have been applied (Gannon,*et al.*, 1980). Again, infusion of the casting material may influence vessel geometry. These techniques are also costly. They do, however, provide accurate representations of the microvascular architecture.

2.8.5-Non canulating flow meters:

The most widely used flowmeter is electromagnetic (Kolin, 1936). The method depends upon the induction of voltage in a conductor (the electrolytes of blood) moving through a

magnetic field at right angles to the lines of force. The magnetic field is produced by a small electromagnet that wraps around the blood vessel. The induced voltage is proportional to the flow rate. An extensive discussion of the theory, history, techniques, advantages, and disadvantages of the electromagnetic flowmeter has recently appeared (Clark, *et al.*, 1980). The use of electromagnetic flow probes has the substantial advantage of providing continuous measurement of total blood flow, although distribution of flow within tissues cannot be ascertained by this method. This method records both mean and phasic flow, calibration is linear, and there is a high frequency response. No cannulation is required, and chronic implantation of these transducers permits measurement of blood flow in a conscious animal (Swan, and Jacobson, 1967). The reliability *in situ* of noninvasive flow meters is problematic. Although they are calibrated under undisturbed conditions, distortions in their measurements may occur with the changes in the spatial relationship of transducer to blood vessels that occasionally develop during experimental conditions. Furthermore, the flowmeter must be calibrated at zero flow. This requires transient occlusion of the vessel at least once before the experiment, a maneuver that may produce reflex, hormonal, or paracrine effects upon blood flow. The use of electrometric determination of the zero flow value obviates mechanical occlusion of the vessel, but the validity of this practice is uncertain.

2.8.6- Indicator dilution methods:

If a marker is infused into the arterial blood of an organ at a constant rate and is thoroughly mixed, then an increase in blood flow will decrease the concentration of the marker (Perry and Parker, 1981). From the Fick's principle, blood flow in an organ can be determined if the arterial and venous concentrations of the marker are determined simultaneously with the total

amount of marker extracted by the organ. The Fick equation, which solves for organ blood flow, becomes the quotient of extraction rate/difference between in flow and outflow concentrations of indicator. Dyes are the most frequently used markers for indicator dilution measurements of blood flow. The concentration of dye is determined by circulating blood into a flow-through curette. The absorption of the dye is recorded on an optical densitometer. Similar methodology may be applied to radioactive indicators, in which case detection of the marker is performed by continuous blood sampling or by the use of external detectors (Wolgast, 1968). Thermal markers have been studied using fine-gauge needle thermocouples to demonstrate a logarithmic relation between temperature and flow; from such determinations gastro-intestinal mucosal blood flow has been extrapolated (Grayson, 1949). Infused cold solutions can be employed in place of a dye or isotopic marker (Perry and Parker, 1981). Indicator dilution techniques have been used extensively to measure mucosal hemodynamic to separate villous and mucosal blood flows (Biber, 1973; Shepherd, 1981), and to determine capillary exchange capacity of the intestine (Shepherd, 1981). These techniques are fairly accurate, harmless, and in some situations noninvasive. Rarely is laparotomy or anesthetization necessary to measure large vessel or organ-blood flow. If the chemical indicator is completely cleared from the tissues on a single pass, background accumulation is not a problem and determinations can be performed repeatedly.

The primary difficulty has been the disappearance of marker from the circulation at rates that are not known to the investigator under all experimental conditions. In addition, any recirculation of marker substances will compound the estimation of vascular concentrations.

2.8.7- Washout techniques

Flow-dependent washout of radiolabelled inert gases (predominantly xenon and krypton) to measure organ blood flow is not a new method (Kety and Schmidt, 1945). These noble gases are highly soluble in lipid membranes hence, their transcapillary exchange after intra-arterial injection or after direct injection into tissues is rapid. Removal of inert gas from a site ("washout") is assumed to be proportional to and dependent upon blood flow (Lundgren, 1980). Mathematical analysis of washout curves for total intestinal or skeletal blood flows may be calculated according to a standard formula (Zierler, 1965) after measurement of the gamma radiation from the inert gas. Concurrent determination of beta radiation with a Geiger-Miiller tube placed at the antimesenteric border and plotting of a monoexponential decay curve allows calculation of blood flow in the muscularis according to other equations (Kety, 1951). The weights of the mucosa-submucosa and muscularis can be determined directly. With this information blood flow in the mucosa-submucosa may be calculated. Methods based on these principles have been used for the study of gastrointestinal blood flow (Bell and Battersby, 1968; Lundgren, 1980). Use of carbon monoxide washout to measure blood flow to the villus, villus countercurrent exchange, and effective villus flow has also been reported (Bond, 1977). Although invasive, these techniques allow repeated measurements and can be applied clinically. Because of their high lipid solubility, inert gases permeate tissues quickly, thereby reducing much of the error caused by variations in surface area, permeability, and capillary filtration pressure. The obvious advantage of this method is the ability to measure total blood flow concomitantly with the distribution of blood flow. Inspired nonradioactive xenon can be excited and estimated with fluorimetric methods, providing a safe clinical application of the washout technique (Nelson, 1981). One problem with this technique arises from the high affinity of noble

gases for air, which may cause marker to be trapped in the gut lumen, thereby introducing errors that could not be factored out with current equations. In addition, the instrumentation needed for measurements not inexpensive, and the analytic problems associated with interpretation of washout curves of inert gases are formidable (Greenway and Murthy, 1972).

2.8.8- Clearance methods

Clearance methods are another special application of the Fick principle in which the marker is cleared from the blood in one passage. The most widely used clearance method is based on the pH partition hypothesis (Jacobson, 1966; Shore, 1957). Its use depends on the ready clearance by the stomach of the un-ionized form of a weak base (like aminopyrine) from the plasma into the gastric lumen. When the molecule comes into contact with the low pH of gastric juice, the base dissociates, thereby losing its lipid-soluble character and being prevented from diffusing readily back into the blood. The trapping of ionized base at low pH accounts for the difference in concentrations of aminopyrine between the gastric juice and plasma phases; the extent of this difference depends upon the pKa of the compound. The rate-limiting step in the accumulation of aminopyrine in the gastric secretions is the route of delivery of the agent, namely mucosal blood flow. Given the assumption that aminopyrine is completely cleared on one passage through the circulation of that tissue which is exposed to acid gastric juice, this technique can be used to estimate gastric mucosal blood flow. This assumption has been questioned (Sonnenberg and Blum, 1980); it is possible that three additional factors influence clearance of aminopyrine, namely a diffusion limit between blood and the gastric lumen, facilitated excretion of marker by bulk flow, and storage within the mucosa. Other substances have been employed to estimate gastric mucosal blood flow including aniline, iodoantipyrine,

phenol red, noble gases, and neutral red. Intestinal blood flow may be estimated by a similar technique using weak acids such as barbital (Fara, 1981).The primary advantage of the clearance technique is that it provides concurrent information regarding blood flow and tissue function, namely gastric acid secretion. Furthermore, the technique can be performed continuously in the anesthetized or conscious subject. The most obvious disadvantage is that it may be used only in an organ with a significant pH gradient between plasma and lumen.

2.8.9- Fractionation of isotopes and microspheres:

This method is based on the assumption that the fractional distribution of an isotope injected into an organ is proportional to the distribution of blood flow within the organ. The technique is derived from the indicator dilution method. As initially used in the gut with 42K and 86Rb (Delaney and Grim, 1974), the method limited the number of possible determinations. Furthermore, the assumption that all tissues have the same extraction ratio of 42K or 86Rb is questionable. A theoretically more attractive method is the study of the tissue distribution of radioactively labelled microspheres (15-5 /tm diameter, labeled with SSSr, ^{14I}Ce, ^{51}Cr, etc). The use of this technique for the measurement of gastric and intestinal blood flows have been reviewed extensively (Shepherd *et al.*, 1981). Briefly, the microspheres are injected into the artery in question. The microspheres are presumed to distribute according to the precapillary distribution of blood flow through the microcirculation and should not be recoverable in the venous return. The spheres become trapped in vessels of smaller diameter than the spheres. After injection the animal is killed and the stomach and intestine are separated into mucosa, submucosa, muscularis, and serosa. From the radioactivity of each tissue layer and the cardiac output, the flow to the tissue can be calculated. This method has been used to measure blood

flow distribution in the splanchnic circulation (Greenway and Murthy, 1972). Nonradioactive spheres used to measure blood flow are counted microscopically, a procedure much less convenient than by radioactive determination.

The nonradioactive technique has been used to measure villus capillary blood flow (Bond and Levitt, 1979). The use of spheres labelled with different isotopes permits more than one determination in each animal. However, serious questions have been raised (Greenway and Murthy, 1972) about the validity of the microsphere technique in assessing the distribution of blood flow when the vessels of the area studied are arranged in series with vessels of another tissue rather than in parallel (as occurs with mucosa and submucosa). Other problems inherent in the technique include non-uniform distribution due to sedimentation of the microspheres, rouleaux formation or plasma skimming, entrapment, shunting of spheres through the layers of the gut into venous blood, and movement of previously lodged spheres. These problems have been discussed extensively (Shepherd *et al.*, 1981). Finally, the microsphere fractionation technique appears to be restricted to measurement in species in which the necessary blunt dissection of mucosa, submucosa, and muscularis can be easily performed.

2.8.10- FUNCTIONAL TECHNIQUES:

These techniques are based on evidence suggesting that transmucosal movement of nutrients such as 0_2, ions, and water can be a function of blood flow to the site of secretion or absorption. Flow per se is not measured.

(a)-Capillary filtration coefficient

The major determinant of the rate of delivery of nutrients and O_2 to the parenchyma is the tone of the precapillary sphincters and arterioles. Hence, it is critical to determine both blood

flow and capillary exchange capacity in order to understand effects of physiological changes or experimental interventions on O_2 and nutrient exchange. One measure of the functional exchange capacity is the capillary filtration coefficient (CFC). This index has been employed in studies of capillary fluid exchange and the control of blood flow and oxygen in take in a variety of gastrointestinal tissues (Jansson *et al.*, 1970, Kvietys, *et al.*, 1980). The methodologies used to determine the CFC include: volumetric or gravimetric techniques for measuring the rate of accumulation or loss of fluid within the tissue, measurement of the rate of lymph flow and exudation of fluid into the intestinal lumen following venous occlusion, indicator dilution techniques using labelled red blood cells or plasma proteins, measurement of the components of the Starling equilibrium (capillary filtration rate, capillary plasma oncotic pressure, interstitial oncotic pressure), and measurement of total weight gained by a tissue after venous pressure elevation (Richards and Granger, 1981). Measurement of CFC gives information about the functional state of exchange vessels in a tissue in terms of the density of perfused capillaries and the availability of blood flow for transvascular fluid and O_2 exchanges. The primary disadvantages of these methods are the difficulty in the separation of measurements of perfused capillary density from microvessel permeability and the invasive nature of most of the techniques. Furthermore, the CFC gives no indication of where within a tissue the transvascular exchange is occurring. Finally, it has been demonstrated that values of the CFC determined by the different methodologies described are variable (Richards and Granger, 1981).

(b)-Clearance of titrated water

This technique has been used to measure blood flow at the site of mucosal absorption and involves perfusion of the gut lumen with $3H_20$ (Mailman, 1981). The absorption rate of $3H_2O$ and the concentration of $3H_20$ in the intestinal effluent, mesenteric vein, and artery must be

measured. The basic assumption of the method is that the absorption of $3H_20$ depends upon blood flow. The equilibrium concentration of $3H_20$ in the venous blood would be the same as the luminal $3H_20$ concentration. Hence absorptive-site blood flow can be calculated from the luminal clearance of $3H_20$. The amount of $3H_20$ absorbed is very nearly proportional to blood flow. This conclusion has been supported by comparing $3H_2O$ clearance with the clearance of other highly permeable compounds such as ^{14}C dimethylsulfoxide, ^{22}Na, and Clearance of $3H_2O$ has been used to assess the relationship between absorption and blood flow in the small intestine of a number of species under a variety of experimental conditions (Mailman, 1981). The major disadvantage of the method is that $3H_20$ clearance is probably not an exclusive function of absorptive-site blood flow and may include such factors as capillary surface area, capillary permeability and filtration pressure, surface area of villi, permeability of epithelial cells, metabolic activity, and osmotic differences.

(c)- Oxygen tension

Determination of gastric mucosal PO_2 is another assessment of nutrient blood flow and involves the use of a gold-filled oxygen microelectrode (Bowen *et al.*, 1978). This approach assumes that the presence of oxygen in tissues is a direct reflection of nutrient microvascular blood flow and the partial pressure of oxygen in the arterial blood. When arterial oxygen tension (PO_2) is held constant, any change in intracellular PO_2 can be assumed to reflect change in either the nutrient microcirculation or in the diffusion of O_2 from capillary to cell. In either case, the available concentration of O_2 is an indication of the effectiveness of nutrient microcirculatory blood flow. With the gold filled microelectrode, one can also measure transcellular electrical potential in the gastric epithelium. The electrode used in this procedure appears neither to interfere with blood flow nor to damage the tissue. However, questions have been raised about

the functional significance of the cellular locus routinely under examination and the validity of the assumption that intracellular O_2 tensions is a reflection of nutrient blood flow to the mucosa.

2.8.11- *In-vitro* vascular segments

Although this in vitro approach does not directly measure blood flow through a particular tissue, changes in vascular tone in response to a vasoactive substance or other experimental manipulation may be taken as presumptive evidence for dilator or constrictor properties of the drug. A number of tissue preparations have been used including 2-5 mm long ring segments, helical strips, or an intact segment of vessel several cm in length. Alterations in vascular tone are measured using force or pressure transducers.

2.8.12-Clinical techniques

A number of methods have been developed for assessment of pathological alterations in the splanchnic circulation, including occlusive and non occlusive ischemic disease, shock, and hemorrhage. These techniques should possess a number of the advantages enumerated earlier, but their primary attributes must be safety for the patient and ease of performance.

(a)-Angiography

Selective angiography is the most widely used clinical estimate of blood flow to splanchnic organs. Quantification of mesenteric artery blood flow has been achieved by the use of reflux angiography employing high-frequency filming equipment and a suitably forceful injector system (Clark *et al.,* 1981). Radio opaque medium is delivered at increasing rates that eventually exceed the blood flow; at this point, the injection fills the vessel completely, having shortly displaced the blood and some of the medium will reflux and be detected radiographically.

The rate of injection at which reflux occurs approximately equals the rate of blood flow. The method has major disadvantages: patient risk from an invasive method and from the nephrotoxicity of the medium, the need for a highly skilled operator, and the expense of the procedure. The method is not quantitative, only a few determinations can be performed with one catheterization, and the medium is vasoactive.

(b)-Video dilution technique

This procedure involves use of intraarterial injection of contrast medium as a dye dilution indicator during fluoroscopy (Lantz *et al.,* 1981). The angiographic densities in the video image are expressed as a specific voltage and may then be calibrated by measuring the total density of the blood vessel within an arbitrary area of the television image. By positioning the densimeter over the vessel in question, the passage of injected medium is recorded as a mass versus time curve. Data accumulated so far correspond to estimations of blood flow with an electromagnetic flowmeter. The major disadvantages of the method include its invasiveness, the toxicity and vasoactivity of the injectate, the need for a skilled professional, its expense, and the problem of uneven mixing of the contrast medium with blood.

(c)-Washout of intraperitoneal xenon

The washout of intraperitoneal xenon provides an estimate of total splanchnic flow by measuring the effective perfusion of the entire peritoneal surface after intraperitoneal injection of ^{133}Xe (Bulkey, 1981). The lipid soluble gas is absorbed into the circulation by passive diffusion. Since ischemic tissue should selectively retain ^{133}Xe activity, this method should highlight areas of hypoperfusion. Washout of ^{133}Xe is determined as described previously, and the washout curves may be analyzed by standard procedures. Because it is based on the principles of inert

gas washout, this method is subject to all the limitations and reservations discussed previously. Furthermore, this modified method does not have the potential for precise quantization provided by the conventional approach. Its usefulness lies in its direct applicability to the diagnosis of occlusive and non-occlusive mesenteric ischemia.

(d)- Fluorescent excitation analysis

Nonradioactive xenon may be administered in inspired air. When excited by X-rays or gamma rays it emits a unique radiation (Nelson, 1981). Once a constant tracer input has been achieved, kinetic analysis allows derivation of parameters that can then be empirically compared to establish blood flow values. The primary advantage of this technique is its safety; it is noninvasive, and xenon is both inert and nonradioactive. However, the technique is at present costly and does require a detailed analysis of the time versus activity xenon curves generated.

2.9 Control of Gastrointestinal blood flow

Gut blood flow is regulated by intrinsic and extrinsic mechanisms. The intrinsic factors include local metabolic control and myogenic control, local reflexes and locally produced vasoactive substances. The extrinsic factors include sympathetic innervations, circulating vasoactive substances and systemic haemodynamic changes (Granger *et al.*, 1980). The total blood flow to the giut wall is unevenly distributed in the four main layers- mucosa, submucosa, muscularis and serosa. The mucosa and submucosa receive most of the blood flow, up to 90%. Changes in total gut blood flow may influence the flow to the different layers to varying extent. Some of the factors which affect intestinal blood flow are discussed below.

2.9.1. Autoregulation

The intestinal vasculature can automatically adjust its resistance in response to alterations in perfusion pressure (Johnson, 1960; Shepherd and Granger, 1973). This reaction occurs in the absence of extrinsic neural and hormonal influences (Johnson and Hanson, 1962; Shepherd and Granger, 1973), and therefore reflects an intrinsic mechanism for modulating microvascular tone at the local level. Because vascular resistance falls as perfusion pressure is reduced, the local vascular response serves to stabilize flow over a wide range of input pressures. Previous studies (Johnson, 1960; Johnson and Hanson, 1962) have demonstrated clearly a tendency of the isolated autoperfused intestine to autoregulate its flow. However, in such preparations the degree of flow autoregulation is not extensive. Inside the body, the intestinal vasculature is subject to the influences of the nerves and circulating hormones.

Previous studies have shown that elevation of portal venous pressure commonly causes constriction of the intestinal resistance vessels (Selkurt and Johnson, 1958). This effect is apparently due to the fact that venous pressure elevation increases pressure in the arterial circuit, particularly that portion adjacent to the capillaries, inducing a myogenic response of these vessels (Johnson, 1959). Therefore, it would be expected that a generalized elevation of pressure within the arterial circuit would also cause constriction of the resistance vessels. In an attempt to test the hypothesis that an increase in arterial pressure would produce a similar change, Selkurt *et al*. (1958) found that under the conditions of their experiments, the intestinal vasculature was ordinarily passive to arterial pressure alteration, showing an increased resistance as pressure was reduced and a decreased resistance as pressure rose. However, in some cases, an active response occurred, the vasculature contracting as the pressure rose and dilating as pressure fell.

In pressure-flow studies on segments of terminal ileum, it was also observed that the resistance decreased with pressure reduction in 72% of the experiments and increased in 28% (Johnson *et al* (1960). Thus, it appears that the resistance vessels of the intestine are not ordinarily passively distensible with changes in arterial pressure. As a result of this vascular reaction, the influence of arterial pressure on blood flow is at least partially counteracted. The reduction of arterial pressure ordinarily causes a decrease in blood flow. This may cause a relative deficiency in oxygen supply and a reduced oxygen consumption with the release of anaerobic metabolites, such as lactic acid. Thus, the dilation with pressure reduction may be secondary to oxygen deprivation (Johnson *et al*., 1960).

A variety of mechanisms has been invoked to explain circulatory autoregulation. One of these is that reduction in arterial pressure and flow causes oxygen deprivation (Anrep, 1912). These experiments demonstrate that oxygen deprivation does not occur when arterial pressure is dropped from 120 to 80 mm Hg. A similar study by Stainsby (1959) on the hind leg of the dog also shows oxygen consumption is not reduced when arterial pressure is decreased from normal to 15-30 mm Hg. Similarly, Berne *et al*. (1957) showed that myocardial oxygen consumption was not changed as the arterial oxygen content was reduced, until a very low venous oxygen level (1-2 vol. %) was reached. Holling and Verel (1957) found no reduction in oxygen consumption when forearm flow was reduced by elevation above heart level. In a number of instances, it was observed that lactic acid had a strong dilating effect but the effect of pressure reduction in the same preparation was to cause an increase in resistance. Also, if venous oxygen is maintained as arterial pressure is reduced, vasodilation is not prevented. On this basis, it appears unlikely that autoregulation is a result of the production of anaerobic metabolites.

A second possibility is that a decrease in tissue oxygen is sufficient to cause vasodilation without reduction of oxygen consumption. However, Berne *et al*. (1957) have demonstrated that myocardial hypoxia causes vasodilation only when coronary sinus oxygen falls below 5.5 vol. %. However, in the intestine, the oxygen content of intestinal venous blood was generally well above this value, and reduction in venous oxygen was shown to be not responsible for the vasodilation seen with pressure reduction (Johnson, 1960).

The third possibility relating to flow changes is that aerobic metabolites may accumulate in the tissues with reduced flows, causing vasodilation. However, Folkow (1949) has shown that resistance decreases in the hind leg a few seconds after pressure reduction, which would seem too brief a period for a metabolite to accumulate. Also, since tissue metabolite concentration is dependent upon both flow and oxygen consumption, changes in the latter should induce vasodilation but this is not the case (Johnson, 1960). This lack of relationship between flow and oxygen consumption has been observed by Mottram (Mottram, 1958) in the human forearm. Blair and co-workers (Blair *et al*., 1959) found the resting blood flow in the forearm was not determined by metabolic requirements. Also, Berne *et al*. (1958) reported that catecholamines increased myocardial oxygen consumption but did not alter blood flow until venous oxygen reached low values. Thus, an increase in tissue metabolite concentrations may not influence flow unless venous oxygen is low.

Other flow dependent mechanisms may be considered such as blood borne constrictor agents (i.e. catecholamines) which would be present in lesser amounts in the tissues with lesser flows. A second possibility is that a nonmetabolic dilator agent is released by the tissues, either continuously, or as a result of reduced flow as postulated for reactive hyperemia (Lewis, 1927). Against these possibilities (and in fact all flow dependent mechanisms) are two arguments. The

first is the observation that in some experiments the response of the vasculature was so great that changes in pressure caused little or no change in flow. In these preparations, the mechanism appeared independent of flow. This degree of response is also commonly seen in the kidney (Ader and Bergman, 1990; Ahren, 2000). The second is that previous experiments have shown that when intestinal flow is reduced by venous pressure elevation, vasoconstriction, rather than the expected vasodilation occurs (Selkurt and Johnson, 1958) despite a presumptive increase in tissue metabolite levels. Moreover, the hypothesis of a blood borne constrictor agent seems untenable in those experiments in which vascular resistance was very low initially (i.e. I .5-2.0 mm Hg/ml/min/100 gm) but decreased with pressure reduction. From this it appears that the mechanism is not flow-dependent, but pressure dependent.

In this instance, again several possibilities exist. One is that a local reflex is responsible. The lack of effect with procaine infusion rules out the reflex explanation, as other experiments do with renal autoregulation (Waugh, 1958). In the kidney, procaine infusions of this intensity eliminated autoregulation (Ochwadt, 1956). However, it appears that in that instance, procaine may directly influence the renal vasculature (Waugh, 1958) which apparently differs from the intestine in this respect.

Vessel caliber could be increased by a reduction in extravascular pressure. This could be effected by a change in intestinal tone or extravascular volume. Neither of these seems greatly influenced by arterial pressure reduction in the intestine. Changes in tissue pressure have been implicated as a possible factor in autoregulation in the isolated kidney by Hinshaw and co-workers (1959). However, Gottschalk (1959) using a similar technique, reported a much lower renal interstitial pressure in kidneys in situ than reported by Hinshaw, and which appeared independent of arterial pressure between 40 and 140 mm Hg. Also, Miles *et al*. (1954) concluded

that renal autoregulation was an active mechanism since cyanide eliminated autoregulation but potentiated the increase in the intrarenal pressure seen with elevation of perfusion pressure.

The one remaining pressure-dependent mechanism which can be invoked is a myogenic response of the arteriolar muscle to a change in wall tension. That smooth muscle will contract when tension is applied is well known (Engelmann, 1869). That this is a characteristic of vascular smooth muscle was first suggested by Bayliss (1902). The work of Folkow (1949) has placed the suggestion of Bayliss on a more firm experimental footing. Waugh (1958) had earlier presented evidence to show that kidney autoregulation is myogenic. Autoregulation in the intestine is also consistent with the myogenic hypothesis (Johnson, 1960). There is some evidence from other types of experiments suggesting a myogenic response of arterial vessels. In vitro studies of the equine carotid artery (Wachholder, 1921) and bovine mesenteric artery (Burgi, 1944) have indicated a contraction of the circular smooth muscle sometimes occurs when internal pressure is increased. Nicoll and Webb (Nicoll and Webb, 1955) in studies of the bat wing have observed constriction with elevation of arteriolar pressure by fluid injection.

Moreover, it has also been shown that the superior mesenteric flow rises during the digestive phase of feeding (Chou *et al.*, 1976; Fronek and Stahlgren, 1968), reflecting an intimate interaction between parenchymal activity and intestinal vascular tone. Norris *et al* (1979) demonstrated for the first time a direct relationship between digestive activity and intestinal pressure-flow relationships. In other words, intrinsic responses of the intestinal vasculature to perfusion pressure alterations are most intense in the fed dog, are moderate in the fasted animal, and are weakest in the hypofunctional isolated intestine. For instance, a typical experiment demonstrating the effect on superior mesenteric flow of stepwise reductions of perfusion pressure in the fasted showed that as perfusion pressure was lowered from 120 to 50 mmHg, a

moderate vasodilation occurred and the fall in intestinal flow was not as large percentagewise as the reduction in perfusion pressure. The autoregulatory dilatation maintained intestinal flow within 17% of the control as perfusion pressure fell from 125 to 75 mmHg. A typical recording of the intestinal vascular response to stepwise lowering of perfusion pressure in the fed dog also showed that within the 85-130 mmHg pressure range, flow essentially was independent of perfusion pressure because autoregulatory changes in vascular resistance compensated for the reduction in pressure differential across the intestinal vasculature. Also, in 30% of the fed dogs, there was a superregulation of flow within the upper pressure range of 85-125 mmHg. In other words, in these animals, flow increased above control as perfusion pressure was reduced. In the fasted group, superregulation of flow did not occur in a single animal. The intensity of flow autoregulation in the intact small intestine of fasted dogs is also greater than that observed for isolated intestinal segments, and it has been suggested that this variability also may result from differences in basal activity of the two preparations (Norris *et al.*, 1979). In fasted and fed dogs, the autoregulatory adjustments of blood flow were complete within 20-80 s (Norris *et al.*, 1979). Several mechanisms may be invoked to explain this modulation of flow autoregulation by the functional status of the small intestine.

One possibility is that the metabolic state of the intestinal parenchyma determines the interstitial concentration of vasodilator metabolites and/or oxygen. Absorption, secretion, and motility are accelerated when food is placed in the lumen of the small intestine. Consequently, oxygen demand and utilization are higher than in the resting state (Molstad *et al.*, 1978; Neter and Wasserman, 1974). Thus, if microvascular sensitivity to changes in tissue oxygenation increases with vasodilator metabolite concentration, the degree of intestinal flow autoregulation

would be a direct function of parenchymal oxygen demand. Such a scheme has been proposed for skeletal muscle (Granger *et al.*, 1976) and myocardium (Gellai *et al.*, 1973).

An alternate explanation revolves around a flow redistribution hypothesis. Recent studies of transmural distribution of blood flow in the small intestine have shown that I) mucosal blood flow is well autoregulated (Bianchi *et al.*, 1970), 2) autoregulation of muscularis blood flow is weak (Lundgren and Svanvik, 1973), and 3) the fraction of total intestinal flow passing through the mucosal circulation is increased during digestion (Chou *et al.*, 1976) . Thus, feeding may enhance flow autoregulation because the intestinal bloodstream may be diverted from a weakly autoregulating region to the intrinsically sensitive mucosal vasculature.

Many of the gastrointestinal hormones released by the presence of food in the gastrointestinal tract are vasoactive. Changes in the blood levels of these hormones may influence the ability of the intestinal vasculature to stabilize flow in the face of perfusion pressure reduction. In addition, plasma glucose levels are probably lower in fasted dogs, and possibly intrinsic vascular reactivity is directly dependent on circulating substrate levels.

A large body of experimental evidence suggests that local control of tissue blood flow involves myogenic, as well as chemical modulation of vascular tone (Johnson, 1960; Shepherd, 1977; Shepherd and Granger, 1973). Indeed, in a recent study (Mortillaro and Granger, 1977) of reactive hyperemia in the small intestine, the local microvascular responses could not be explained in terms of a unitary metabolic or myogenic mechanism. Instead, the vascular behavior implied the operation of both feedback mechanisms in the small intestine. In the present study, the superregulation phenomenon observed in some fed preparations is difficult to explain without invoking a myogenic mechanism (Johnson, 1977). Thus, the effects of feeding on autoregulation of superior mesenteric flow may result from increased myogenic sensitivity to changes in

transmural pressure. This change in sensitivity could be induced by alterations in the concentration of vasoactive metabolites and/or hormones.

2.9.2 Myogenic Control of Blood flow

Vasoconstriction in response to an abrupt increase in transmural or intravascular pressure defines the myogenic response. This response represents the inherent property of vascular smooth muscle (VSM) to contract in response to a stretch stimulus, i.e., a contraction that occurs in the absence of extrinsic neural, metabolic, or hormonal influences (Davis and Hill, 1999). The physiological relevance of the myogenic response is substantial as it contributes to pressure-flow autoregulation (Johnson, 1968) and is a key participant in setting basal vascular tone (Folkow, 1952). The mechanistic basis of the myogenic response is calcium-dependent activation of the actin-myosin motor unit, as evidenced by a brisk rise in VSM intra-cellular Ca^{2+} concentration ($[Ca^{2+}]i$) in response to the mechanostimulus of pressure and by subsequent phosphorylation of myosin light chain kinase (D'Angelo *et al.*, 1997; Meininger and Davis, 1992; Zou *et al.*, 1995). However, other factors also play a role in the initiation and maintenance of myogenic vasoconstriction (5). These factors include PKC (Dessy *et al.*, 2000; Han *et al.*, 2001; Hill *et al.*, 1990; Horowitz *et al.*, 1996; Karibe *et al.*, 1997; Osol *et al.*, 1991) and reactive oxygen species (Rudolf *et al.*, 2007).

2.9.3 Enteric Nervous System

The gastrointestinal tract has a nervous system all its own called the enteric nervous system. It lies entirely in the wall of the gut, beginning in the oesophagus and extending all the way to the anus. The number of neurons in this enteric nervous system is about 100 million,

almost exactly equal to the number in the entire spinal cord; this demonstrates the importance of the enteric nervous system for controlling gastrointestinal function. It especially controls gastrointestinal movements and secretion (Guyton and Hall, 2000).

The enteric nervous system is composed mainly of two plexuses: (1) an outer plexus lying between the longitudinal and circular muscle layers, called the myenteric plexus or Auerbach's plexus, and (2) an inner plexus called the submucosal plexus or Meissner's plexus, that lies in the submucosa. The myenteric plexus controls mainly the gastrointestinal movements, and the submucosal plexus controls mainly gastrointestinal secretion and local blood flow (Guyton and Hall, 2000).

The demands for mucosal blood flow in the gastrointestinal microcirculation are significantly greater than in other peripheral vascular beds (Granger *et al.*, 1989; Jodal and Lundgren, 1989). Mucosal oxygen and transcapillary fluid and solute transport needs vary significantly, depending on the physiological state of the intestine. For example, vascular perfusion to intestinal mucosa increases by up to 100% during digestion (Fara, 1984; Hulten, 1969). The rate of vascular perfusion to the mucosa is directly controlled by the contractile state of submucosal arterioles, the major resistance vessels in the gastrointestinal tract (Granger *et al.*, 1989). Although multiple regulatory systems are important in modulating this tone (Granger *et al.*, 1989; Jodal and Lundgren, 1989; Vanner and Surprenant, 1991), neural regulatory mechanisms are poised to rapidly respond to the widely fluctuating demands as stimuli are constantly changing within the lumen of the intestine.

Although extrinsic neural reflexes have been implicated in the regulation of intestinal blood flow, functional studies (Granger *et al.*, 1989; Jodal and Lundgren, 1989; Lundgren*et al.*, 1989) have demonstrated that important neural vasodilator reflexes exist within the intestine.

These studies suggest that enteric neural reflexes can respond to increased mucosal requirements for blood flow as chyme moves through the lumen. For example, in in-vivo studies (Granger *et al.,* 1989; Jodal and Lundgren, 1989) both local mechanical and chemical stimulation of the mucosa have been shown to initiate neurally mediated reflex vasodilation. This action is mediated by enteric nerves because these responses were not blocked by extrinsic denervation (Biber *et al.,* 1973; Lundgren*et al.,* 1989). The neuronal pathways mediating these enteric neural reflexes could not, however, be deduced from these in vivo studies. In-vitro studies (Neild *et al.,* 1990) of guinea pig ileum have demonstrated that submucosal cholinergic vasodilator neurons innervating submucosal arterioles appear to be the major "final common pathway" mediating enteric vasodilator reflexes. In-vitro studies (Vanner and Surprenant, 1993) have also shown that mechanical stimulation of the mucosa can activate these neurons. This reflex, however, was mediated entirely by submucosal neurons because the myenteric plexus had been dissected from this preparation. Nonetheless, there is evidence that implies that vasodilator reflexes are also mediated by the myenteric plexus. For example, in-vivo studies show a close relationship between intestinal motility and blood flow (Fioramonti and Bueno, 1984); such actions may well be coordinated by neural connections between the myenteric and submucosal plexus. The existence of multiple enteric neural pathways is consistent with the parallel and overlapping nature of other effector systems within the intestine that regulate blood flow (Granger *et al.,* 1989; Jodal and Lundgren, 1989; Vanner and Surprenant, 1993). Which of these systems predominates appears to depend on the physiological and pathophysiological state of the intestine.

The functional role of these neurons was later reported by Vanner (2000). Multiple neural projections from neurons in the myenteric plexus with axons terminating in the submucosal

plexus have been described in immunohistochemical studies (Meedeniya *et al.*, 1998; Portbury *et al.*, 1995). In-vitro electrophysiological studies have also demonstrated that most submucosal neurons receive synaptic inputs after stimulation of the myenteric plexus, but the functional role of these neurons was not known. The functional role of these neurons was later reported by Vanner (2000), who demonstrated that stimulation of myenteric neurons activated submucosal neural pathways, causing vasodilation of submucosal arterioles. These vessels are the major resistance within the intestine, controlling blood flow to the mucosa (Lundgren *et al.*, 1989). These findings provided direct functional evidence that the myenteric plexus could play a major role in regulating mucosal function through the modulation of vasodilator reflexes in the submucosal plexus. In addition, there is evidence that some vasodilator neurons may have a dual secretomotor function (Jiang *et al.*, 1993), raising the possibility that these pathways might also coordinate secretion.

Moreover, angiotensin II (ANG II) immunoreactivity (IR) is expressed in the enteric nervous system. Two enzymes catalyze the conversion of angiotensin I (ANG I) to ANG II, which is the biologically active form in the intestine. One of the enzymes, angiotensinconverting enzyme (ACE), is expressed in a variety of tissues and organs. In the gastrointestinal tract, ACE is localized in the brush border of the small intestinal epithelium where it might be involved in digestion of peptides, as well as the generation of ANG II (Stevens *et al.*, 1988; Yoshioka *et al.*, 1987). Synaptosomal fractions from dog ileal myenteric, deep muscular, and submucosal plexuses contain ACE. Mast cell α-kinases are a second set of converting enzymes. α-Chymase is the major non-ACE producer of ANG II in humans and dogs (Fukami *et al.*, 1998; Siddiqui and Miner, 2004; Caughey *et al.*, 2000). Release of α-chymase accounts for the appearance of ANG II as one of the main products associated with degranulation of mast cells (Caughey *et al.*,

2000). Significantly elevated levels of ANG II are found in mucosal biopsies from patients with Crohn's colitis, which suggests that elevated levels might be associated with inflammatory states, which include mast cell hyperplasia (Jaszewski *et al.*, 1990).

The predictable hypertensive action of systemically administered ANG II is well known. Systemic dosing with ANG II evokes vasoconstriction and reduced blood flow in the intestinal mesenteric vasculature in parallel with whole body hypertension. Elevated vascular resistance and decreased flow in the inferior mesenteric vascular bed leads to ischemic colitis in pigs receiving pathophysiological doses of ANG II (Bailey *et al.*, 1986).

2.9.4 Autonomic Nervous System

The sympathetic nervous system contributes importantly to regulation of vascular tone in the systemic circulation (Floras and Hara, 1993; Lindqvist *et al.*, 1993; Yamamoto *et al.*, 1992) and in isolated vascular beds (Boddi *et al.*, 1996; Edfeldt and Lundvall, 1994; Lundvall and Edfeldt, 1994). In situations such as exercise or environmental stress, sympathetic regulation of vascular tone is highly activated, and a higher degree of variability can be expected in total peripheral resistance than during basal conditions. This higher variability in total peripheral resistance is reflected in a higher variability of arterial blood pressure (Blanc *et al.*, 1991; Lundvall and Edfeldt, 1994; Macor *et al.*, 1996).

Gore and Bohlen (Bohlen and Gore, 1977; Gore and Bohlen, 1975) have shown that from a purely anatomic standpoint, the regional vasculatures in the intestinal wall do not become separate parallel vascular beds until the terminal arterioles are reached. Furthermore, on the basis of pressure drops in the intestinal microcirculat ion (Bohlen and Gore, 1977; Gore and Bohlen, 1975), approximately 65-70% of the total intestinal vascular resistance occurs before the regional

intestinal vasculatures begin. Therefore, it is conceivable that a large portion of the intestinal vascular responses to noradrenergic stimulation occurs before the regional vasculatures begin. Indeed, Bohlen and Gore (1976) have shown that the large and intermediate-diameter arterioles are primarily responsible for reduction of microvascular pressures during direct sympathetic stimulation. These observations do not discount the existence or importance of differences in regional responses to noradrenergic stimulation previously reported for intestine (Dressel and Wallentine, 1966; Folkow *et al.*, 1964; Svanik, 1973). However, they do indicate that a complex scheme of events, involving both the series and parallel sections of the intestinal vasculature, may occur during direct sympathetic stimulation.

The vascular events in the total intestine and the muscular and mucosal layers had been studied during direct sympathetic stimulation at different frequencies. Bohlen *et al*. (1978) showed that at a stimulation frequency of 8Hz, blood velocity and flow in the intestinal serosal or muscular layer and mucosal layer are decreased. The reduction in serosal and mucosal flow is caused primarily by vascular activity within the respective vasculatures rather than by responses of larger vessels in series. This statement is based on the indication that the reduction in flow to the serosal and mucosal layers can be almost totally attributed to the increased resistance of each parallel vasculature. However, during stimulation at 16 Hz, the resistance of the serosal vasculature, R, is 96% of the control resistance; yet the blood flow is approximately 30--35% of the control flow during stimulation. The mucosal resistance is 55% of control; yet the mucosal flow is approximately 50% of the control flow. Based on the resistances in the serosal and mucosal vasculatures, their flows during 16-Hz stimulation should be equal to or greater than those during the resting state. Therefore, the fact that flows in the serosal and mucosal layers are less than predicted indicates that the larger vessels in series with the parallel vasculatures have

constricted. In this context, the major expression of responses to direct sympathetic stimulation shifts from the parallel vasculatures at low and moderate rates of stimulation to the larger vessels in series with the parallel vasculatures as the rate of sympathetic stimulation is progressively increased (Bohlen *et al.*, 1978).

The failure of neural activity to increase serosal and mucosal resistance during 16-Hz stimulation may be related to the autoregulatory escape phenomenon (Folkow *et al.*, 1964) that accompanies intense direct adrenergic stimulation of the intestinal vasculature. This phenomena is characterized by an increase in total intestinal vascular resistance during intense adrenergic stimulation followed by a return toward the control resistance as stimulation continues.

The absence of constrictor activity in the mucosal and serosal vasculature at high rates of sympathetic stimulation may be caused by a frank override of sympathetic activity by local regulatory mechanisms. However, Richardson and Johnson (Richardson and Johnson, 1969) have demonstrated that the escape phenomenon will occur at arterial inflow pressures below the range of pressures at which the intestinal vasculature regulates blood flow. These data tend to discount both a metabolic and myogenic component in the escape phenomena. Richardson and Johnson (1969) proposed that autoregulatory escape occurs by the dilation of vessels which are initially constricted by sympathetic stimulation. In essence, they proposed that a vessel may lose the constrictor tone initiated by sympathetic stimulation. This loss of neural vascular tone need not imply that mechanisms identical to the metabolic or myogenic autoregulatory phenomena are involved. However, there is little doubt that the generation of increased vascular tone by sympathetic activity requires an energy source within vascular smooth muscle if vasoconstriction is to be sustained. In this context, the constriction of microvessels and subsequent reduction in blood flow during sympathetic stimulation may decrease delivery of blood to the point that the

energy available for constriction is inadequate to support sustained smooth muscle activity. There is also a possibility that previously quiescent myogenic behavior is activated as microv ascular pressures decrease during intense sympathetic stimulation (Henrich, 1973; Svanik, 1973).

On the other hand, stimulation of the parasympathetic nerves increases local blood flow at the same time that it increases glandular secretion. This increased flow probably results secondarily from the increased glandular activity and not as a direct effect of nervous stimulation (Guyton and Hall, 2005).

2.9.5 Endothelin

The expected vascular response to infusion of endothelin-1 (ET-1) is a transient dilation that occurs over 30–60 s followed by a prolonged constriction; the maximal constrictor response is normally observed by 15 min but varies somewhat between tissues (McMurdro *et al.,* 1993; Sakurai *et al.,* 1992; Warner *et al.,* 1994; Davenport *et al.,* 1995). The vasoconstrictor action of ET-1 is primarily mediated through activation of ET_A receptors (Davenport *et al.,* 1995; Moreland *et al.,* 1995; Riezebos *et al.,* 1994; Warner *et al.,* 1993; Warner *et al.,* 1994) and, in some cases, also ET_B receptors located on vascular smooth muscle cells (Bird and Waldron, 1993 ; Ihara *et al.,* 1992; McMurdro *et al.,* 1993; Warner *et al.,* 1993). In addition, activation of ET_B receptors located on endothelial cells by ET-1 (Opgenorth, 1995) results in the release of the vasodilator substances prostacyclin and/or nitric oxide (NO) (Opgenorth, 1995).

The gut circulation appears to be more susceptible to the vasoconstrictor actions of ET-1 than other peripheral vascular beds. Specifically, the gut vasoconstrictor response to ET-1 is more intense than that observed for iliac flow in anesthetized monkeys (Clozel *et al.,* 1989), renal and carotid flows in anesthetized cats (Toothhaker, 1991), and flow in all

nongastrointestinal tissues in the anesthetized rat (Maclean *et al.*, 1989). This sensitivity of the gut vasculature to ET-1 may be of considerable importance in light of the fact that *1*) ET-1 levels are elevated in a number of instances, including chronic hypoxia (Cargill *et al.*, 1995; Rerri *et al.*, 1995), a variety of surgical interventions (Fukada *et al.*, 1995; Hiroyasu *et al.*, 1997; TeVelthuis *et al.*, 1996), and sepsis (Miura *et al.*, 1996), and *2*) this tissue has a high resting O_2 uptake (20–25 $ml \cdot kg^{-1} \cdot min^{-1}$) that is normally met by a high blood flow rate (300–500 $ml \cdot kg^{-1} \cdot min^{-1}$) (Connolly *et al.*, 1997; Dodd *et al.*, 1987; Nelson *et al.*, 1987; Samsel *et al.*, 1994). If blood flow is reduced to the point that gut O_2 delivery falls below critical values (30–40 $ml \cdot kg^{-1} \cdot min^{-1}$), O_2 uptake will be compromised (Nelson *et al.*, 1987).

King-VanVlack *et al*. (1999) showed that O_2 uptake fell in proportion to the fall in blood flow during ET-1 administration and that little, if any, compensatory increase in gut O_2 extraction occurred to offset this flow limitation. The latter observation is in striking contrast to the response normally observed in this tissue during local or whole body stagnant hypoxia. ET-1 caused a significant increase in vascular resistance and consequently, gut O_2 uptake and blood flow decreased by 32% and 37% respectively (King-VanVlack *et al.*, 1999). When gut blood flow returned to control levels during ET_A receptor blockade, so did gut O_2 uptake. A similar pattern emerged as gut blood flow decreased further (64%) with ET_B receptor blockade, gut O_2 uptake also decreased (62%). Subsequent ET_A receptor blockade resulted in small but significant increases in both blood flow and gut O_2 uptake, but both values remained significantly less than those observed in the control period. It was surprising that little, if any, compensatory increase in gut O_2 extraction occurred to offset this flow limitation. The average values for gut O_2 extraction ratio ranged from a minimum of 0.34 during control to a maximum value of 0.41 during ET-1 infusion. Previous studies using the same in situ canine gut loop preparation have established

that the gut relies heavily on compensatory O_2 extraction responses during periods of reduced O_2 supply (Dodd *et al.,* 1987) such that under conditions of low flow, gut O_2 extraction increased to values ranging from 0.60 to 0.70 (Connolly *et al.,* 1997; Samsel *et al.,* 1994). Peak and/or critical values for O_2 extraction in this gut preparation during ischemia have been reported to reach 0.80 (Nelson *et al.,* 1987). It is not unreasonable to postulate that the failure of the gut to increase O_2 extraction during ET-1 infusion and ET_A and ET_B receptor blockade was the result of redistribution of gut blood flow away from exchange vessels.

Another possibility is that ET-1 administration may have reduced gut O_2 demand through a direct action on cellular metabolism. The latter seems unlikely in light of in vitro studies that have demonstrated that ET-1 causes contraction of guinea pig ileal longitudinal smooth muscle via activation of ET_B receptors located on the longitudinal smooth muscle cells (Bolger *et al.,* 1992; Hori *et al.,* 1994; Miasiro *et al.,* 1995; Yoshinaga *et al.,* 1992). This direct inotropic action of ET-1, if present in situ, would be expected to result in an increase rather than a decrease in gut metabolism and O_2 demand. Thus, it has been suggested that ET-1 infusion may result in microvascular plugging independent of changes in vessel diameter, which would significantly reduce the number of capillaries receiving nutritive flow with a resultant decrease in O_2 extraction (King-VanVlack *et al.,* 1999).

On the other hand, ET-1-induced vasoconstriction in the gut vasculature was mediated through ET_A receptors. This conclusion was based on the findings that gut vascular resistance returned to levels that were not significantly different from control and gut blood flow also returned to control values when ET_A receptor blockade was superimposed during ET-1 infusion. Furthermore, subsequent ET_B receptor blockade did not alter gut blood flow or gut vascular resistance. If the constrictor actions of ET-1 had been partially mediated by ET_B receptors, then

further changes in both gut blood flow and vascular resistance would have been expected with ET_B receptor blockade (King-VanVlack *et al.,* 1999). These results are consistent with those of Warner *et al*. (1993), in which the maximal increase in perfusion pressure following administration of ET-1 (10^{-9} M) in the in vitro-perfused rat mesentery bed was reduced 70% by ET_A receptor blockade with BQ-123, whereas no further decrease in perfusion pressure was observed with ET_A and ET_B blockade with PD-142893. Also, in the newborn, ET-1 administration had been shown to cause vasoconstriction and an unopposed ET-1-induced vasoconstriction could clearly compromise intestinal oxygenation in newborn intestine (Craig *et al.,* 2000).

Furthermore, exercise results in a significant redistribution of tissue blood flow, which greatly increases blood flow in active muscles but decreases it in the splanchnic circulation (Laughlin *et al.,* 1982). It had been reported that the circulating plasma ET-1 concentration is increased by exercise in humans (Maeda *et al*., 1994), and that the expression of ET-1 mRNA in the kidneys was markedly higher in exercised rats than in control rats (Maeda *et al.,* 1998). These findings suggest that endogenously generated ET-1 may contribute to the regulation of vascular tonus during exercise. Maeda *et al*. (2002) have shown that the magnitudes of decrease in the blood flow to visceral organs and increase in the blood flow to active muscles during exercise were significantly depressed by the administration of the ET_A-receptor antagonist TA-0201. Therefore, it was suggested that ET-1–mediated vasoconstriction participates in the decrease of blood flow in visceral organs during exercise, thereby contributing to the increase of blood flow in active muscles during exercise. Indeed, it had beenpreviously observed that ET-1 mRNA expression was increased in visceral organs such as the kidney by exercise (Maeda *et al.,* 1998). ET_A-receptor blockade caused no significant change in mean blood pressure and systemic

vascular resistance during exercise, although the magnitudes of decrease in visceral blood flow and increase in visceral vascular resistance were significantly depressed by ETA-receptor blockade. On the other hand, the magnitude of decrease in active muscle vascular resistance during exercise was significantly depressed by ET_A-receptor blockade. This finding implies that the ET_A-receptor blockade might cause vasoconstriction in active muscle to maintain blood pressure during exercise. The finding that ET_A-receptor blockade did not cause a decrease in systemic resistance suggests that alternate endogenous mechanisms caused vasoconstriction to compensate for the loss of ET-1–mediated vasoconstriction in the splanchnic organs, and that this effect was prominent in active skeletal muscle (Maeda *et al.*, 2002).

2.9.6 Nitric Oxide

In the mammalian mesenteric vasculature, it is well established that the endothelium releases numerous vasoactive molecules, such as nitric oxide (NO), prostaglandins, endothelium-derived hyperpolarizing factor (EDHF), and endothelium-derived contracting factors, which contribute to the regulation of vascular tone (Hutri-Kahonen *et al.,* 1999). In addition, mesenteric arteries are innervated by sympathetic vasoconstrictor and primary sensory vasodilator nerves (Zheng *et al.,* 1997). NO is clearly important in maintaining the vasodilator tone of mesenteric arteries, in as much as inhibition of nitric oxide synthase (NOS) causes vasoconstriction and increases blood pressure (Gardiner *et al.,* 1990). Endothelial NOS (eNOS) is the predominant isoform responsible for NO generation in mesenteric arteries, but there is also evidence that neural NOS (nNOS) in perivascular nitrergic nerves generates NO to provide neurally derived vasodilation (Ahlner *et al.,* 1991; Leckstrom *et al.,* 1993; Toda and Okamura, 1990; Tsuchiya *et al.,* 1994).

In contrast to mammals, much less is known about the role of NO in the regulation of mesenteric vascular tone in amphibians. In the small intestine, NADPH-diaphorase histochemical staining and nNOS immunoreactivity (nNOS-IR) have been demonstrated in perivascular nerves (Li *et al.*, 1992; Li *et al.*, 1993; Murphy *et al.*, 1993; Olsson, 2002), but isoform-specific localization of NOS within the amphibian mesenteric vasculature has not been performed. There is evidence in amphibians that NO is involved in capillary fluid regulation, inasmuch as inhibition of NOS has been reported to decrease hydraulic conductivity (Rumbaut *et al.*, 1995) and capillary permeability (He *et al.*, 1997; Rumbaut *et al.*, 2002) in the mesenteric circulation of the leopard frog *Rana pipiens*. Furthermore, substance P (SP) increased microvascular permeability in *R. pipiens* by increasing NO production (Nguyen *et al.*, 1995). Although there is no definitive evidence establishing the site of NO production within the mesenteric circulation of amphibians, it was assumed, on the basis of the mammalian paradigm, that the capillary endothelium was the source of NO (Rumbaut *et al.*, 1995).

NO signals via activation of soluble guanylate cyclase (GC) and generation of the second messenger cyclic GMP (cGMP), which in blood vessels generally mediates vasodilation (Schmidt *et al.*, 1993). Intriguingly, NO-mediated vasodilation is not ubiquitous in all vertebrates, because it is not observed in chondrichthyans and lungfishes; the first group in which it is generally observed is the bony fishes (Olson and Donald, 2009). NO-mediated vasodilation is always found in amphibian blood vessels, inasmuch as the NO donor SNP is a generic vasodilator (Broughton *et al.*, 2002; 2005; Jennings and Donald, 2008). The presence of an NO/cGMP signaling system was confirmed in toad mesenteric arteries, in as much as sodium nitroprusside (SNP) and 3-morpholinosydnonimine (SIN-1) caused vasodilation, which was

blocked by the soluble guanylate cyclase (GC) inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ).

However, the type and location of NOS isoforms and, therefore, the potential source of endogenous NO in toad mesenteric arteries are not known until recently when Brett and John (2010) investigated these using NADPH-diaphorase histochemistry and immunohistochemistry. They observed no positive NADPH staining in the endothelium of the mesenteric arteries, which provides further evidence that amphibian vascular endothelial cells do not express eNOS/NOS. Recent genomic and expressed sequence tag (EST) analyses have demonstrated that endothelial nitric oxide synthase (eNOS) is apparently not present in fish but first appears in amphibians (Putta *et al.*, 2004). In contrast to the endothelium, positive NADPH staining was always observed in perivascular nerves of mesenteric blood vessels, and nNOS-IR nerves showed a pattern very similar to that of NADPH positive nerves. The presence of nitrergic nerves in toad mesenteric arteries is consistent with previous studies in large systemic blood vessels ((Broughton *et al.*, 2002; 2005) and the pulmocutaneous vasculature (Jennings and Donald, 2008) of these species.

Previously, acetylcholine (ACh) was found to cause the release of NO from perivascular nitrergic nerves in toad systemic vasculature ((Broughton *et al.*, 2002; 2005), presumably by neuronal signaling that activates neuronal nitric oxide synthase (nNOS). In toad mesenteric arteries, application of ACh also mediated vasodilation that was solely attributed to NO generation of cGMP, because it was completely blocked by the soluble GC inhibitor ODQ and the nonspecific NOS inhibitors L-Nitro-N-Arginine-Methyl-Ester (L-NAME). Given the presence of nitrergic nerves in the mesenteric arteries and the fact that removal of the endothelium had no effect on the ACh-induced vasodilation, it is then probable that ACh is

inducing NO neurotransmission and subsequent vasodilation. This is further supported by the observation that preincubation of the arteries with the more selective nNOS inhibitor *N*5-(1-imino-3-butenyl)-l-ornithine (vinyl-L-NIO) (5) significantly decreased the response to Ach (Brett and John, 2010).

Another interesting point of difference between mammals and toad is the interaction between adrenergic and nitrergic mechanisms in vascular control. In various mammalian blood vessels, there is evidence that endothelium-independent, NO-mediated vasodilation induced by nicotine is dependent on perivascular adrenergic nerves, because the vasodilation is abolished by guanethidine and chemical sympathectomy (El-Mas *et al.*, 2008; Lee *et al.*, 2000; Si *et al.*, 2001; Zhang *et al.*, 1998). It is proposed that nicotine binds to receptors on adrenergic terminals, causing the release of norepinephrine (NE), which then binds to adrenergic receptors on adjacent nitrergic nerves to initiate the release of NO. In rat mesenteric arteries, a different mechanism is found. In these vessels, it is postulated that NO is acting presynaptically to regulate NE release, because in endothelium-denuded mesenteric arteries, inhibition of nNOS augmented the adrenergic vasoconstriction upon nerve stimulation, which was concluded to be due to attenuation of NO inhibiting NE release from adrenergic nerves (Hatanaka *et al.*, 2006). It appears in toad mesenteric arteries that the nicotine-induced NO vasodilation is a direct effect that is not dependent on interaction with adrenergic nerves, because pretreatment of the arteries with guanethidine did not attenuate the NO vasodilation, as it does in mammals; in fact, a larger vasodilation was observed. It appears that nNOS expression in vertebrate perivascular nerves occurred in parallel with the development of sympathetic innervation, thus providing a mechanism for rapid vasodilation to oppose sympathetic vasoconstriction (Brett and John, 2010).

In addition to a direct effect on vascular smooth muscle, there is mounting evidence to indicate that NO may also modulate other vascular control mechanisms. For example, NO has been shown to counterbalance or attenuate adrenergic vascular tone. In vitro studies on isolated vessel segments and vascular strips indicate that contractile responses to transmural nerve stimulation (Cohen *et al.,* 1988; Toda *et al.,* 1991) and α-adrenoceptor agonists (Martin *et al.,* 1986; Topouzis *et al.,* 1991) are augmented after NO synthesis inhibition or removal of the endothelium. Nase and Matthew (1996) have demonstrated that endogenous NO activity can attenuate sympathetic neurogenic constriction in the intestinal microvasculature. After local inhibition of NO synthase activity with N^G-Monomethyl-L-Arginine (L-NMMA), the magnitude and rate of arteriolar constriction in response to perivascular nerve stimulation were significantly enhanced in the intact intestinal microcirculation; showing a possible physiological importance of endogenous NO as a modulator of sympathetic neurogenic constriction.

Histochemical studies in the rat, guinea pig, and human intestine show extensive vascular localization of NO synthase, demonstrating a strong potential for NO generation in this vascular bed (Cuffari *et al.,* 1992; Nichols *et al.,* 1993). This endogenous NO may limit the influence of sympathetic adrenergic nerves on arteriolar tone. The magnitude of sympathetic constriction at 3, 8, and 16 Hz was increased in the presence of L-NMMA. The enhanced constriction in the presence of L-NMMA was maintained throughout the entire stimulation period. Furthermore, the finding that L-arginine completely reversed the effect of L-NMMA on sympathetic constriction indicates that L-NMMA was selectively inhibiting NO synthase under that experimental condition (Nase and Matthew, 1996). Furthermore, the effects of the inhibitor of nitric oxide (NO) synthesis, NG-nitro-L-arginine methyl ester (L-NAME), on systemic arterial blood pressure and jejunal motility, blood flow, and oxygen uptake have also been reported in

anaesthetized dogs. L-NAME (cumulative doses of 0.1-20 mg /kg, i.v.) dose-dependently increased blood pressure, vascular resistance and jejunal motility and decreased jejunal blood flow. Thus, Endogenous nitric oxide may play a role in regulating motility and blood flow in the resting canine jejunum (Alemayehu *et al.*, 1994).

In diabetics, abnormal endothelium-dependent relaxation has been reported and several mechanisms have been proposed to explain this. These include abnormalities in signal transduction, reduced synthesis of endothelium-derived relaxing factor (EDRF), accelerated inactivation of nitric oxide, and generation and release of competing vasoconstrictor substances (Johnstone *et al.*, 1993). Faulty signal transduction has been variably attributed to decreased expression of inhibitory G proteins, reduced phosphoinositol metabolism, and increased activation of protein kinase C (Gawler *et al.*, 1987; Greene *et al.*, 1987; Lee *et al.*, 1989). Abnormalities in the endothelial milieu might hasten the inactivation of nitric oxide. These include high levels of oxygen-derived free radicals, advanced glycosylation end-products, and transport barriers such as thickened basement membranes (Greglewski *et al.*, 1988; Pieper and Gross, 1988; Brownlee *et al.*, 1988; Bucala *et al.*, 1991; Superstein *et al.*, 1968). Several groups of investigators have reported that endothelial generation and release of vasoconstrictor prostanoids compete with, and thereby attenuate, endothelium-dependent relaxation in diabetes (Tesfamariam *et al.*, 1989; Mayhan, 1989). In these studies, endothelium-dependent relaxation has been restored by the cyclooxygenase inhibitor indomethacin and by a prostaglandin H_2 and thromboxane A_2 receptor antagonist.

2.9.7 Insulin

In an attempt to study the role of the gut in glucose homeostasis, Alada and Oyebola (1996) injected insulin into normal dogs and measured jejunal blood flow. They observed that insulin caused a transient increase in both blood flow and systemic blood pressure which were said to be due to stimulation of sympathetic nerves by insulin. However, in diabetic dogs, insulin had no effect on blood flow (Alada *et al.*, 2005). This is consistent with the report that in lean, insulin-sensitive subjects, insulin leads to a doubling of muscle blood flow, with half of the maximal effect occurring at the highly physiologic insulin concentration of 46 µU/ml. It was shown that insulin's ability to vasodilate skeletal muscle vasculature is directly proportional to its ability to stimulate glucose uptake (insulin sensitivity) (Laakso *et al.*, 1992). In other words, insulin sensitivity and vasodilatations are coupled such that the most insulin-sensitive subjects exhibit the greatest degree of vasodilatation. On the other hand, insulin resistant subjects such as obese patients or those with hypertension or noninsulin–dependent diabetes mellitus exhibit blunted vasodilatory responses to insulin (Laakso *et al.*, 1990; Baron *et al.*, 1993).

In an attempt to test whether insulin-mediated vasodilation is endothelium-derived nitric oxide (EDNO)-dependent, leg blood flow (LBF) was measured in healthy volunteers during euglycemic hyperinsulinemia (insulin levels 220µU/ml) alone and during a superimposed infusion of L- monomethyl arginine (L-NMMA, an inhibitor of nitric oxide synthesis) into the femoral artery. During hyperinsulinemia, LBF increased approximately two-fold. With superimposed infusion of L-NMMA, LBF fell by 50% to the basal rate. Therefore, these data indicate that insulin-mediated vasodilation is largely (if not exclusively) EDNO-dependent (Baron and Clark, 1997). To verify that insulin actually caused the increased production of nitric

oxide (NO), Baron (1997) measured the oxidative end-products NO_2/NO_3 (Nox) in the femoral venous effluent under each condition. Insulin caused doubling of rate of production of venous Nox, which was completely abrogated by L-NMMA. In human umbilical vein endothelial cells, insulin also caused a dose-dependent release of NO, as measured with a NO amperometric probe (Zeng and Quon, 1996)). Thus, evidence suggests that insulin stimulates the release of EDNO via direct interaction with the endothelium. The above report is also consistent with the report that NG-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase caused significant decrease in jejunal blood flow (Alemayehu *et al.*, 1994).

2.9.8 Feeding

The control of gastrointestinal blood flow, especially after feeding, has received much attention in mammals, but details still remains elusive despite the fact that several studies have focused directly on the cardiovascular response to feeding (Chou and Coatney, 1994; Chou *et al*., 1984; Granger and Kvietys, 1981; Granger *et al*., 1980; Kvietys and Granger, 1982; Matheson *et al*., 2000). So far it has been shown in mammalian species that mechanical stimuli might contribute (Biber, 1973), but chemical stimuli are almost certainly more important in the control of the gastrointestinal blood flow control. The chemical stimulus is dependent on the composition of the feed and it has been noted that different nutrients are not equally essential in inducing the postprandial hyperemia (Chou *et al*., 1972; Chou *et al*., 1978; Kvietys *et al*., 1981; Siregar and Chou, 1982). The exact mechanism controlling the hyperemia in mammals is under debate but may involve direct effects from the absorbed nutrients on the vasculature (Chou *et al*., 1985), endocrine factors (Biber, 1973; Chou *et al*.,1977; Chou *et al*., 1984; Fara *et al*., 1972), non-metabolic vasoactive factors (Chou *et al*., 1989; Chou and Siregar, 1982; Sawmiller and

Chou, 1988; Sawmiller and Chou, 1990), metabolic vasoactive factors (Bohlen, 1980a; Bohlen, 1980; Bohlen, 1998; Pawlik *et al*., 1980) and neural mechanisms (Biber, 1973; Kato *et al*., 1989; Takagi *et al*., 1988). There are, however, large variations in mammals reported in the literature, which, to some extent, can be explained by large interspecies differences. Nevertheless, the results indicate that metabolic factors pertaining to a change in the partial pressure of oxygen or the osmolarity of the gut are of major importance in triggering the postprandial gastrointestinal hyperemia (Bohlen, 1980; Bohlen, 1998). However, this does not exclude a possible importance of neural mechanisms in, for example, controlling the response to a change in the partial pressure of oxygen within the gastrointestinal tract (Surprenant, 1994; Vanner and Surprenant, 1996).

In fish, little is known about most aspects of the postprandial regulation of gastrointestinal blood flow and little attention has been directed to a possible neural regulation of gastrointestinal blood flow in teleosts. It has been previously shown, in two separate teleost species, that despite the obvious differences between mammalian and piscine species, there are numerous similarities in the postprandial cardiovascular response. For instance, the mechanical distension that occurs as food enters the stomach induces a pressor response (i.e. increased dorsal aortic blood pressure) that may facilitate an efficient shunting of blood from the systemic circulation to the gastrointestinal tract when hydrolyzed food components induce a subsequent intestinal hyperemia (Seth and Axelsson, 2009; Seth *et al*., 2008). Furthermore, it has alsobeen shown that the nutrient components are not equally important and that a balanced diet, resembling the natural diet, induces the most profound hyperemia (Seth *et al*., 2009). The reason for this and the mechanisms of control behind the hyperemia remain to be established in fish. It is probable that the postprandial gastrointestinal hyperemia is influenced by, or depends on, neural components, either extrinsic (sympathetic and/or parasympathetic) or intrinsic (enteric) to

the gastrointestinal tract. A few studies have revealed an extrinsic neural component that directly influences the postprandial hyperemia in rats (Rozsa and Jacobson, 1989) or has indirect effects *via* cholecystokinin in cats (Biber *et al*., 1974), as well as *via* a non-cholinergic, non-adrenergic neural mechanism in mongrel dogs (Kato *et al*., 1989; Takagi *et al*., 1988). Other studies in mammals have, however, concluded that there is no neural component or it is at least of minor importance compared with the metabolic component (Nyhof and Chou, 1981; Nyhof and Chou, 1983; Nyhof *et al*., 1985; Vanner and Surprenant, 1996). Even less is known about a possible role for the enteric nervous system (Furness, 2006; Olsson and Holmgren, 2009; Olsson *et al*., 2009), intrinsic to the gut, in the modulation of the hyperemic response although it has been implicated as possible factor in connecting the metabolic response to the status of the vasculature (Bohlen, 1998).

More recently, the involvement of the extrinsic and intrinsic innervation of the gastrointestinal tract, i.e. the sympathetic, parasympathetic and enteric innervation, respectively, in the control of the postprandial hyperemia in *rainbow trout* was examined. This was done by sectioning the vagal and splanchnic innervation of the gastrointestinal tract, as well as by blocking the enteric portion with the voltage-gated sodium-channel-inhibitor tetrodotoxin (TTX). The results showed the importance of the extrinsic innervation in maintaining and regulating gut blood flow during normal conditions. However, it is probably of little importance to the gastrointestinal hyperemia after feeding (Seth and Axelsson, 2010).

It is well known that the presence of digested or hydrolyzed food components in the intestine is the principal determinant of the postprandial hyperemia in mammals (Chou *et al*., 1978; Chou *et al*., 1985; Fara, 1984; Gallavan and Chou, 1985; Kvietys *et al*., 1980; Siregar and Chou, 1982; Sit *et al*., 1980) and in fish (Seth *et al*., 2009). The information concerning what

controls this hyperemia is much more limited, especially the neural contribution. This is despite the fact that several studies have focused on the neural reflexes controlling the intestinal circulation in mammals as reviewed by, for example Vanner and Surprenant (Vanner and Surprenant, 1996).

The results indicate that the intrinsic innervation of the gut is more important than extrinsic innervation in the control of postprandial hyperemia. This is reasonable since it seems that most of the submucosal arterioles are innervated directly by the enteric nervous system and the extrinsic innervation mainly innervates the larger superficial arteries and arterioles in fish (Olsson and Holmgren, 2001; Olsson and Holmgren, 2009; Olsson *et al*., 2009) and mammals (Furness, 2006). Extrinsic parasympathetic nerves do innervate the submucosal arterioles but only indirectly via enteric neurons, whereas some extrinsic sympathetic nerves innervate the submucosal arteriols directly (Holtzer, 2006).

Two of the main determinants of the postprandial hyperemia in mammals is almost certainly the oxygen tension (P_{O2}) (Bohlen, 1980) and the increased sodium concentration (hyperosmolarity) in the tissue (Bohlen, 1982; Bohlen, 1998; Chou *et al*., 1972). The decrease in oxygen tension as a result of the cost of nutrient uptake, enzyme secretion and assimilation as well as the subsequent increase in osmolarity could be sensed either in the mucosa and/or submucosa by some sort of chemoreceptor or in the venules by oxygen sensors such as hemoglobin (Dietrich *et al*., 2000; Ellsworth *et al*., 1995) or H_2S (Olson, 2008; Olson, 2009; Olson *et al*., 2008). However, there is a lack of knowledge about how a low P_{O2} or the hyperosmolarity is communicated rapidly from the mucous to the submucosal arterioles where a large portion of any change in resistance and flow occurs (Gore and Bohlen, 1977), since

the change in P_{O2} or osmolarity is less profound in the submucosa than in the mucosa (Bohlen, 1998).

It has therefore been suggested that the signal is relayed from the mucosa, where the vascular effects could be both direct and indirect, *via* enteric nerves from the myenteric plexuses of fish (Seth and Axelsson, .The signal is subsequently sent to the submucosal vasculature, *via* nitrergic perivascular nerves releasing vasoactive factors such as, nitric oxide (NO) (Jennings *et al*., 2004), endothelial-dependent prostaglandins (Kagstrom and Holmgren, 1997), neuropeptide Y (Shahbazi *et al*., 2002) and vasoactive intestinal polypetide (Jensen *et al*., 1991; Kagstrom and Holmgren, 1997). In mammals there is also a muscarinic-receptor-mediated release of endothelial NO (Vanner *et al*., 1993). However, given that it is still debatable whether or not NO is synthesized and released only from nerves in fish or if there is also an endothelial subform (Olson and Donald, 2009; Olson and Villa, 1991), prostaglandins could function as an endothelially derived vasorelaxing factor in fish, comparable to that of NO in mammals (Jennings *et al*., 2004; Shahbazi *et al*., 2002).

Even though the mechanism by which the enteric nervous system controls the postprandial hyperemia in fish is unknown, it must be of fundamental importance given that response was completely abolished when blocking the enteric nervous system. A similar importance or involvement of the enteric nervous system has been shown in several mammalian studies as well (Chou *et al*., 1972; Neild *et al*., 1990; Surprenant, 1994). On the other hand, other studies show little importance of neural control mechanisms, whether extrinsic or intrinsic (Nyhof and Chou, 1983; Nyhof *et al*., 1985). The reason for this difference is not clear, but could depend on species differences and the stimulus used to evoke the hyperemia. For example, in vessel structures where the arterioles and the venules lie in close proximity the signal could

perhaps diffuse from the venule to the arteriole (Bohlen, 1998b) or the signal could propagate as a series of events within the vascular wall (Collins *et al*., 1998; Ellsworth *et al*., 2009).

Other studies indicate that mechanical stimuli to the intestinal mucosa induce a tetradotoxin-sensitive (TTX-sensitive) vasodilatory reflex intrinsic to the gut (Vanner, 1993). However, it is unclear whether this vasodilatory reflex is primary or secondary to a change in gut motility, although changes in gut motility are usually associated with a subsequent rearrangement in blood flow within the gut, with only minor effects on the net flow (Chou, 1982; Chou and Gallavan, 1982; Chou and Grassmick, 1978). TTX could perhaps also have interfered with the nutrient uptake in the intestine, but dibucaine (an amide local anesthetic) not TTX is generally used to block intestinal nutrient uptake (Nyhof and Chou, 1983). Certain types of chemical stimuli, such as capsaicin, induce an extrinsically mediated vasodilation as it can be blocked with extrinsic nerve sectioning (Vanner, 1994; Vanner and Bolton, 1996), but most studies show little importance of extrinsic innervation in maintaining a postprandial intestinal hyperemia (Nyhof and Chou, 1981; Nyhof and Chou, 1983; Nyhof *et al*., 1985; Takagi *et al*., 1988; Vanner and Surprenant, 1996; Vatner *et al*., 1970). By contrast, the increase in gastric blood flow observed in dogs during feeding is most probably mediated *via* a vagal reflex pathway, as it can be blocked with topical administration of local anesthetic to the vagi (Takagi *et al*., 1988). This suggests that the gastric and the intestinal hyperemia are not equally controlled, but few studies have focused on the reflex pathways that are elicited by physiological stimuli during feeding.

In order to study a possible involvement of an acetylcholine-mediated release of NO, or any related compound, animals were pretreated with atropine. However, the injection of atropine produced a rather unexpected response as it caused a substantial increase in blood flow, which

was larger than any postprandial change in blood flow. This effect could be due to a parasympathetic vasoconstrictor tonus on the gastrointestinal vasculature, which is unlikely considering that the parasympathetic nervous system imposes a strong vasodilator tone in the gastrointestinal tract, at least in mammals (Holzer, 2006). It could also indicate that there is a change in the tonus of the smooth musculature of the gastrointestinal tract leading to a change in the transmural pressure of the vasculature. This is also unlikely considering that there is a considerable vasorelaxation in isolated gastrointestinal vessels when adding atropine (Seth *et al*., 2010). The most likely explanation is that the effect instead represents a pharmacological artifact. There are a few reports in mammals of a non-cholinergic, non-adrenergic vascular effect of atropine, possibly mediated *via* K^+ channels, at least in adrenergically stimulated vessels (Liu *et al*., 2004). This calls for caution when interpreting the results using atropine in fish as well as mammals.

2.9.9 Adrenergic receptors

Adrenergic receptors have been shown to mediate changes in intestinal blood flow. For instance, adrenaline has been reported to cause an increase in canine jejunal blood flow which was maximal at 5 minutes. However, this increase was abolished by propranolol, showing that the increase was mediated by beta adrenoceptor (Grayson and Oyebola, 1983). Also, in the large intestine, propranolol has been reported to abolish adrenalin-induced increase in colonic blood flow while prazosin had no effect (Alada *et al.*, 2001).

Furthermore, contrasting responses of the intestinal vasculature to adrenergic blockade have been reported in dogs and rabbits during nicotine infusion. For instance, while nicotine infusion after beta blockade in dogs caused a huge increase in blood flow and abolished the reduction in flow to below basal levels post-infusion in dogs (Grayson and Oyebola, 1985),

nicotine infusion in rabbits not only failed to cause an increase in blood flow, but actually resulted in a highly significant decrease in jejunal blood flow throughout the 90 minutes of observation (Oyebola *et al.*, 2009). This suggests that nicotine most probably resulted in vasoconstriction of the rabbit jejunal blood vessels leading to the profound decrease in blood flow. This also suggests that alpha adrenoceptors mediate vasoconstrictor effect in the rabbit intestinal vessels. Hence, blocking of beta adrenoceptor activity by propranolol allowed unopposed alpha receptor effect leading to vasoconstriction when the animal was challenged with nicotine (Oyebola *et al.*, 2009). Furthermore, while pre-treatment with prazosin caused a significant increase in resting blood flow in rabbits, it had no effect on resting blood flow in dogs (Grayson and Oyebola, 1985). This result confirms the earlier suggestion that alpha adrenoceptors mediate the vasoconstrictor response to adrenoceptor agonists in the rabbit jejunal vascular bed. Blockade of the alpha receptors with prazosin therefore resulted in unopposed beta adrenoceptor activity leading to vasodilation and increased blood flow. The decrease in blood flow caused by nicotine infusion in rabbits pretreated with prazosin was not observed in dogs similarly treated (Grayson and Oyebola, 1985). That is, blood flow did not change during nicotine infusion in dogs pretreated with prazosin.

2.10 Adrenergic Mechanism in the Gastrointestinal Tract

The concept of adrenergic receptors was introduced by Ahlquist (1948). There are three types of receptors in the vessel wall designated α, β, and γ. Genomic clones have been obtained for nine subtypes of adrenergic receptors, including three α_1-subtypes (α_{1a}, α_{1b}, α_{1c}), three α_2 subtypes (α_{2a}, α_{2b}, α_{2c}) and three β-receptor subtypes (β_1, β_2, β_3) (Bylund, 1992; Graham et al.,1996).The alpha receptor is innervated by adrenergic sympathetic nerve fibres and will cause constriction of the smooth muscle when stimulated by adrenaline or noradrenaline. The beta

receptors will cause dilatation of smooth muscle and it is not innervated. The beta receptor responds to adrenaline and isoprenaline but not noradrenaline. The γ-receptor is innervated by cholinergic sympathetic fibres, and will cause dilatation which can be prevented by atropine. Adrenaline can thus act through the α-receptor to cause constriction and through the β-receptor to cause dilatation. It is considered that β-receptors have a lower threshold than α-receptors and cause dilatation, but that large doses of adrenaline will act on α-receptors and obscure the effects of β-receptors. Most vessels have α-receptors but need not have β or γ receptors. Ahlquist and Levy (1959) showed that the canine ileum has both α- and β-receptors. In addition, adrenoceptors of both α- and β-subtype have been recognized at different levels of the gastrointestinal tract where they are involved in in the regulation of motility and secretion. In particular, α_1-adrenoceptors are located postjunctionally on smooth muscle cells and, to a lesser extent, on intrinsic neurons, while α_2-adrenoceptors may be present both presynaptically and postsynaptically. β_1 and β_2 –adrenoceptors are found mainly on smooth muscle cells, but the former may be present on enteric neurons (Bulbring and Tomita, 1987; Ek *et al.*, 1986).

2.11 Importance of the Hexoses used

Glucose ($C_6H_{12}O_6$), a simple sugar (monosaccharide), is the most important carbohydrate in biology because it is used by cells as a source of energy and metabolic intermediate. Glucose is one of the main products of photosynthesis and starts cellular respiration.

After absorption into a cell, glucose can be used immediately for release of energy to the cell, or it can be stored in the form of glycogen, which is a large polymer of glucose. All cells of the body are capable of storing atleast some glycogen, but certain cells can store large amounts, especially liver cells, which can store up to 5 to 8 % of their weight as glycogen, and muscle cells, which can store up to 1 to 3 percent glycogen. The glycogen molecules can be polymerized

to almost any molecular weight, the average molecular weight being 5 million or greater; most of the glycogen precipitates in the form of solid granules. This conversion of the monosaccharides into a high-molecular-weight precipitated compound (glycogen) makes it possible to store large quantities of carbohydrates without significantly altering the osmotic pressure of the intracellular fluids. High concentrations of low-molecular-weight soluble monosaccharides would play havoc with the osmotic relations between intracellular and extracellular fluids (Guyton and Hall, 2005).

Galactose and glucose are the two monosaccharide sugar components that make up the disaccharide sugar, lactose. Lactose is found primarily in milk and milk products.In the human body, glucose is changed into galactose via hexoneogenesis to enable the mammary glands to secrete lactose. However, most galactose in breast milk is synthesized from galactose taken up from the blood, and only 35±6% is made by *de novo* synthesis (Sunehag *et al.*, 2002). Moreover, the metabolism of galactose also leads to glucose production. Thus, galactose is a gluconeogenic substrate.

Fructose, or fruit sugar, is a simple monosaccharide found in many plants. It is one of the three dietary monosaccharides, along with glucose and galactose, that are absorbed directly into the blood stream during digestion.

Pure, dry fructose is a very sweet, white, odorless, crystalline solid and is the most water-soluble of all the sugars (Hyvonen and Koivistoinen, 1982). From plant sources, fructose is found in honey, tree and vine fruits, flowers, berries and most root vegetables. In plants, fructose may be present as the monosaccharide and/or as a component of sucrose. Sucrose is a disaccharide with a molecule of glucose and a molecule of fructose bonded together with a glycosidic linkage. Commercially, fructose is usually derived from sugar cane, sugar beets and

corn and there are 3 commercially important forms. Crystalline fructose is the monosaccharide, dried and ground, and of high purity. High-fructose corn syrup (HFCS) is a mixture of glucose and fructose as monosaccharides. Sucrose (table sugar) is the third form. All forms of fructose, including fruits and juices, are commonly added to foods and drinks for palatability, taste enhancement and improved browning of some foods, such as baked goods. Fructose is also converted to glucose at high rates in the liver (Levine and Haft, 1970).

2.12 Pharmacology of drugs used

2.12.1 Prazosin

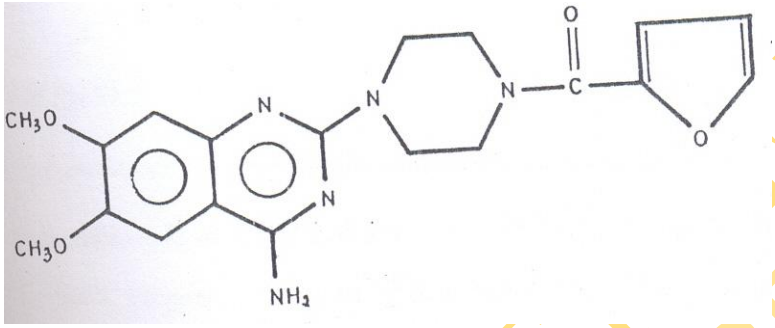


Figure 2-6: The structure of Prazosin (Hoffman and Lefkowitz, 1996)

Chemistry

Chemically, prazosin is 1- (4-amino-6, 7-dimethyloxy-2-quinozoliny1-4 2-furoyl) piperazine. It is a white crystalline substance, slightly soluble in water and isotonic saline, and has a molecular weight of 419.87.

Information about the deposition of prazosin is limited. More than 99% of the drug is metabolized and there may be substantial first-pass metabolism. Bioavailability average is 57%. In normal man, the half-lifeof the drug is about 3 hours (Bateman *et al.*, 1979), this value is probably not altered in renal failure.

Pharmacological Actions

Prazosin is an antihypertensive agent that appears to exert its vasodilator action through blockade of postsynaptic receptors. Administration of prazosin causes reversal of pressure responses to noradrenaline. Prazosin appears to be a rather selective α-1 blocking agent.

It probably has little effect on β-receptors and prazosin does not cause enhanced neural release of noradranaline. This may explain the relatively modest degree of tachycardia associated with the administration of this adrenergic antagonist.

Prazosin reduced vascular tone in both resistance and capacitance vessels. This is associated with a reduction in venous return and cardiac output. The hemodynamic effect associated with prazosin, namely, decreased arterial blood pressure, reduction in arterial and venous tones, and relatively little change in cardiac output, heart rate, or right atrial pressure, are similar to the hemodynamic consequences of direct acting vasodilators, such as sodium nitroprusside (Graham and Pettinger, 1979).

2.12.2 Propranolol

Chemistry

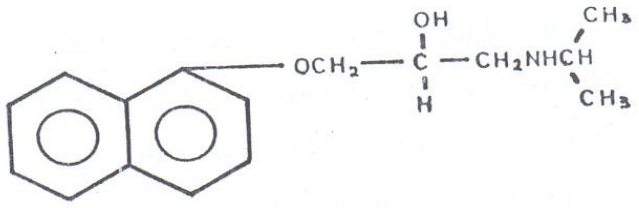


Figure 2-7: The structure of Propranolol (Hoffman and Lefkowitz, 1996)

Propranolol is a nonselective β-adrenergic competitive blocker and does not exhibit any intrinsic agonistic properties.

Absorption, fate and Excretion

Propranolol is almost completely absorbed following oral administration. However, much of the administered drug is metabolized by the liver during its first passage through the portal circulation and only up to about one third reaches the systemic circulation.

Propranolol is bound to plasma protein to the extent of 90 to 95% (Evans *et al.*, 1973), and it is virtually completely metabolized before excretion in the urine (Hayes and Cooper, 1971). The half-life for elimination of propranolol in man is about 4hours.

Pharmacological Actions

The most important effects of β-adrenergic blocking drugs are on the cardiovascular system, predominantly due to actions on the heart. Propranolol decreases heart rate and cardiac output, prolongs mechanical systole and slightly decreases blood pressure in resting subjects.

Propranolol inhibits in man the rise in plasma fatty acids induced by sympathomimetic amines or by enhanced sympathetic nervous system activity inhibits the lipolytic action of catecholamines on isolated adipose tissue of several species. The actions of propranolol on carbohydrate metabolism are more complicated. The hyperglycemic response to adrenaline is reduced by propranolol in most species.

Propranolol also increases the activity of the human uterus, more in the non-pregnant than in pregnant state. On the respiratory system, propranolol consistently increases airways resistances. This effect is small and of no clinical significance in normal individuals.

2.12.3 L-Nitro-Arginine methyl esther (L-NAME)

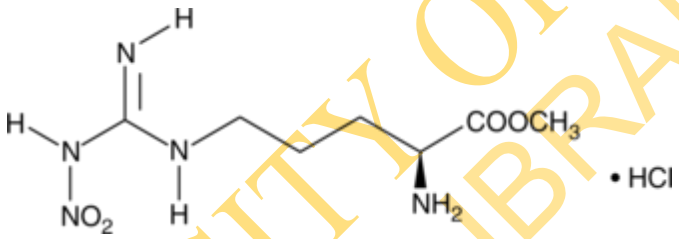


Figure 2-8: Structure of L-NAME (Griffith and Kilbourn, 1996)

Description

L-NAME requires hydrolysis of the methyl ester by cellular esterases to become a fully functional inhibitor, L-N-Nitro-Arginine (L-NNA) (Griffith and Kilbourn, 1996). L-NAME is metabolized to L-NNA in canine blood and plasma in vitro. L-NNA is therefore the active metabolite of L-NAME and the whole amount of LNA added to blood was detectable in blood after 4 hrs of incubation suggesting that L-NA undergoes no further metabolism (Krejcy *et al.*, 1992). L-NNA exhibits some selectivity for inhibition of neuronal and endothelial isoforms. It

exhibits K_i values of 15 nM, 39 nM, and 4.4 µM for nNOS (bovine), eNOS (human), and iNOS (murine), respectively (Buckner *et al.,* 1988). The reported K_i value for the inhibition of iNOS ranges from 4-65 µM.L-NAME inhibits cGMP formation in endothelial cells with an IC_{50} of 3.1 µM (in the presence of 30 µM arginine) and reverses the vasodilation effects of acetylcholine in rat aorta rings with an EC_{50} of 0.54 µM.

Pharmacological actions

The effects of the inhibitor of nitric oxide (NO) synthesis, NG-nitro-L-arginine methyl ester (L-NAME), on systemic arterial blood pressure and jejunal motility, blood flow, and oxygen uptake have been reported in anaesthetized dogs. L-NAME (cumulative doses of 0.1-20 mg /kg, i.v.) dose-dependently increased blood pressure, vascular resistance and jejunal motility and decreased jejunal blood flow. Thus, Endogenous nitric oxide may play a role in regulating motility and blood flow in the resting canine jejunum (Alemayehu *et al.,* 1994). L-NAME has also been reported to increase vascularpressures and cardiac output and decreased blood flow tobrain (by 18%), heart (by 36%), kidney (by 46%), and intestine (by 52%) (Charles *et al.,* 1998).

CHAPTER THREE

3.0 MATERIALS AND METHODS

The following materials were used for the study:

(a) **Animals:** Male *Mongrel* dogs weighing between 9-16kg were used for the study.

(b) **Drugs:**

(i) D-glucose (BDH Chemicals Limited, Poole, England).

(ii) D-fructose (BDH Chemicals Limited, Poole, England)

(iii) D-galactose (BDH Chemicals Limited, Poole, England)

(iv) Sodium Heparin (Panpharma S.A., France)

(v) Sodium pentobarbitone (Sagatal). (May and Baker Ltd U.K).

(vi) Propranolol Hydrochloride (Inderal). (Zeneca Ltd, UK).

(vii) Prazosin hydrochloride (Minipress). (Pfizer Inc., New York).

(viii) L-Nitro Arginine Methyl Esther (L-NAME) (Sigma, U.S.A)

(ix) Infusion pump (Palmer, England)

(x) Physiographic two channel recorder (Gemini Model 7070, Ugo Basil, Italy)

3.1: Surgical procedure

Each animal was fasted for 18-24 hours before the start of the experiment. Anaesthesia was induced by an intravenous injection of 30mg/kg – body weight of sodium pentobarbitone. Light anaesthesia was maintained with supplementary doses of sodium pentobarbitone as necessary. The animal was laid supine and firmly secured on the dissecting table. The trachea was intubated with a trachea endoscope and the animal was allowed to breathe room air spontaneously. The right and left femoral arteries and veins were exposed, incisions were made

and cannulae were inserted into them. The cannula placed in the left femoral vein was connected to the infusion pump (Palmer, England) for infusion of the hexoses while that placed in the left femoral artery was connected to the Physiographic two channel recorder (Gemini Model 7070, Ugo Basil, Italy) to monitor blood pressure continuously. Arterial blood samples (0.05ml) were collected from the right femoral artery while the right femoral vein was used for administration of the drugs or normal saline.

Through a midline laparatomy, the jejunum was identified and a vein draining the proximal segment of the jejunum was cannulated. At the end of the surgical procedure, sodium heparin 300 i.u was administered intravenously to prevent blood clotting. The abdomen was then closed in two layers with interrupted sutures. After all surgical procedures were completed, a 60-90 minutes stabilization period was observed.

3.2- Experimental Design of the study:

A total of ninety eight (98) male *Mongrel* dogs weighing 9-16kg were used for the study. The dogs were divided into eight (8) groups and treated as discussed below.

3.2.1 Control group

Five dogs were used. Blood samples were obtained from the femoral artery and jejunal vein for the measurement of basal arterial and venous glucose concentrations. Basal blood pressure and jejunal blood flow were also taken. An intravenous infusion of normal saline (0.01ml/kg/min) was then given through the femoral vein for 20 minutes. Measurements of blood pressure, jejunal blood flow, arterial and venous glucose concentrations were then carried out at 5min, 10min, 15min, 20min, 25min, 30min, 45min, 60min, 75min, and 90min post infusion.

3.2.2 Glucose group

Fifteen dogs were used. The dogs were divided into three subgroups of five dogs each. Blood samples were obtained from the femoral artery and jejunal vein for the measurement of basal arterial and venous glucose concentrations. Basal blood pressure and jejunal blood flow were also taken. An intravenous infusion of glucose (0.15-, 0.55- or 1.1 mg/kg/min) was then given through the femoral vein for 20 minutes. Measurements of blood pressure, jejunal blood flow, arterial and venous glucose concentrations were then carried out at 5min, 10min, 15min, 20min, 25min, 30min, 45min, 60min, 75min, and 90min post infusion.

3.2.3 Fructose group

Fifteen dogs were used. The dogs were divided into three subgroups of five dogs each. Blood samples were obtained from the femoral artery and jejunal vein for the measurement of basal arterial and venous glucose concentrations. Basal blood pressure and jejunal blood flow were also taken. An intravenous infusion of fructose (0.15-, 0.55- or 1.1 mg/kg/min) was then given through the femoral vein for 20 minutes. Measurements of blood pressure, jejunal blood flow, arterial and venous glucose concentrations were then carried out at 5min, 10min, 15min, 20min, 25min, 30min, 45min, 60min, 75min, and 90min post infusion.

3.2.4 Galactose group

Fifteen dogs were used. The dogs were divided into three subgroups of five dogs each. Blood samples were obtained from the femoral artery and jejunal vein for the measurement of basal arterial and venous glucose concentrations. Basal blood pressure and jejunal blood flow were also taken. An intravenous infusion of galactose (0.15-, 0.55- or 1.1 mg/kg/min) was then given through the femoral vein for 20 minutes. Measurements of blood pressure, jejunal blood

flow, arterial and venous glucose concentrations were then carried out at 5min, 10min, 15min, 20min, 25min, 30min, 45min, 60min, 75min, and 90min post infusion.

3.2.5 Pre-treatment with β-adrenoceptor blocker

Twelve dogs were used. The dogs were divided into three subgroups of four animals each.Each animal was first given i.v. injection of propranolol (0.5mg/kg). Thirty minutes after the injection, basal recordings were made and the effects of a 20-minute infusion of glucose (1.1 mg/kg/min), fructose (1.1 mg/kg/min) or galactose (1.1 mg/kg/min) on blood pressure, blood flow, and arterial and venous blood glucose levels were studied.

3.2.6 Pre-treatment with α-adrenoceptor blocker

Twelve dogs were used. The dogs were divided into three subgroups of four animals each.Each animal was first given i.v. injection of prazosin (0.2 mg/kg). Thirty minutes after the injection, basal recordings were made and the effects of a 20-minute infusion of glucose (1.1 mg/kg/min), fructose (1.1 mg/kg/min) or galactose (1.1 mg/kg/min) on blood pressure, blood flow, and arterial and venous blood glucose levels were studied.

3.2.7 Pre-treatment with combined α- and β- adrenoceptor blockers

Twelve dogs were used. The dogs were divided into three subgroups of four animals each.Each animal was first given i.v. injection of prazosin (0.2 mg/kg) and propranolol (0.5 mg/kg). Thirty minutes after the injection, basal recordings were made and the effects of a 20-minute infusion of glucose (1.1 mg/kg/min), fructose (1.1 mg/kg/min) or galactose (1.1 mg/kg/min) on blood pressure, blood flow, and arterial and venous blood glucose levels were studied.

3.2.8 Pre-treatment with Nitric oxide synthase blocker

Twelve dogs were used. The dogs were divided into three subgroups of four animals each.Each animal was first given i.v. injection of L-NAME (35 mg/kg). Thirty minutes after the injection, basal recordings were made and the effects of a 20-minute infusion of glucose (1.1 mg/kg/min), fructose (1.1 mg/kg/min) or galactose (1.1 mg/kg/min) on blood pressure, blood flow, and arterial and venous blood glucose levels were studied.

3.3: Experimental procedure

Blood pressure was recorded continuously throughout the duration of the experiment. After stabilization, basal measurements of jejunal venous blood flow, and glucose levels for arterial and venous blood glucose concentrations were made. After the basal measurements, the effects of a 20-minute intravenous infusion of nomal saline (0.01 ml/kg/min), glucose (0.15 mg/kg/min, 0.55 mg/kg/min or 1.1 mg/kg/min), fructose (0.15 mg/kg/min, 0.55 mg/kg/min or 1.1 mg/kg/min) or galactose (0.15 mg/kg/min, 0.55 mg/kg/min or 1.1 mg/kg/min) were studied.These measurements were repeated at 5 min,10 min,15 min,20 min,25 min,30 min,45 min,60 min,75 min, and 90 minutes post-infusion of the hexoses.

Arterial and venous blood samples for glucose estimation were obtained from the femoral and jejunal venous cannulae respectively. Jejunal blood flow was determined by timed collection of the effluent from the jejunal venous cannula as previously described (Alada and Oyebola, 1996). The vascular resistance of jejunal perfusion was calculated by dividing the mean arterial blood pressure by the corresponding jejunal blood flow. Blood glucose was determined by the glucose oxidase method. Arterio-venous glucose difference was calculated as the difference between arterial and venous blood glucose concentrations while glucose uptake (mg/min) was

calculated as the product of the arterio-venous glucose difference and jejunal blood flow per minute.

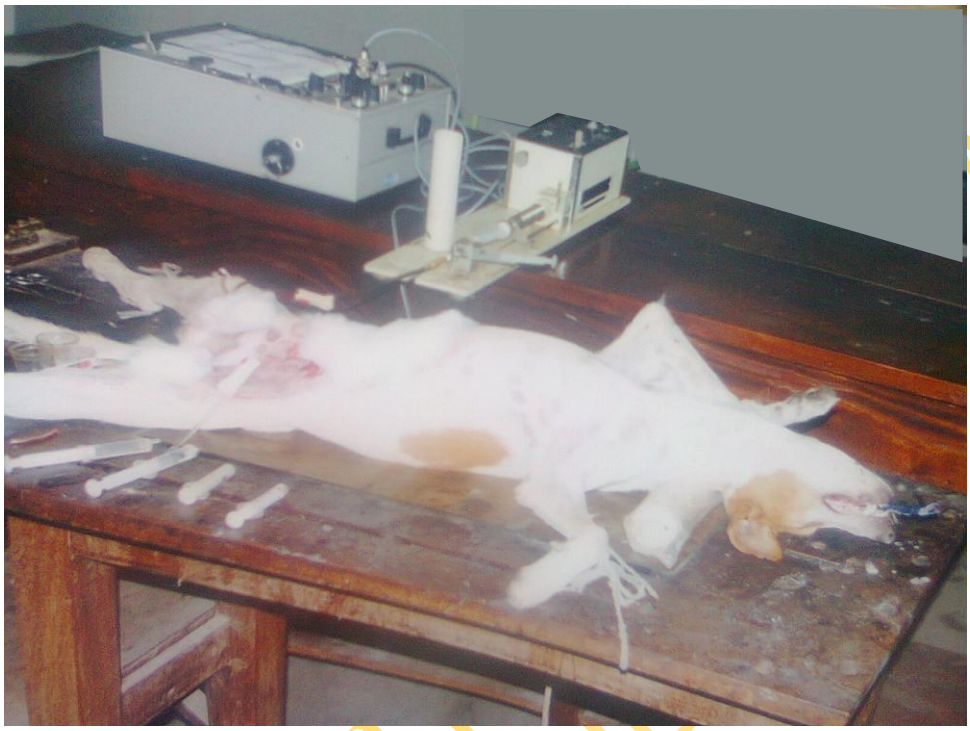


Plate 3-1: Experimental set-up showing the cannulae in situ, the 2-channel recorder (Ugobasil) and infusion pump in action.

3.4 Measurement of blood pressure and vascular resistance.

The arterial blood pressure was recorded continuously from cannulated right femoral artery with the pressure transducer placed at heart level. The pressure was recorded on a two-channel recorder (Gemini Model 7070 UgoBasil). Mean arterial blood pressure (MABP) was derived from the relation: MABP = DIASTOLIC PRESSURE + 1/3 PULSE PRESSURE (Ganong, 1987). Vascular resistance was calculated by dividing the blood pressure by blood flow.

3.5 Measurement of Blood Glucose

Each sample of blood collected was analyzed for blood glucose concentration using the modified glucose oxidase method (Trinder, 1967) using a glucometer. Result of blood glucose measurement using glucometer correlates excellently with the results obtained from standard laboratory methods (Ajala *et al.*, 2003; Devreese and Leroux-Roels, 1993). Apart from this, glucometers are easy to use and can measure blood glucose in very small blood samples. The glucometer used in this study is the One TOUCH BASIC-plus. The system consists of three main products; the ONE TOUCH BASIC- plus glucose meter, ONE TOUCH Test strips and ONE TOUCH Normal control solution. This product has been designed, tested and proven to produce accurate blood glucose test results (Tattersall, 1985; Life Scan File, 2000).

After calibration of the glucose meter, a test strip was removed from the vial and was inserted on the glucose meter. Then, blood sample was applied gently to the test spot (0.05ml), after 45seconds the blood test result was obtained in the meter display window.

- Principles of determination of blood glucose.

Glucose and oxygen react in the presence of glucose oxidase yielding glucoronic acid and hydrogen peroxide. Hydrogen peroxide subsequently oxidizes the dyes in a reaction mediated by peroxidase producing a blue colored form of the dyes. The intensity of this blue colour is proportional to the glucose concentration in the sample.

- Reagent Composition

Each cm^2 of test strips contains the following reactive ingredients in the approximate concentrations listed below:

Glucose oxidase ---------- 14IU

Peroxidase ---------- 11IU

3-Metyl 1-2-benzothiazolinone

Hydrazone hydrochloride ---------- 0.06mg

3-dimethylaminobenzoic acid ---------- 0.12mg

Each test strip vial cap contains up to3g of silica gel.

3.6 STATISTICAL ANALYSIS

The results were expressed as mean ± SEM. Statistical analysis of the obtained data was carried out using ANOVA and Students't-test. P values of less than 0.05 were taken as statistically significant.

CHAPTER

RESULTS

The results of all the experiments carried out are presented in tables 4-1 to 4-16 and figures 4-1 to 4-25. All the values given in the tables are mean ± SEM and asterisks in all the tables indicate values that are significantly different from control values of the variables measured.

4.1 GLUCOSE EXPERIMENTS.

4.1.1 Effects of glucose infusion on Mean arterial blood pressure (MABP), intestinal blood flow (IBF) and vascular resistance in dogs.

The infusion of 0.15 mg/kg/min, 0.55 mg/kg/min and 1.1 mg/kg/min of glucose had no effect on mean arterial blood pressure in dogs. However, the three doses of glucose caused significant increases in blood flow to the intestine (figure 4-1). Blood flow increased from a basal value of 10.80 ± 0.37 ml/min to 12.60 ± 0.24 ml/min (17% increase); from 10.80 ± 0.37 ml/min to 13.60 ± 1.12 ml/min (26% increase) and from 10.00 ± 0.32 ml/min to 14.40 ± 0.93 ml/min (44% increase) during the infusion of 0.15 mg/kg/min, 0.55 mg/kg/min and 1.1 mg/kg/min of glucose respectively. The increases in blood flow lasted for 20 minutes after which blood flow returned to the basal level.

The three doses of glucose also caused significant decreases in vascular resistance (figure 4-1). Vascular resistance decreased from a basal value of 8.02 ± 0.49 R.U to 6.19 ± 0.52 R.U (23% reduction); from 8.33 ± 0.32 R.U to 7.04 ±0.27 R.U (15% reduction) and from 8.57 ± 0.24 R.U to 6.19 ± 0.68 R.U (28% reduction) during the infusion of the three doses of glucose respectively. The decreases lasted for about 30minutes after which the vascular resistance returned to basal values.

4.1.2 Effects of glucose infusion on blood glucose, (A-V) glucose difference and intestinal glucose uptake (IGU) in dogs.

The effects of the intravenous infusion of different doses (0.15 mg/kg/min, 0.55 mg/kg/min and 1.1mg/kg/min) of glucose on blood glucose levels are shown in table 4-1. All the doses of glucose produced immediate increases in both arterial and venous blood glucose levels. These increases were dose-dependent. At the peak of the response, glucose infusion (0.15 mg/kg/min) increased arterial blood glucose from a basal level of 94.20 ± 1.07 mg/dl to 117.60 ± 3.06 mg/dl (about 23% increase) twenty minutes into the infusion period. Arterial blood glucose also rose from 99.80 ± 2.85 mg/dl to 125.00 ± 1.70 mg/dl (25% increase) following infusion of 0.55 mg/kg/min of glucose. The highest increase in arterial blood glucose was recorded during the infusion of 1.1 mg/kg/min of glucose as blood glucose rose from a basal level of 99.40 ± 0.87 mg/dl to 141.20 ± 5.65 mg/dl (42 % increase). However, while blood glucose returned to basal values after one hour post-infusion of 0.15 mg/kg/min and 0.55mg/kg/min, the increase in blood glucose was sustained throughout the experiment at 1.1mg/kg/min. Similarly, there were significant increases in venous blood glucose levels. Venous blood glucose levels increased from 89.40 ± 1.91mg/dl to 110.60 ± 3.61mg/dl (24% increase); 96.20 ± 2.87 mg/dl to 117.40± 2.09 mg/dl (22% increase) and from 95.20 ± 1.02 mg/dl to 118.80 ±4.75 mg/dl (25% increase) following the infusion of 0.15 mg/kg/min, 0.55 mg/kg/min and 1.1 mg/kg/min respectively. The venous blood glucose levels were however lower than the arterial blood glucose levels throughout the experiment.

The different doses of glucose also produced significant increases in (A-V) glucose (figure 4-2). While the doses of 0.15 /kg/min and 0.55mg/kg/min produced slight but significant increases in (A-V) glucose, 1.1mg/kg/min produced a more significant effect. (A-V) glucose

increased from a basal level of 3.80 ±0.37 mg/dl to 5.80 ± 0.58 mg/dl (53% increase) at 0.15 mg/kg/min of glucose. It also increased from 3.60 ± 0.40 mg/dl to 7.80 ± 0.37 mg/dl (117 % increase) and from 4.20 ± 0.37 mg/dl to 24.20 ±2.13 mg/dl (476 % increase) when glucose was infused at 0.55 mg/kg/min and 1.1 mg/kg/min respectively. The increases in (A-V) glucose were also immediate and sustained at the corresponding periods of the increases in the glucose levels.

The three doses of glucose used produced immediate and sustained increases in intestinal glucose uptake in a dose-dependent manner (figure 4-2). Following infusion of glucose, intestinal glucose uptake increased from 41.40 ± 5.27 mg/min to 72.00 ± 8.35 mg/min (74% increase); 38.60 ± 3.68 mg/min to 107.20 ± 14.45 mg/min (164 % increase) and from 44.40 ± 4.25 mg/min to 342.20 ± 40.77 mg/min (670% increase) for 0.15 mg/kg/min, 0.55 mg/kg/min and 1.1 mg/kg/min of glucose respectively.

Table 4-1: Effects of intravenous infusion of glucose (0.15 mg/kg/min,0.55 mg/kg/min and 1.1 mg/kg/min) on arterial and venous glucose concentrations in dogs.Values are expressed as mean ± SEM (N=5), (*P<0.05, **P<0.01)

	Dose	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Arterial blood glucose	0.15 mg/kg/min	94.20	107.00	114.20	115.80	113.40	116.40	117.60	111.00	96.40	92.20	92.80
		±1.06	±1.58*	±2.92**	±3.34**	±3.11**	±2.87**	±3.06**	±5.19	±1.69	±1.43	±1.46
	0.55 mg/kg/min	99.80	109.40	117.60	121.40	122.60	124.00	125.00	119.80	104.60	104.80	101.00
		±2.85	±1.47**	±1.17**	±0.69**	±1.29**	±2.70**	±1.70**	±3.01**	±2.86**	±2.63	±2.45
	1.1 mg/kg/min	99.40	115.80	119.60	126.00	131.80	134.40	141.20	131.60	132.40	119.80	113.20
		±0.87	±1.32**	±2.42**	±1.70**	±4.79***	±5.85**	±5.65***	±7.37**	±6.42**	±1.71**	±1.85**
Venous blood glucose	0.15 mg/kg/min	89.40	100.80	108.00	109.40	106.80	109.80	110.60	105.00	91.40	87.60	88.40
		±1.91	±0.58**	±3.11**	±3.17**	±3.34*	3.21**	±3.61**	±5.41	±1.29	±1.72	±0.87
	0.55 mg/kg/min	96.20	104.60	111.60	115.00	115.80	116.40	117.40	114.20	99.60	99.60	95.80
		±2.87	±1.99*	±1.50**	±0.84**	±1.24**	±2.82*	±2.09*	±2.99**	±2.64	±2.73	±2.27
	1.1 mg/kg/min	95.20	101.20	101.40	102.00	107.60	112.20	118.40	113.20	116.40	105.80	99.00
		±1.02	±1.53*	±2.89*	±2.19*	±3.50*	±4.05*	±4.75*	±4.31*	±5.24*	±1.39*	±1.97*

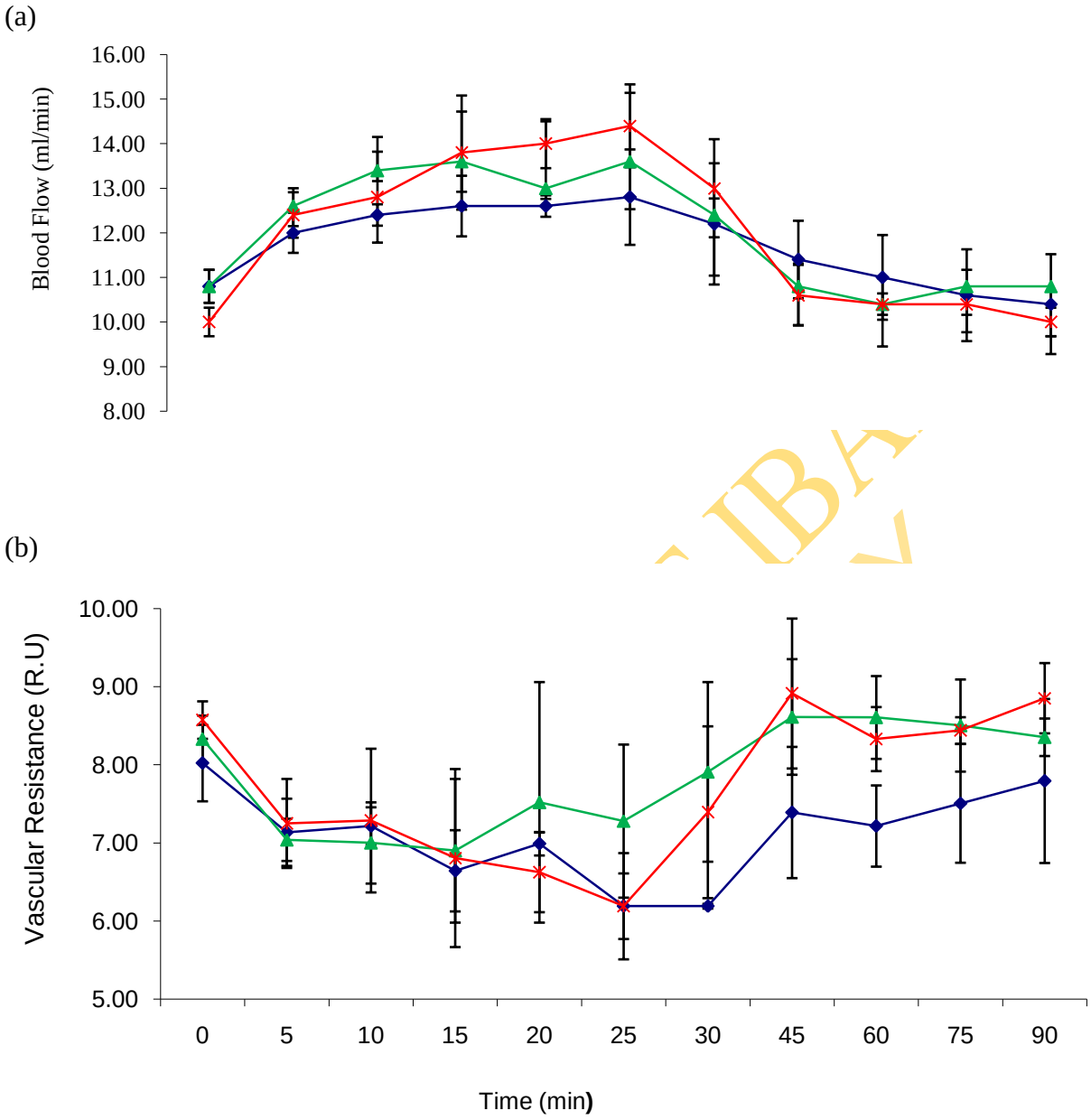


Figure 4-1: Effects of intravenous infusion of glucose (0.15 (♦-♦),0.55 (▲-▲) 1.1 (×-×) mg/kg/min) on blood flow (a) and vascular resistance (b) in dogs.

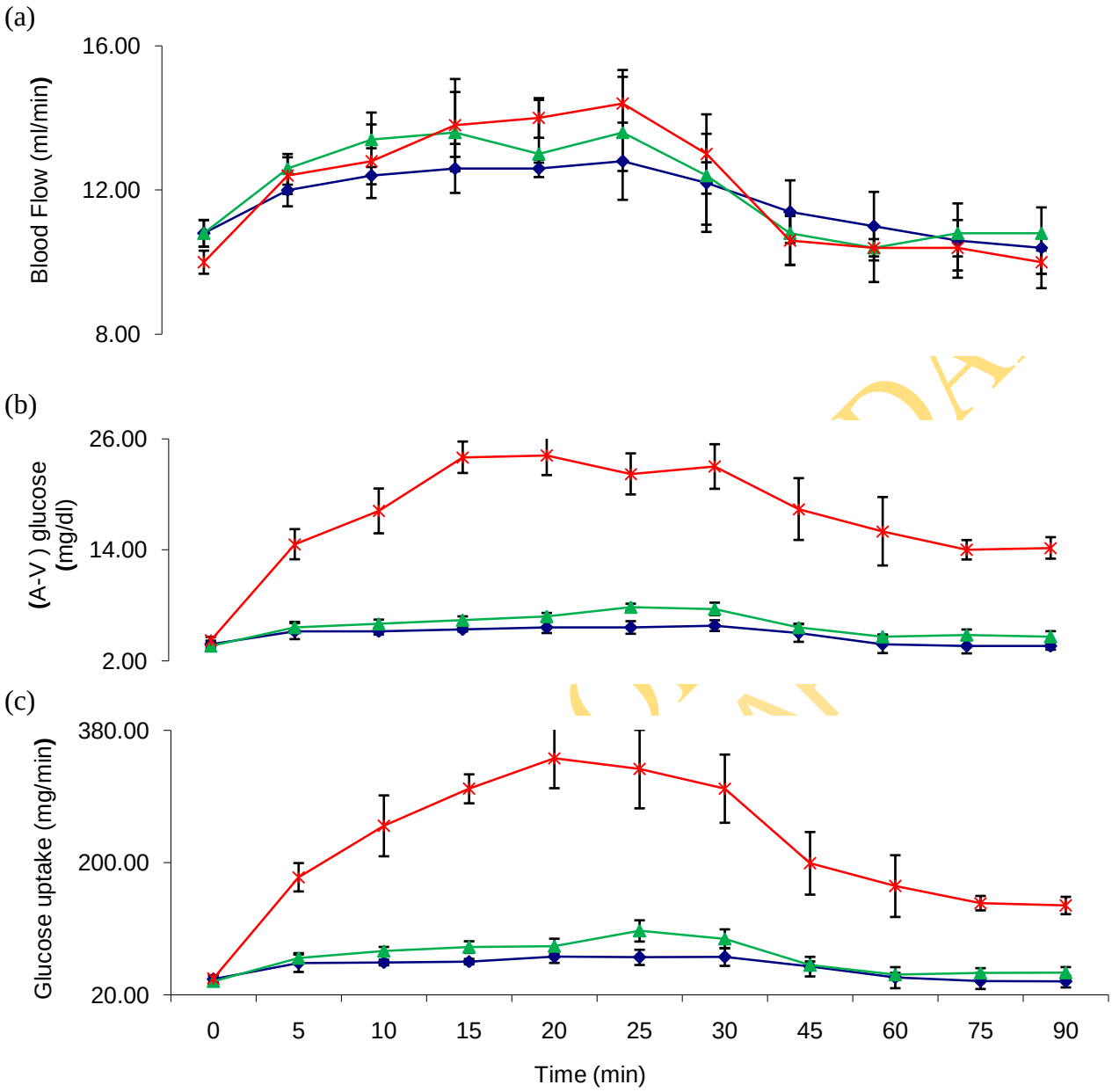


Figure 4-2: Effects of intravenous infusion of glucose (0.15 (◆-◆), 0.55 (▲-▲) 1.1 (×- ×) mg/kg/min) on blood flow (a), (A-V) glucose (b) and glucose uptake (c).

4.1.2.1 Effects of glucose infusion on Mean Arterial blood pressure, blood flow and vascular resistance in dogs pre-treated with adrenergic receptor blockers

The effects of propranolol on mean arterial blood pressure and blood flow during glucose infusion are shown in figure 4-3. Propranolol significantly decreased basal mean arterial blood pressure from 88.44 ± 0.29 mmHg to 83.42 ± 1.62 mmHg. Glucose however did not have any significant effect on blood pressure in propranolol-treated dogs. Propranolol also decreased basal blood flow from 10.00 ± 0.32 ml/min in the untreated dogs to 8.50 ± 0.28 ml/min in the dogs pretreated with propranolol. However, there was no significant change in vascular resistance after pre-treatment with propranolol.

The effects of pre-treatment with prazosin on mean arterial blood pressure, blood flow and vascular resistance during glucose infusion are shown in figure 4-5. Prazosin significantly decreased the basal mean arterial blood pressure from 88.44 ± 0.29 mmHg in the untreated dogs to 70.42 ± 3.46 mmHg (20% reduction) in prazosin-treated dogs. Prazosin also caused a transient decrease in blood flow at 5 mins. Moreover, there was a decrease in vascular resistance at the 20^{th} minute of glucose infusion. Vascular resistance decreased from a basal value of 7.53 ± 0.65 R.U to 4.69 ± 0.53 R.U at the 20^{th} minute. Thereafter, vascular resistance decreased again from the 45^{th} minute of the observation period to the end of the experiment.

The effects of pre-treatment with prazosin and propranolol on blood pressure, blood flow and vascular resistance are shown in figures 4-7. Pre-treatment with the combination of both prazosin and propranolol significantly decreased basal mean arterial blood pressure from 88.44 ± 0.29mmHg in the untreated dogs to 80.17 ± 1.3mmHg (9% reduction) in the dogs pre-treated with prazosin and propranolol. Basal blood flow also decreased from 10.00 ± 0.32ml/min in untreated dogs to 8.75 ± 0.48ml/min (13 % decrease) in dogs pre-treated with prazosin and

propranolol. Blood flow also decreased further from the basal value of 8.75 ± 0.48ml/min to 6.50 ± 0.65mins (about 26% decrease) at 20mins during glucose infusion after pre-treatment with prazosin and propranolol. The decrease in blood flow lasted for about 30mins after which it returned to the basal level. There was no change in basal vascular resistance after pre-treatment with prazosin and propranolol. However, there was an increase in vascular resistance in the post-infusion period (i.e. between 20^{th} and 30^{th} min) after which vascular resistance returned to the basal level. Vascular resistance increased from the basal value of 9.25 ± 0.54 R.U to 12.84 ± 1.15 R.U (about 39% increase) at 20mins.

4.1.2.2 Effects of glucose infusion on blood glucose, (A-V) glucose and intestinal glucose uptake in dogs pre-treated with adrenergic receptor blockers

Pre-treatment of the dogs with propranolol followed by glucose infusion produced significant decreases in both arterial and venous blood glucose (Table 4-2). Pretreatment with propranolol also reduced significantly (A-V) glucose in response to glucose infusion. For instance, pretreatment with propranolol caused about 400% reduction in glucose-induced increase in (A-V) glucose. Pre-treating the dogs with propranolol also significantly reduced the glucose-induced increase in intestinal glucose uptake. The increase in intestinal glucose uptake decreased from 670% for untreated dogs to 200% for propranolol-treated dogs (figure 4-4).

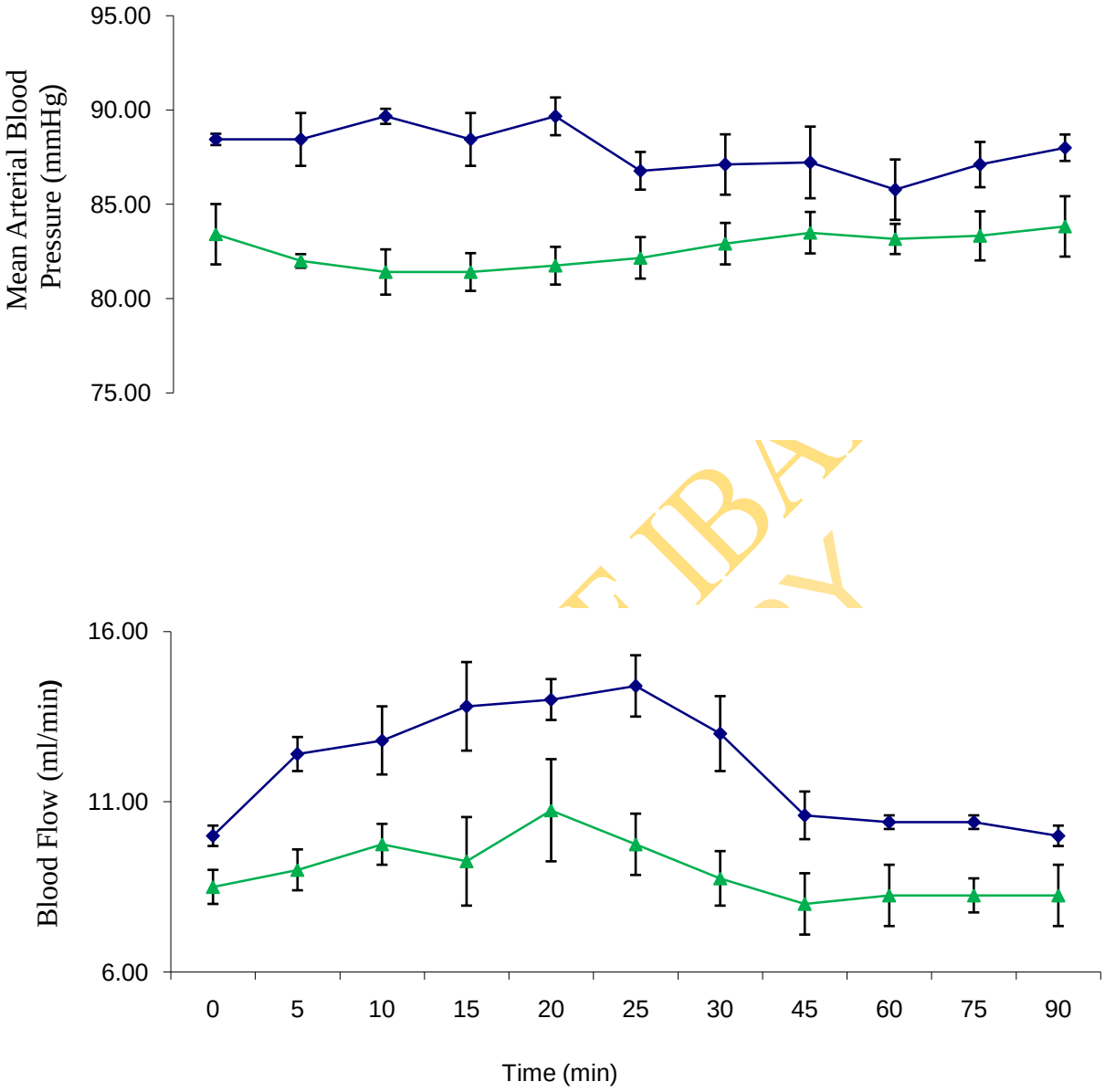
Although, pre-treatment of the dogs with prazosin did not abolish glucose-induced increases in blood glucose, it caused significant decreases in arterial and venous blood glucose (table 4-3). However, α-adrenergic pre-treatment had no significant effect on (A-V) glucose and glucose uptake (figure 4-6).

Pretreatment with the combination of propranolol and prazosin also caused significant decreases in arterial and venous blood glucose levels (table 4-4), (A-V) glucose and intestinal glucose uptake (figure 4-8).

Table 4-2: Effects of intravenous infusion of glucose (Glu) (1.1 mg/kg/min) on arterial and venous glucose Concentrations (mg/dl) before and after pre-treatment with propranolol (Pro) (0.5 mg/kg). (*p<0.05)

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Glu	Arterial blood glucose	99.40 ±0.87	115.80 ±1.32	119.60 ±2.42	126.00 ±1.70	131.80 ±4.79	134.40 ±5.85	141.20 ±5.65	131.60 ±7.37	132.40 ±6.42	119.80 ±1.71	113.20 ±1.85
Pro+Glu		97.50 ±3.23	103.50 ±2.53*	109.25 ±1.49*	114.00 ±2.16*	119.00 ±4.04	117.50 ±4.33	109.25 ±5.65*	106.75 ±4.77*	102.25 ±3.22*	98.50 ±1.55*	95.50 ±1.66*
Glu	Venous blood glucose	95.20 ±1.02	101.20 ±1.53	101.40 ±2.89	102.00 ±2.19	107.60 ±3.50	112.20 ±4.05	118.40 ±4.75	113.20 ±4.31	116.40 ±5.24	105.80 ±1.39	99.00 ±1.97
Pro+Glu		93.75 ±3.57	97.50 ±1.94	100.00 ±1.08	104.50 ±1.55	108.00 ±3.46	109.75 ±5.07	100.00 ±4.38*	99.50 ±2.96*	96.25 ±2.17*	92.25 ±1.11*	89.75 ±1.49*

(a)



(b)

Figure 4-3: Effects of intravenous infusion of glucose on mean arterial blood pressure (a) and Blood Flow (b) in untreated (♦-♦) and dogs pretreated with propranolol (▲-▲).

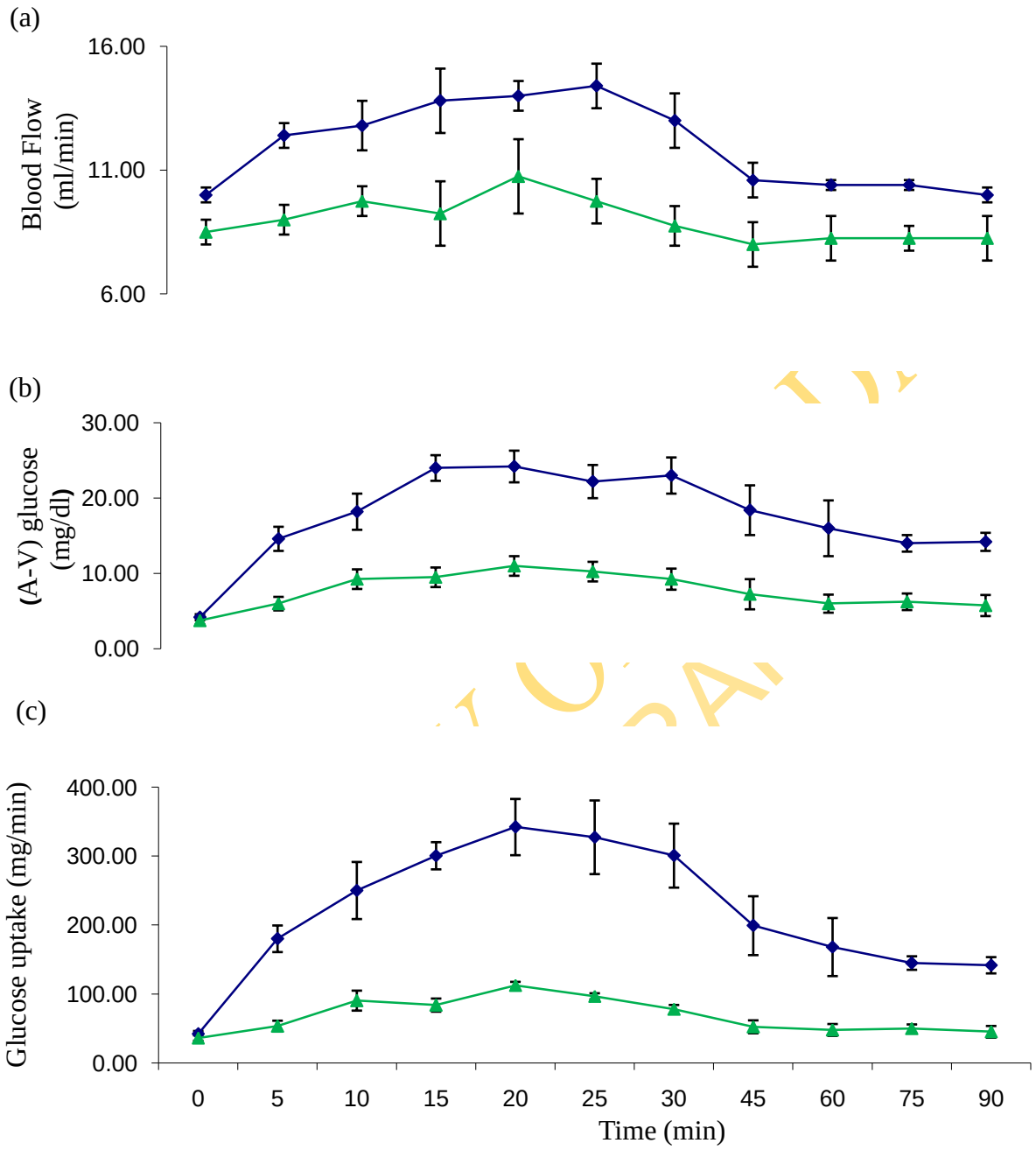
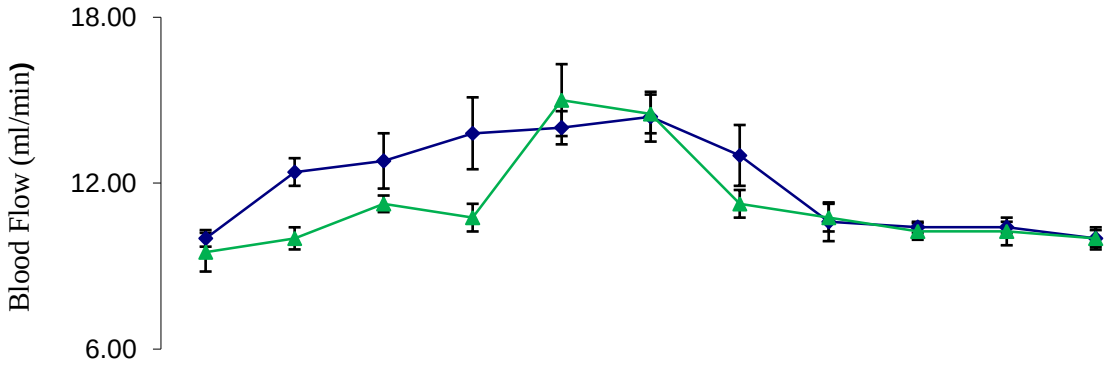


Figure 4-4: Effects of intravenous infusion of glucose on blood flow (a), (A-V) glucose (b) and glucose uptake uptake (c) in untreated (◆-◆) and dogs pre-treated with propranolol (▲-▲).

Table 4-3: Effects of intravenous infusion of glucose (Glu) (1.1 mg/kg/min) on arterial and venous glucose concentrations (mg/dl) before and After pre-treatment with prazosin (pra) (0.2 mg/kg). (*p<0.05).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Glu	Arterial blood glucose	99.40 ±0.87	115.80 ±1.32	119.60 ±2.42	126.00 ±1.70	131.80 ±4.79	134.40 ±5.85	141.20 ±5.65	131.60 ±7.37	132.40 ±6.42	119.80 ±1.71	113.20 ±1.85
Pra+Glu		97.25 ±1.11	104.25 ±2.17*	110.00 ±1.41*	114.25 ±2.17*	122.50 ±2.63	122.50 ±1.04	119.50 ±0.50*	116.25 ±1.03	114.25 ±1.49*	105.25 ±1.89*	101.75 ±1.49*
Glu	Venous blood glucose	95.20 ±1.02	101.20 ±1.53	101.40 ±2.89	102.00 ±2.19	107.60 ±3.50	112.20 ±4.05	118.40 ±4.75	113.20 ±4.31	116.40 ±5.24	105.80 ±1.39	99.00 ±1.97
Pra+Glu		93.25 ±0.75	94.50 ±2.02	97.25 ±0.95	98.00 ±1.58	100.25 ±4.13	107.00 ±1.47	105.50 ±0.65*	104.25 ±1.31	103.50 ±1.71*	96.25 ±1.93*	93.00 ±1.91

(a)



(b)

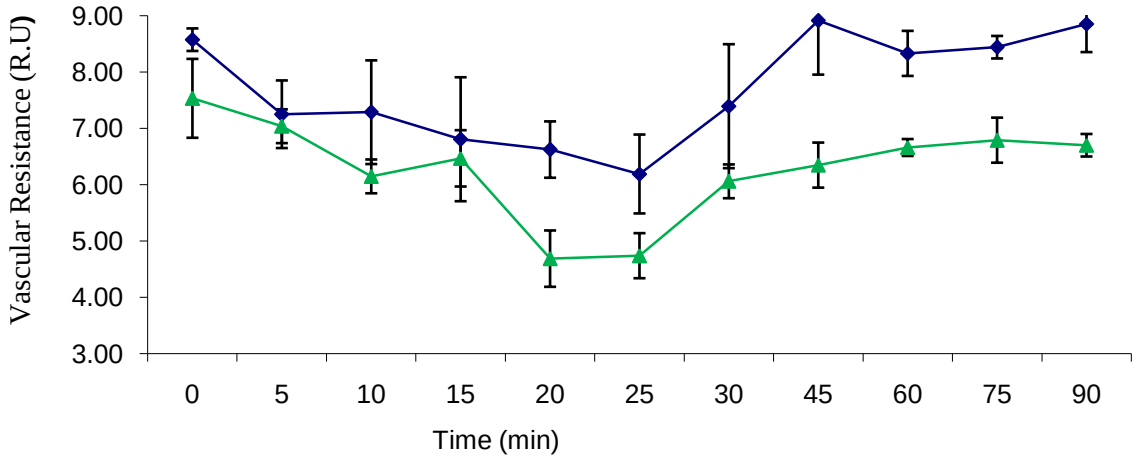


Figure 4-5: Effects of intravenous infusion of glucose (1.1 mg/kg/min) on blood flow (a) and vascular resistance (b) in untreated (◆-◆) and dogs pretreated with Prazosin **(▲-▲).**

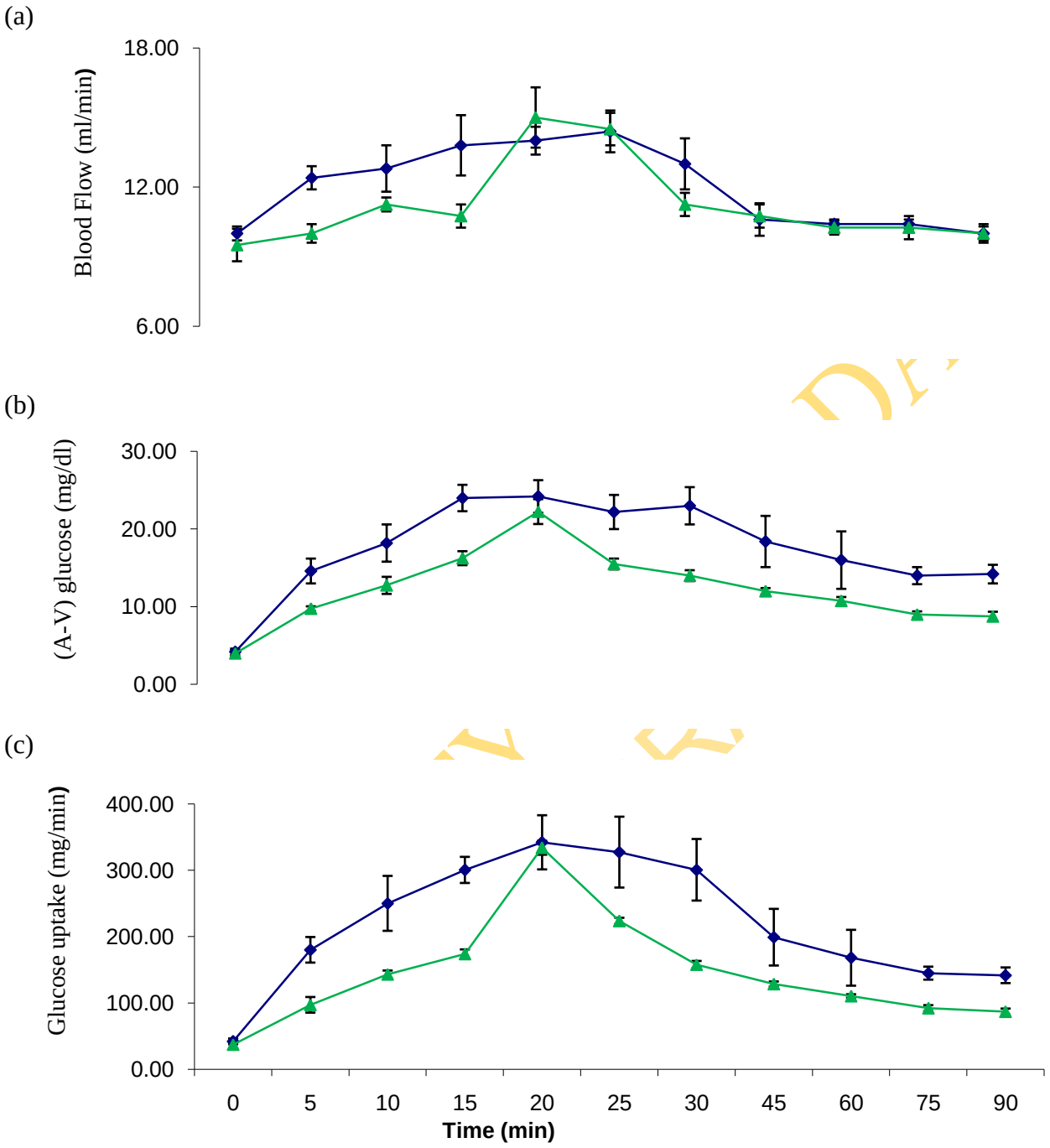
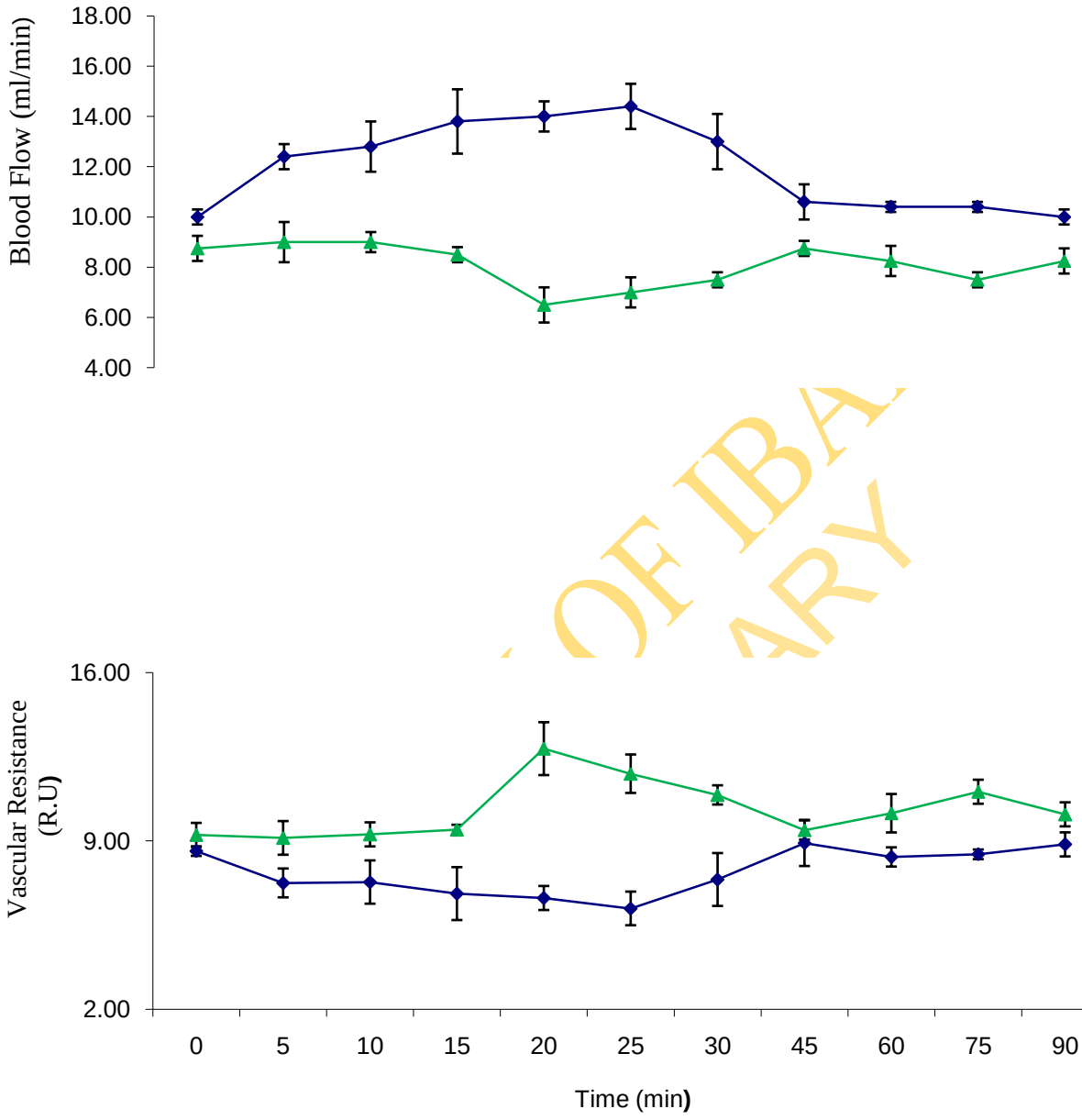


Figure 4-6: Effects of intravenous infusion of glucose (1.1 mg/kg/min) on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated (◆-◆) and dogs pretreated with Prazosin (▲-▲).

Table 4-4: Effects of intravenous infusion of glucose (Glu) (1.1mg/kg/min) on arterial and venous glucose concentrations(mg/dl) before and after pretreatment with propranolol (Pro) (0.5 mg/kg) and prazosin (pra) (0.2 mg/kg).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Glu	Arterial blood glucose	99.40 ±0.87	115.80 ±1.32	119.60 ±2.42	126.00 ±1.70	131.80 ±4.79	134.40 ±5.85	141.20 ±5.65	131.60 ±7.37	132.40 ±6.42	119.80 ±1.71	113.20 ±1.85
Pro+pra+Glu		95.50 ±2.10	101.75 ±2.69*	108.00 ±1.41*	112.75 ±1.60*	123.75 ±2.39	123.00 ±1.22	118.75 ±2.39	116.00 ±2.00	103.25 ±1.97*	99.00 ±0.58*	95.75 ±0.75*
Glu	Venous blood glucose	95.20 ±1.02	101.20 ±1.53	101.40 ±2.89	102.00 ±2.19	107.60 ±3.50	112.20 ±4.05	118.40 ±4.75	113.20 ±4.31	116.40 ±5.24	105.80 ±1.39	99.00 ±1.97
Pro+pra+Glu		91.50 ±2.33	94.25 ±3.20	98.75 ±1.97*	103.25 ±1.89*	112.50 ±3.07*	112.50 ±1.66*	111.00 ±1.96	109.25 ±2.10	98.25 ±2.02	93.00 ±0.71	90.75 ±0.48

(a)



(b)

Figure 4-7: Effect of intravenous infusion of glucose on blood flow (a) and vascular resistance (b) in untreated (◆-◆) and dogs pretreated with propranolol and Prazosin (▲-▲).

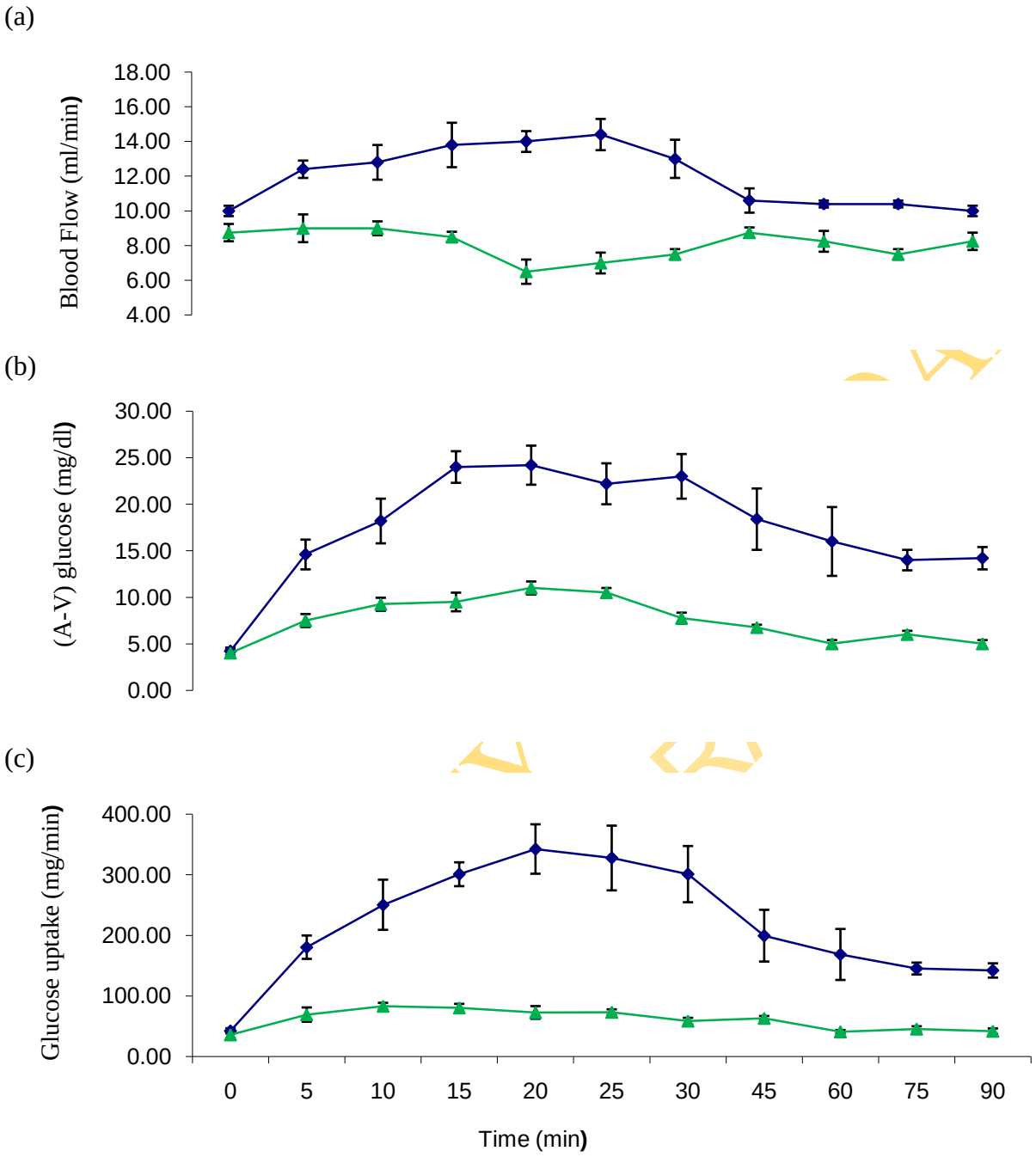


Figure 4-8:Effects of intravenous infusion of glucose on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated (◆-◆) and dogs pretreated with propranolol and Prazosin (▲-▲).

4.1.3 Effects of L-NAME

4.1.3.1 Effects of glucose on Mean Arterial blood pressure, blood flow and vascular resistance in dogs pre-treated with L-NAME.

The effects of infusion of glucose on mean arterial blood pressure, blood flow and vascular resistance are shown in figure 4-9. After pretreatment with L-NAME, there was an increase in basal mean arterial blood pressure. Basal meanarterial blood pressure was 88.44 ± 0.29 mmHg in the untreated dogs while it was 95.67 ± 1.14 mmHg in dogs treated with L-NAME. The decrease was sustained throughout the 90mins observation period. There was also a significant decrease in both basal and glucose-stimulated increases in blood flow. While blood flow increased from a basal value of 10.00 ± 0.32ml/min to 14.40 ± 0.93ml/min in untreated dogs at 25 mins, blood flow decreased from a basal value of 6.75 ± 0.41ml/min to 3.50 ± 0.25ml/min (about 48% reduction) at 25 mins in L-NAME treated dogs. There was also a significant increase in the resting value of vascular resistance in L-NAME-treated dogs. Basal vascular resistance was 8.57 ± 0.24 R.U in untreated dogs while it was 14.39 ± 1.03 R.U in L-NAME-treated dogs. Vascular resistance increased further and attained a peak of 29.87 ± 1.67 R.U (108% increase) at 30mins. The increase was sustained throughout the 90mins observation period.

4.1.3.2 Effects of glucose infusion on Blood glucose, (A-V) glucose and intestinal glucose uptake in dogs pretreated with L-NAME.

The effects of glucose infusion on blood glucose, (A-V) glucose and intestinal glucose uptake after pre-treatment with L-NAME are shown in table 4-5 and figure 4-10. Pre-treatment with L-NAME caused significant decreases in both arterial and venous blood glucose levels. However, there was no change in the basal blood glucose levels before and after pretreatment

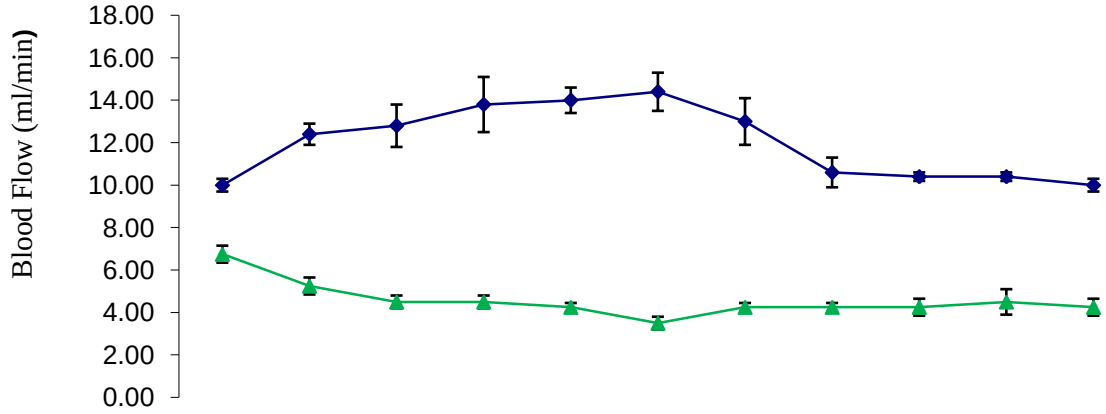
with L-NAME. Figure 4-10 also shows that there was no significant difference in (A-V) glucose before and after pretreatment with L-NAME. In other words, L-NAME did not affect the increase in (A-V) glucose produced by glucose infusion.

However, L-NAME caused a significant decrease in basal glucose uptake from 42.20 ± 4.25mg/min in the untreated dogs to 28.75± 3.82mg/min in the L-NAME-treated dogs. L-NAME also produced a significant decrease (about 450%) in glucose -induced increase in intestinal glucose uptake.

Table 4-5: Effects of intravenous infusion of glucose (Glu) (1.1 mg/kg/min) on arterial and venous glucose Concentrations (mg/dl) before and after pre-treatment with L-Nitro-Arginine-Methyl-Esther (L-NAME) (35 mg/kg). (*P<0.05).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Glu	Arterial blood glucose	99.40 ±0.87	115.80 ±1.32	119.60 ±2.42	126.00 ±1.70	131.80 ±4.79	134.40 ±5.85	141.20 ±5.65	131.60 ±7.37	132.40 ±6.42	119.80 ±1.71	113.20 ±1.85
L-NAME+Glu		98.00 ±3.63	107.25 ±3.82	108.75 ±2.69*	112.25 ±2.29*	121.00 ±5.45	116.50 ±1.19*	113.25 ±2.93*	114.50 ±1.94	110.50 ±4.11*	108.25 ±3.45*	105.50 ±4.73
Glu	Venous blood glucose	95.20 ±1.02	101.20 ±1.53	101.40 ±2.89	102.00 ±2.19	107.60 ±3.50	112.20 ±4.05	118.40 ±4.75	113.20 ±4.31	116.40 ±5.24	105.80 ±1.39	99.00 ±1.97
L-NAME+Glu		94.00 ±3.92	90.00 ±3.51*	89.75 ±2.84*	91.75 ±2.06*	100.75 ±5.22	95.50 ±1.50*	93.25 ±2.72*	96.75 ±2.21*	94.25 ±4.33*	95.00 ±3.49*	93.25 ±2.87

(a)



(b)

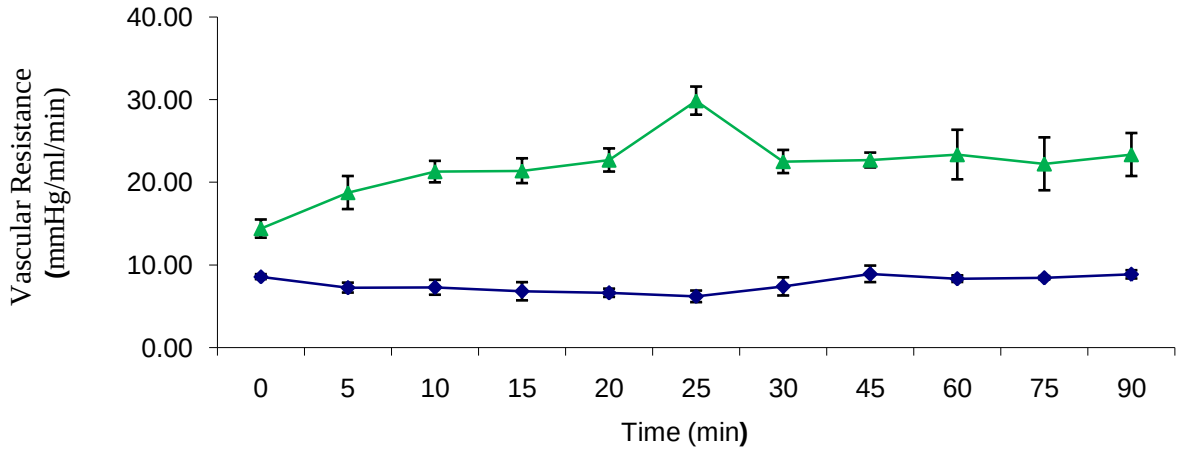


Figure 4-9: Effects of intravenous infusion of glucose on blood flow (a) and vascular resistance (b) in untreated (◆-◆) and dogs pre-treated with L-NAME (▲-▲).

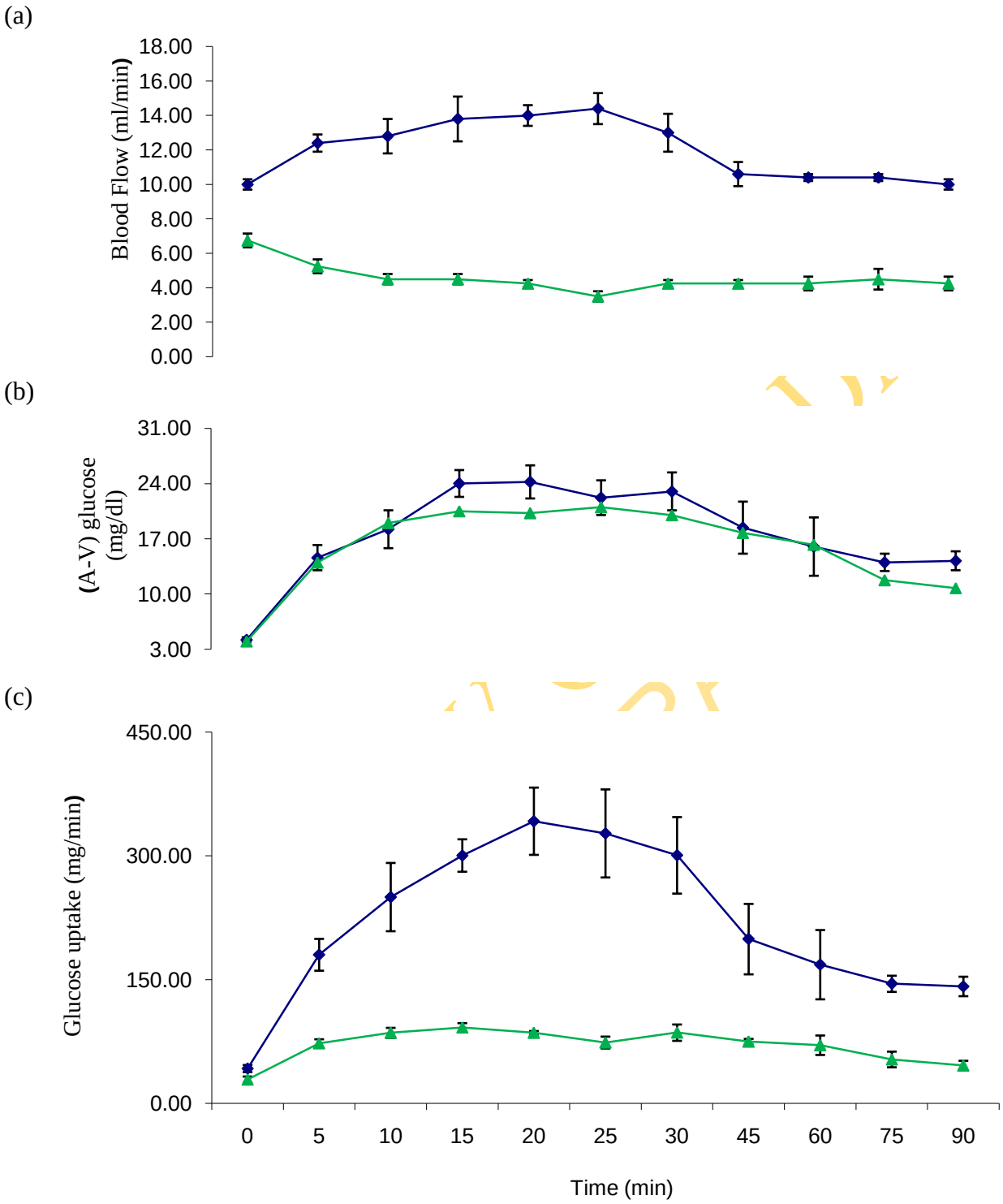


Figure 4-10: Effects of intravenous infusion of glucose on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated ( ) and dogs pretreated with L-NAME ( ).

4.2 FRUCTOSE EXPERIMENTS

4.2.1.1 Effects of Fructose infusion on Mean Arterial Blood Pressure, intestinal blood flow and vascular resistance in dogs.

The different doses of fructose had no effect on mean arterial blood pressure, blood flow and vascular resistance in dogs

4.2.1.2 Effects of Fructose on blood glucose, (A-V) glucose and intestinal glucose uptake (IGU)

While the lowest dose (0.15 mg/kg/min) of fructose did not have any effect on arterial blood glucose levels, higher doses (0.55 mg/kg/min) and 1.1 mg/kg/min) caused significant increases in arterial blood glucose levels. Also, there was no significant change in venous blood glucose levels in response to the lower doses (0.15 mg/kg/min and 0.55 mg/kg/min). However, at a dose of 1.1 mg/kg/min, fructose infusion produced a significant reduction in venous blood glucose levels when compared with the basal (table 4-6).

At higher doses of 0.55 mg/kg/min and 1.1 mg/kg/min, fructose produced significant increases in (A-V) glucose. For instance, at 0.55 mg/kg/min of fructose, (A-V) glucose increased from 4.00 ± 0.32 mg/dl to 15.80 ± 1.02 mg/dl while at a dose of 1.1 mg/kg/min, (A-V) glucose increased from 2.83 ± 0.60 mg/dl to 30.83 ± 1.83 mg/dl. The lowest dose (0.15 mg/kg/min) of fructose however did not produce any significant effect on (A-V) glucose.

The effects of the different doses of fructose on intestinal glucose uptake in dogs mimicked that of (A-V) glucose in this experiment. That is, at the doses of 0.55 mg/kg/min and 1.1 mg/kg/min, fructose infusion produced significant increases in intestinal glucose uptake. 0.55 mg/kg/min of fructose caused about 324% increase in glucose uptake while 1.1mg/kg/min

caused about 670% increase in intestinal glucose uptake. The lowest dose of fructose (0.15 mg/kg/min) however did not affect the intestinal glucose uptake.

Table 4-6: Effects of intravenous infusion of fructose (0.15 mg/kg/min, 0.55 mg/kg/min and 1.1 mg/kg/min) on arterial and venous glucose concentrations in dogs. Values are expressed as mean ± SEM (N=5), (*P<0.05, **P<0.01)

	Dose	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Arterial blood glucose	0.15 mg/kg/min	99.00 ±1.87	98.00 ±1.87	98.00 ±1.70	97.20 ±1.93	95.80 ±1.16	95.20 ±1.83	94.60 ±1.33	96.60 ±2.66	91.80 ±1.02*	89.20 ±2.63*	80.00 ±2.74*
	0.55 mg/kg/min	100.00 ±2.70	104.40 ±3.75	105.40 ±3.56	100.80 ±5.03	103.40 ±4.07	105.60 ±3.83	115.00 ±3.24*	103.80 ±4.02	108.80 ±2.42	113.80 ±2.08*	117.40 ±3.37*
	1.1 mg/kg/min	97.60 ±1.78	110.60 ±1.25**	112.00 ±2.72**	108.80 ±3.01*	114.20 ±1.88**	103.60 ±3.70	110.80 ±1.28	103.40 ±2.18	109.60 ±1.21	107.40 ±1.78	98.80 ±2.31
Venous blood glucose	0.15 mg/kg/min	93.80 ±2.20	93.80 ±2.27	93.20 ±2.01	92.20 ±2.01	90.80 ±1.98	89.60 ±2.40	88.40 ±2.04	89.00 ±2.85	89.60 ±1.36	85.60 ±2.80*	76.60 ±2.93*
	0.55 mg/kg/min	95.80 ±2.60	99.00 ±3.56	95.80 ±3.79	93.00 ±4.97	92.20 ±4.07	94.80 ±3.18	99.20 ±2.85	90.40 ±3.89	93.60 ±3.93	102.00 ±3.24	103.00 ±3.79
	1.1 mg/kg/min	94.20 ±2.06	88.80 ±1.36	89.80 ±4.65	90.60 ±1.12	101.40 ±1.60*	91.60 ±2.25	85.80 ±2.91*	91.40 ±0.93	89.80 ±0.86	85.40 ±1.21*	94.00 ±2.39

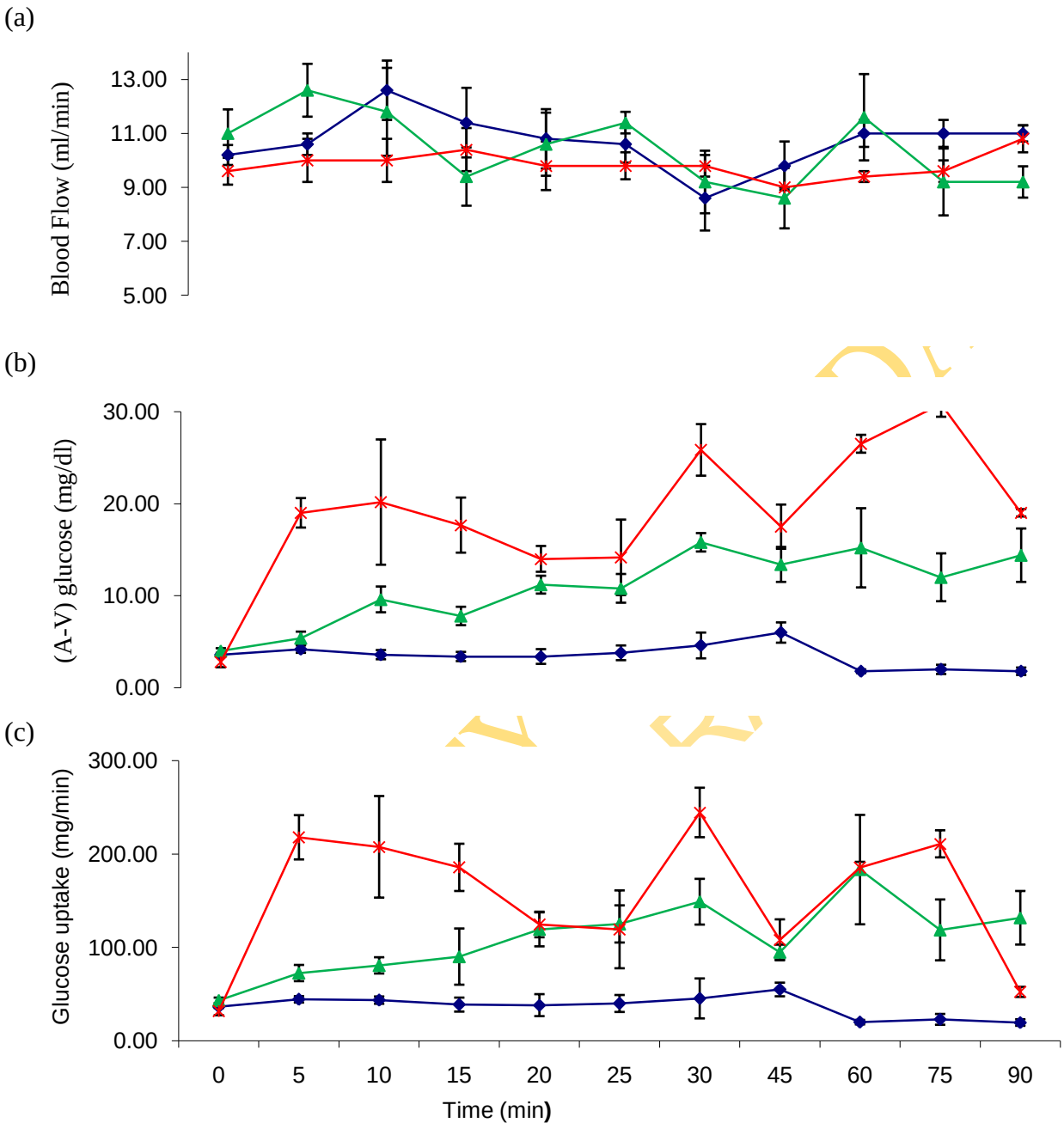


Figure 4-11: Effects of intravenous infusion of fructose (0.15- (◆-◆) ,0.55- (▲-▲) 1.1 (×-×) mg/kg/min) on blood flow (a) (A-V) glucose (b) and glucose uptake (c).

4.2.2 Effects of pretreatment with adrenergic receptor blockers

4.2.2.1 Effects of fructose infusion on mean arterial blood pressure, blood flow and vascular resistance in dogs pretreated with adrenergic receptor blockers

Propranolol decreased significantly the basal mean arterial blood pressure and blood flow. Basal mean arterial blood pressure decreased from 85.67 ± 0.67 mmHg in untreated dogs to 83.42 ± 1.62 mmHg. Basal blood flow also decreased from 9.60 ± 0.51 ml/min in the untreated dogs to 7.67 ± 0.33 ml/min in propranolol-treated dogs. It however had no effect on the basal vascular resistance. Administration of fructose however did not affect the three haemodynamic parameters in dogs pretreated with propranolol (figure 4-12).

The effects of pretreatment of the dogs with prazosin on mean arterial blood pressure, blood flow and vascular resistance followed by infusion of fructose are shown in figure 4-14. Pretreatment of the dogs with prazosin caused a significant reduction ($P<0.05$) in resting mean arterial blood pressure. Basal mean arterial blood pressure decreased from 85.67 ± 0.67 mmHg in untreated dogs to 79.33 ± 2.33 mmHg in prazosin-treated dogs. Prazosin treatment however had no effect on basal blood flow but caused significant reduction in blood flow during fructose infusion. However, there was no significant change in the vascular resistance before and after pretreatment with prazosin following fructose infusion.

The effects of pretreatment with the combination of propranolol and prazosin on mean arterial blood pressure, blood flow and vascular resistance are shown in figure 4-16. Pretreatment with both adrenergic receptor blockers caused significant decreases in the basal mean arterial blood pressure. Basal mean arterial blood pressure decreased from 85.67 ± 0.67 mmHg in untreated dogs to 79.02 ± 1.08 mmHg in the treated dogs. Basal blood flow also decreased from 9.60 ± 0.51 ml/min in untreated dogs to 7.77 ± 0.39 ml/min in the treated dogs.

There was no significant change in vascular resistance following pretreatment with the two adrenergic blockers

4.2.2.2 Effects of fructose on blood glucose, (A-V) glucose and intestinal glucose uptake in dogs pretreated with adrenergic receptor blockers

The effects of fructose on arterial and venous blood glucose levels in dogs pretreated with propranolol are shown in table 4-7. Propranolol significantly reduced fructose-induced increases in arterial blood glucose. However, propranolol had no significant effect on the venous blood glucose levels in response to fructose infusion. Figure 4-13 shows the effects of fructose infusion on (A-V) glucose and intestinal glucose uptake in dogs pretreated with propranolol. Propranolol abolished the increase in (A-V) glucose produced by fructose. Propranolol also abolished the fructose-induced increase in intestinal glucose uptake (IGU).

The effects of fructose infusion on arterial and venous glucose levels in dogs pretreated with prazosin are not shown in table 4-8. Prazosin significantly reduced the fructose – induced rise in arterial blood glucose levels. Prazosin however, did not affect the venous blood glucose levels following fructose infusion. When the (A-V) glucose was calculated as shown in 4-15b, prazosin had little or no effect on (A-V) glucose during fructose infusion. There was also no significant difference in the effect of fructose on intestinal glucose uptake before and after pretreatment with prazosin (figure 4-15c).

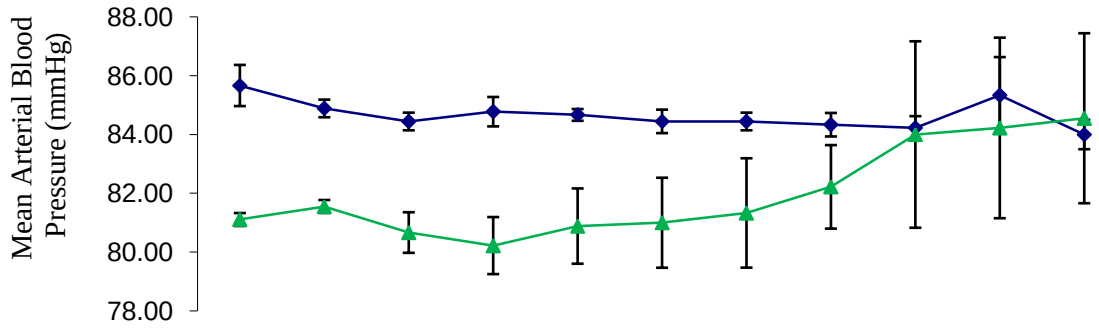
When the animals were pretreated with a mixture of propranolol and prazosin and then infused with fructose, there was no significant difference in the arterial blood glucose levels between the untreated and the pretreated groups (table 4-9). There was also no significant difference in the venous blood glucose levels between the untreated and the treated

animals.There was also no significant change in (A-V) glucose and intestinal glucose uptake in dogs pretreated with propranolol and prazosin (figure 4-17).

Table 4-7: Effects of intravenous infusion of fructose (Fru) (1.1mg/kg/min) on arterial and venous glucose concentrations (mg/dl) before and after pre-treatment with propranolol (Pro) (0.5 mg/kg). (*p<0.05).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Fru	Arterial blood glucose	97.60 ±1.78	110.60 ±1.25	112.00 ±2.72	108.80 ±3.01	114.20 ±1.88	103.60 ±3.70	110.80 ±1.28	103.40 ±2.18	109.60 ±1.21	107.40 ±1.78	98.80 ±2.31
Pro+Fru		95.25 ±2.32	97.75 ±3.57*	106.00 ±1.35	101.75 ±4.44	98.50 ±1.55*	97.25 ±1.49	97.00 ±0.71*	95.50 ±0.65*	90.00 ±3.54*	91.75 ±2.02*	94.50 ±0.65
Fru	Venous blood glucose	94.20 ±2.06	88.80 ±1.36	89.80 ±4.65	90.60 ±1.12	101.40 ±1.60	91.60 ±2.25	85.80 ±2.91	91.40 ±0.93	89.80 ±0.86	85.40 ±1.21	94.00 ±2.39
Pro+Fru		91.50 ±2.78	92.25 ±3.88	90.50 ±1.32	92.75 ±4.21	88.25 ±1.93*	87.25 ±1.25	87.00 ±0.71	87.00 ±0.41*	81.50 ±3.43*	82.75 ±2.36	90.25 ±0.85

(a)



(b)

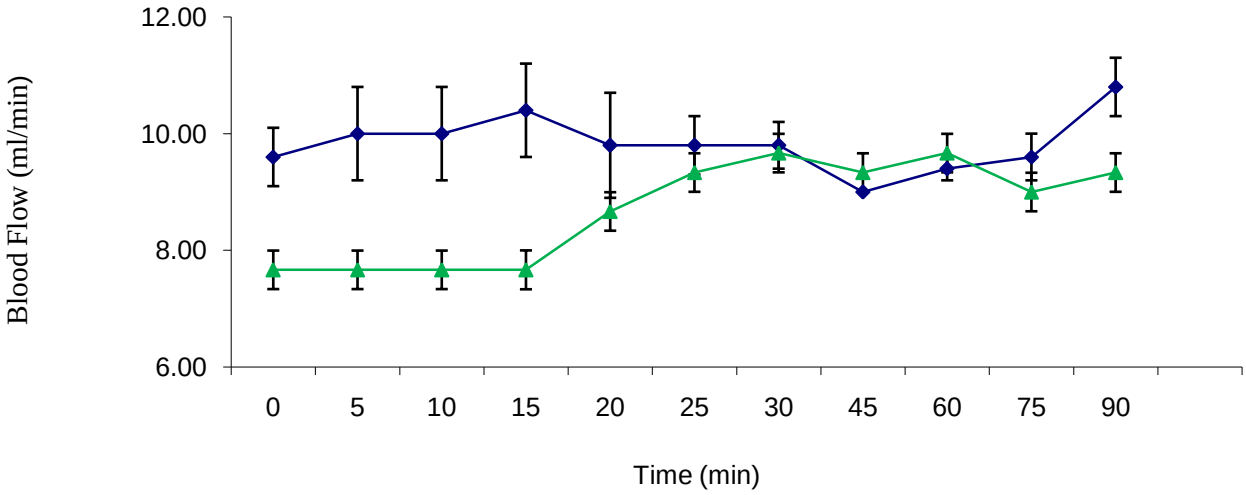


Figure 4-12: Effects of intravenous infusion of fructose on mean arterial blood pressure (a) and blood Flow (b) in untreated (◆-◆) and dogs pre-treated with propranolol (▲-▲) .

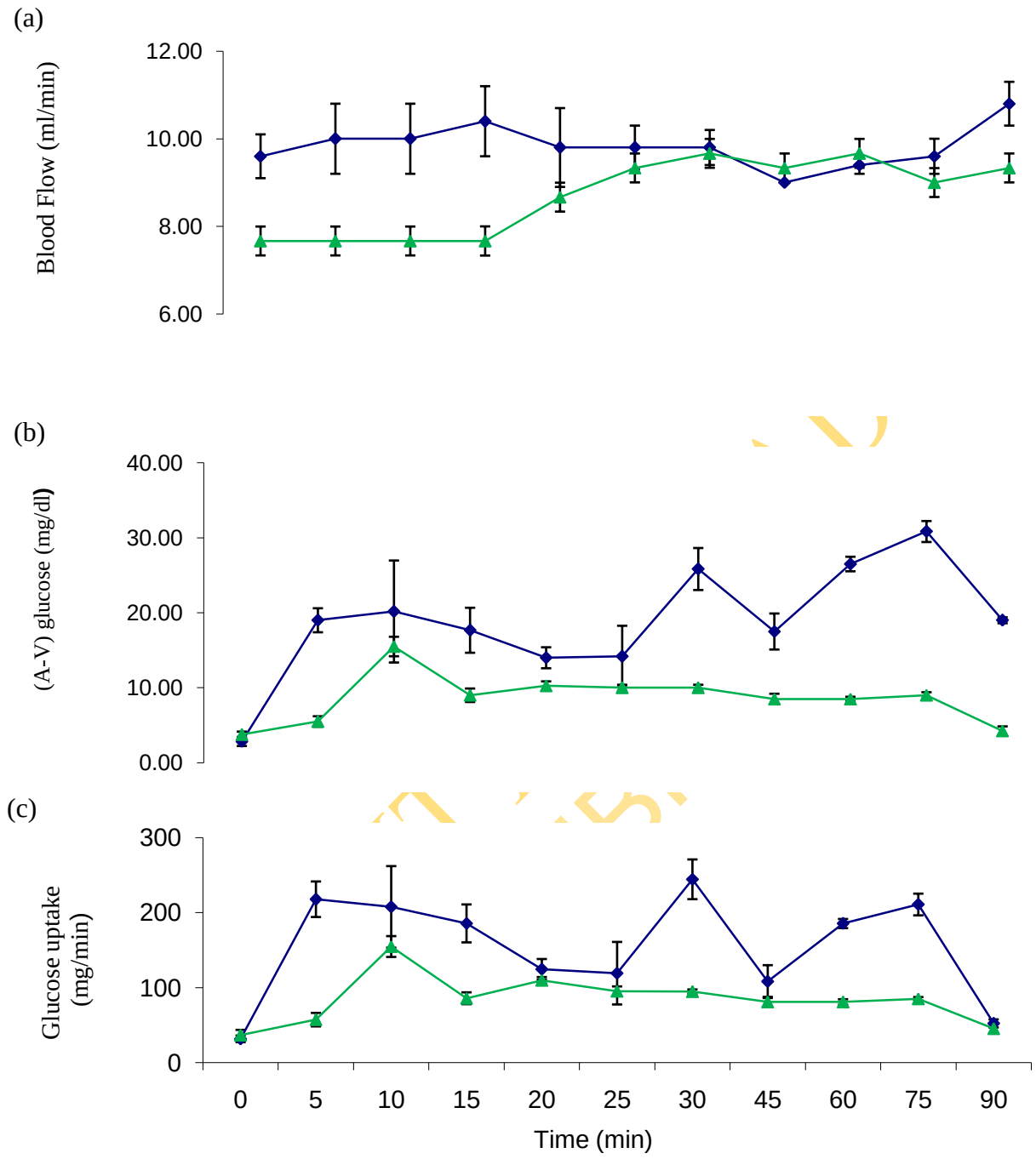
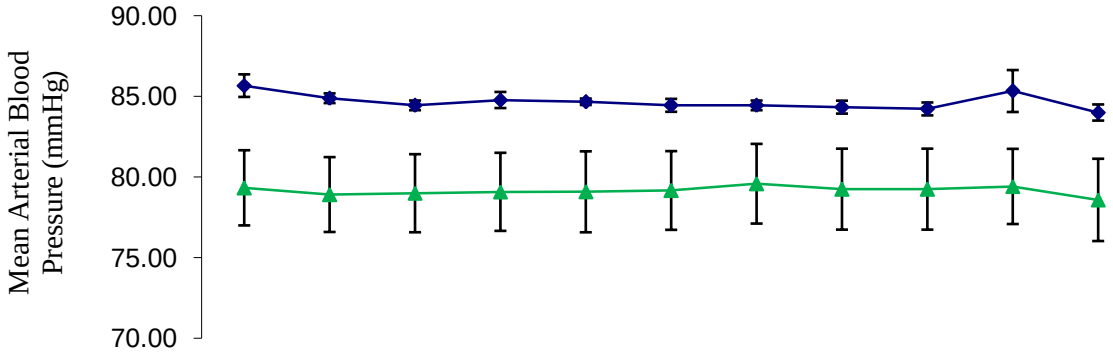


Figure 4-13: Effects of intravenous infusion of fructose on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated (◆-◆) and dogs pretreated with propranolol (▲-▲) .

Table 4-8: Effects of intravenous infusion of fructose (Fru) (1.1mg/kg/min) on arterial and venous glucose concentrations (mg/dl) in dogs pre-treated with prazosin (Pra) (0.2 mg/kg). (*p<0.05).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Fru	Arterial blood glucose	97.60 ±1.78	110.60 ±1.25	112.00 ±2.72	108.80 ±3.01	114.20 ±1.88	103.60 ±3.70	110.80 ±1.28	103.40 ±2.18	109.60 ±1.21	107.40 ±1.78	98.80 ±2.31
Pra+Fru		99.75 ±2.32	100.75 ±1.11*	99.00 ±2.16	97.00 ±3.58*	102.75 ±2.10*	100.75 ±1.49	94.75 ±1.03*	94.50 ±1.32*	92.75 ±0.85*	93.50 ±0.96*	94.75 ±1.03
Fru	Venous blood glucose	94.20 ±2.06	88.80 ±1.36	89.80 ±4.65	90.60 ±1.12	101.40 ±1.60	91.60 ±2.25	85.80 ±2.91	91.40 ±0.93	89.80 ±0.86	85.40 ±1.21	94.00 ±2.39
Pra+Fru		96.00 ±3.03	79.50 ±2.40*	87.75 ±2.56	87.75 ±3.25	78.00 ±2.94*	81.25 ±2.75*	83.75 ±1.70	81.00 ±2.86*	82.75 ±1.44*	81.75 ±1.25	81.25 ±1.70*

(a)



(b)

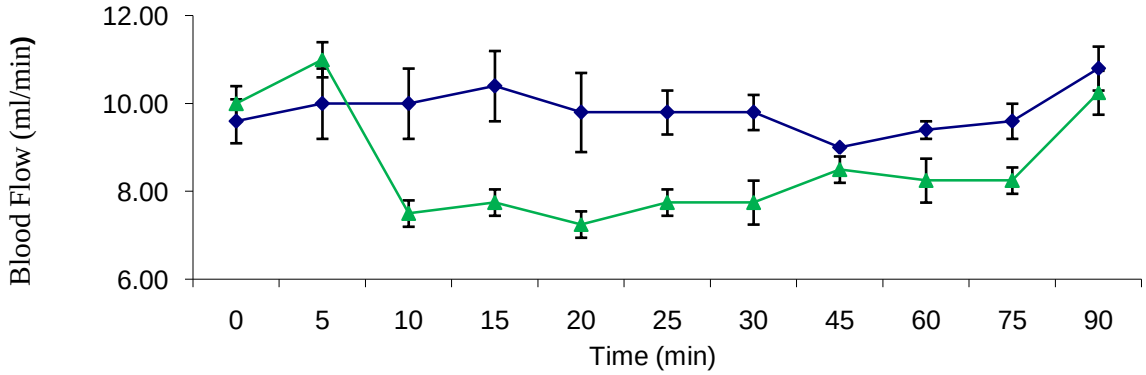
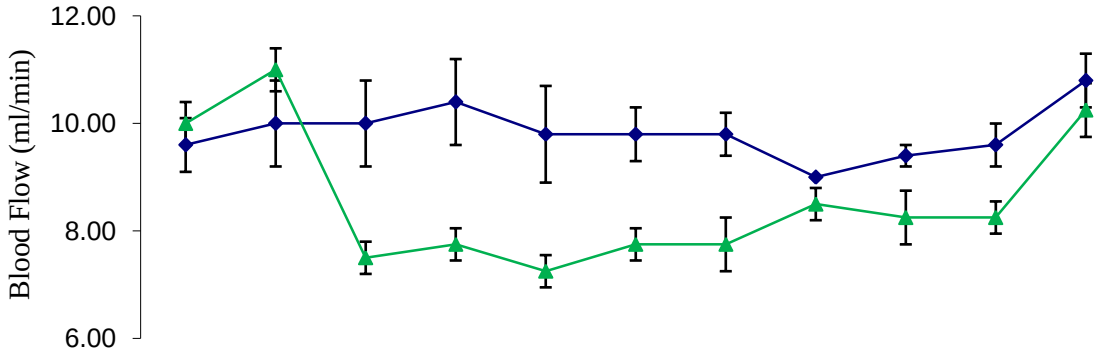
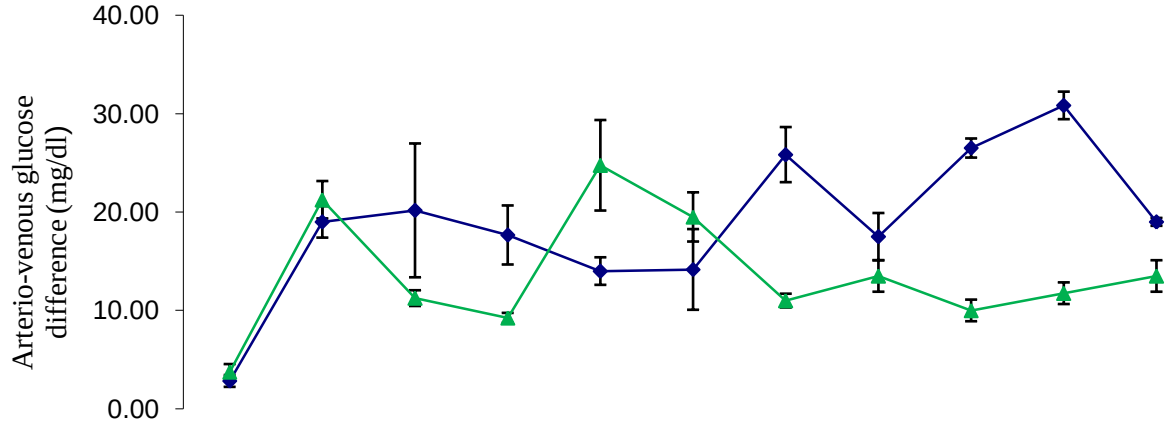


Figure 4-14: Effect of intravenous infusion of fructose(1.1 mg/kg/min) on mean arterial blood pressure (a) and blood Flow (b) in untreated (◆- ◆) and dogs pre-treated with prazosin **(▲-▲).**

(a)



(b)



(c)

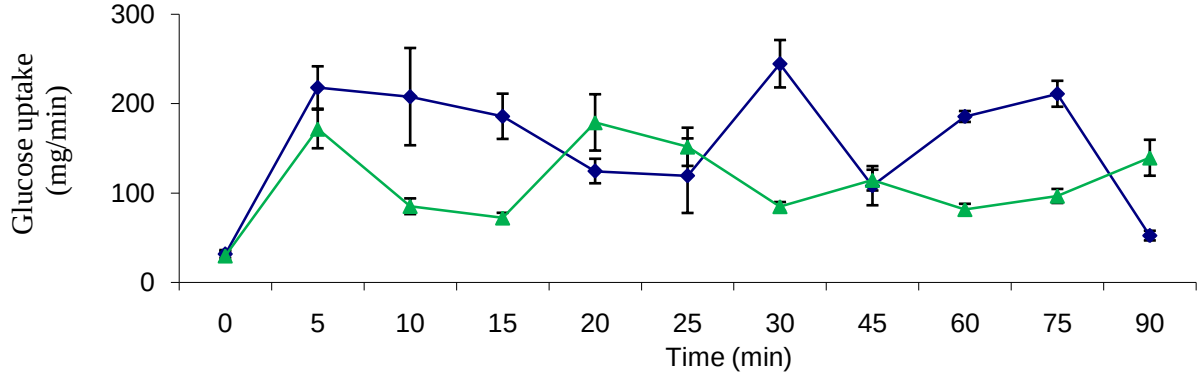
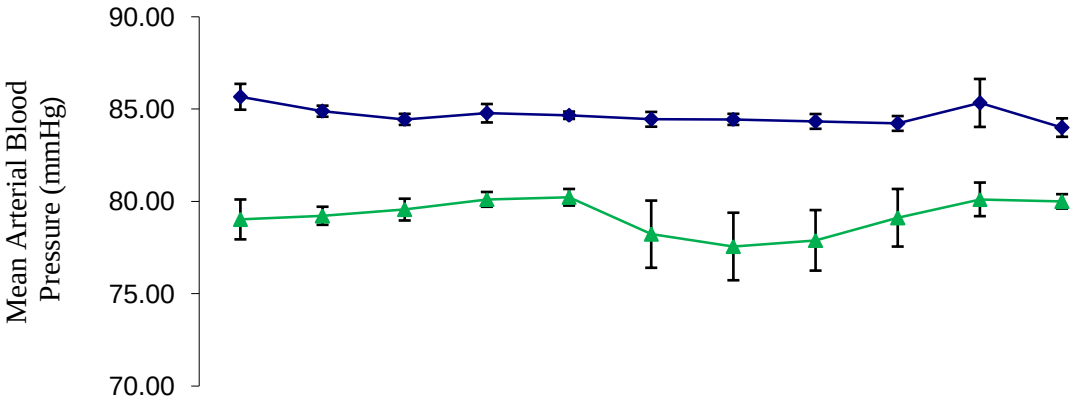


Figure 4-15: Effect of intravenous infusion of fructose on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated (◆-◆) and dogs pre-treated with prazosin (▲-▲).

Table 4-9: Effects of intravenous infusion of fructose (Fru) (1.1 mg/kg/min) on arterial and venous glucose concentrations (mg/dl) before and after pre-treatment with propranolol (Pro) (0.5 mg/kg) and prazosin (Pra) (0.2 mg/kg). (*p<0.05).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Fru	Arterial blood glucose	97.60 ±1.78	110.60 ±1.25	112.00 ±2.72	108.80 ±3.01	114.20 ±1.88	103.60 ±3.70	110.80 ±1.28	103.40 ±2.18	109.60 ±1.21	107.40 ±1.78	98.80 ±2.31
Pro+Pra+Fru		98.25 ±2.78	100.75 ±2.17*	116.00 ±4.34	104.00 ±2.48	106.25 ±1.80*	102.50 ±1.76	101.00 ±0.58*	98.50 ±2.47	100.00 ±2.04*	100.25 ±1.49*	99.25 ±0.85
Fru	Venous blood glucose	94.20 ±2.06	88.80 ±1.36	89.80 ±4.65	90.60 ±1.12	101.40 ±1.60	91.60 ±2.25	85.80 ±2.91	91.40 ±0.93	89.80 ±0.86	85.40 ±1.21	94.00 ±2.39
Pro+Pra+Fru		94.25 ±2.66	94.25 ±2.29*	96.25 ±3.20	93.75 ±2.84	90.50 ±1.76*	87.50 ±1.89	81.25 ±1.11	80.00 ±2.48*	79.25 ±1.97*	80.50 ±2.06	77.50 ±4.05*

(a)



(b)

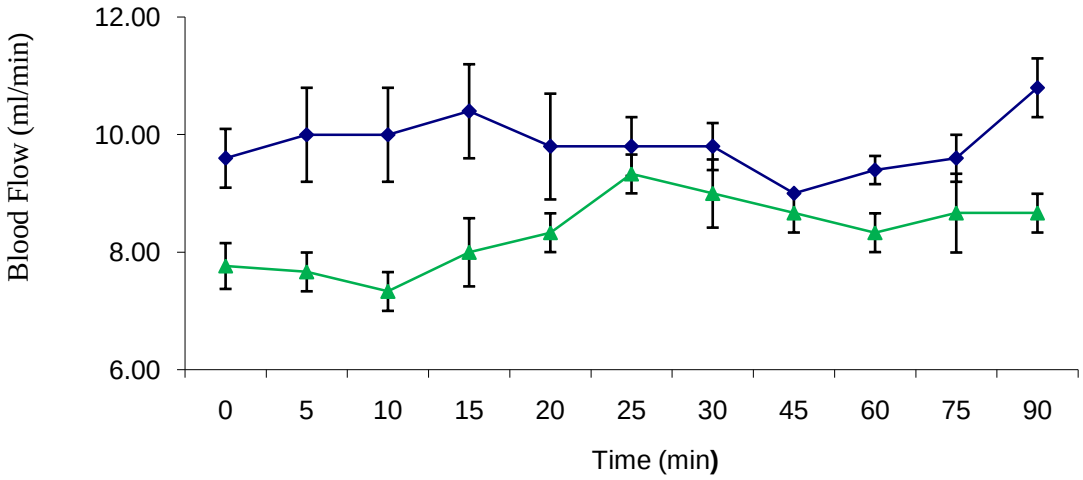
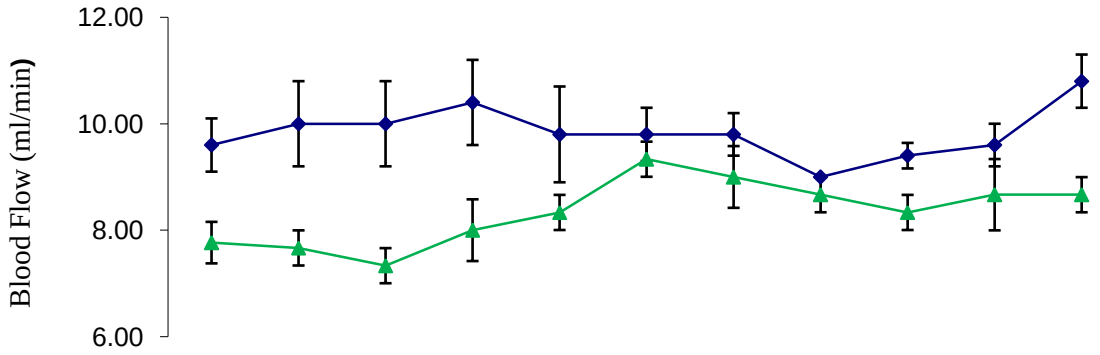
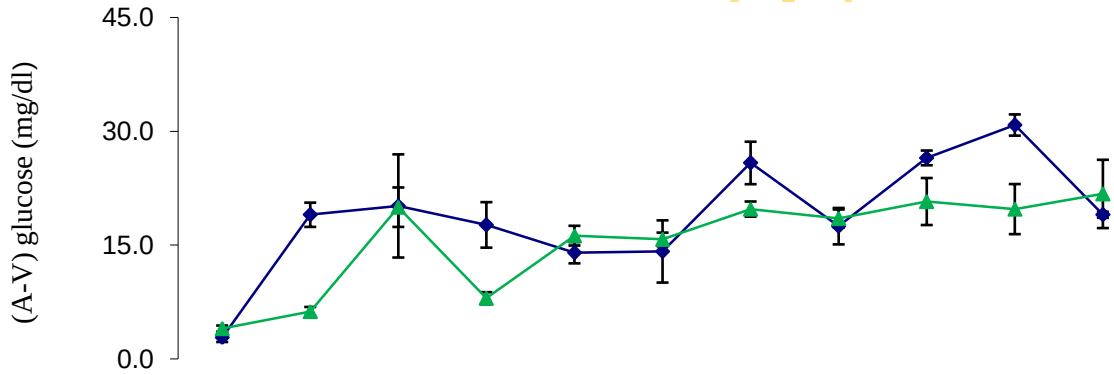


Figure 4-16: Effects of intravenous infusion of fructose on mean arterial blood pressure (a) and blood Flow (b) in untreated (◆-◆) and dogs pre-treated with propranolol and prazosin (▲-▲).

(a)



(b)



(c)

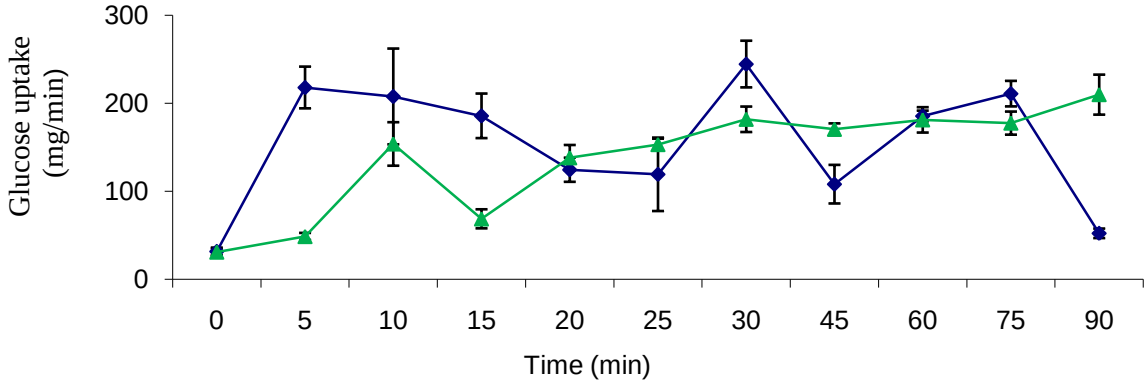


Figure 4-17: Effect of intravenous infusion of fructose on blood flow (a), (A-V)glucose (b) and glucose uptake (c) in untreated (◆-◆)dogs pre-treated with propranolol and prazosin **(▲-▲).**

4.2.3 Effects of L-NAME

4.2.3.1 Effects of fructose on mean arterial blood pressure, blood flow and vascular resistance in dogs pretreated with L-NAME

The effects of fructose on mean arterial blood pressure, blood flow and vascular resistance in untreated and L-NAME-treated dogs are shown in figure 4-18. L-NAME treatment produced a significant difference in the resting mean arterial blood pressure. For instance, the resting mean arterial blood pressure increased from 85.67 ± 0.67 mmHg in untreated dogs to 98.83 ± 1.45 mmHg in the dogs pretreated with L-NAME. Fructose infusion however did not affect the mean arterial blood pressure in L-NAME-treated dogs. This increase in mean arterial blood pressure was sustained throughout the 90 minute observation period. L-NAME also caused significant reduction in both the basal intestinal blood flow and when fructose was infused (figure 4-15). However, the basal vascular resistance increased from 9.35 ± 0.91 R.U in the untreated dogs to 16.68 ± 1.01 R.U in L-NAME-treated dogs. When fructose was infused, vascular resistance increased further to 27.90 ± 3.13 R.U and remained elevated throughout the observation period.

4.2.3.2 Effects of fructose on blood glucose, (A-V) glucose and intestinal glucose uptake in L-NAME- treated dogs

The effects of fructose infusion on arterial and venous blood glucose levels in L-NAME-treated dogs are shown in table 4-10. Pretreatment of the dogs with L-NAME reduced significantly the increase in arterial blood glucose levels produced by fructose infusion. There was however no significant effect of fructose on the venous blood glucose levels in L-NAME-pretreated dogs. Pretreating the dogs with L-NAME did not have any significant difference in (A-V) glucose following fructose infusion (figure 4-19b). In figure 4-19c, pretreatment of the

dogs with L-NAME however completely abolished fructose-induced increases in intestinal glucose uptake.

Table 4-10: Effects of intravenous infusion of fructose (Fru) (1.1 mg/kg/min) on arterial and venous glucose concentrations (mg/dl) before and after pre-treatment with L-Nitro-Arginine-Methyl-Esther (L-NAME) (35 mg/kg).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Fru	Arterial blood glucose	97.60 ±1.78	110.60 ±1.25	112.00 ±2.72	108.80 ±3.01	114.20 ±1.88	103.60 ±3.70	110.80 ±1.28	103.40 ±2.18	109.60 ±1.21	107.40 ±1.78	98.80 ±2.31
L-NAME+Fru		96.25 ±2.69	107.50 ±3.28*	106.25 ±1.49	101.50 ±0.65	102.25 ±1.31*	101.00 ±1.96	101.00 ±2.42*	101.25 ±1.31	97.75 ±1.25*	95.25 ±1.44*	94.25 ±2.10
Fru	Venous blood glucose	94.20 ±2.06	88.80 ±1.36	89.80 ±4.65	90.60 ±1.12	101.40 ±1.60	91.60 ±2.25	85.80 ±2.91	91.40 ±0.93	89.80 ±0.86	85.40 ±1.21	94.00 ±2.39
L-NAME+Fru		92.25 ±2.66	92.00 ±3.37	90.75 ±2.32	89.25 ±1.18	91.50 ±1.76*	89.50 ±1.89	85.25 ±1.03	89.75 ±1.49	85.00 ±0.58*	80.00 ±1.47*	83.00 ±1.73*

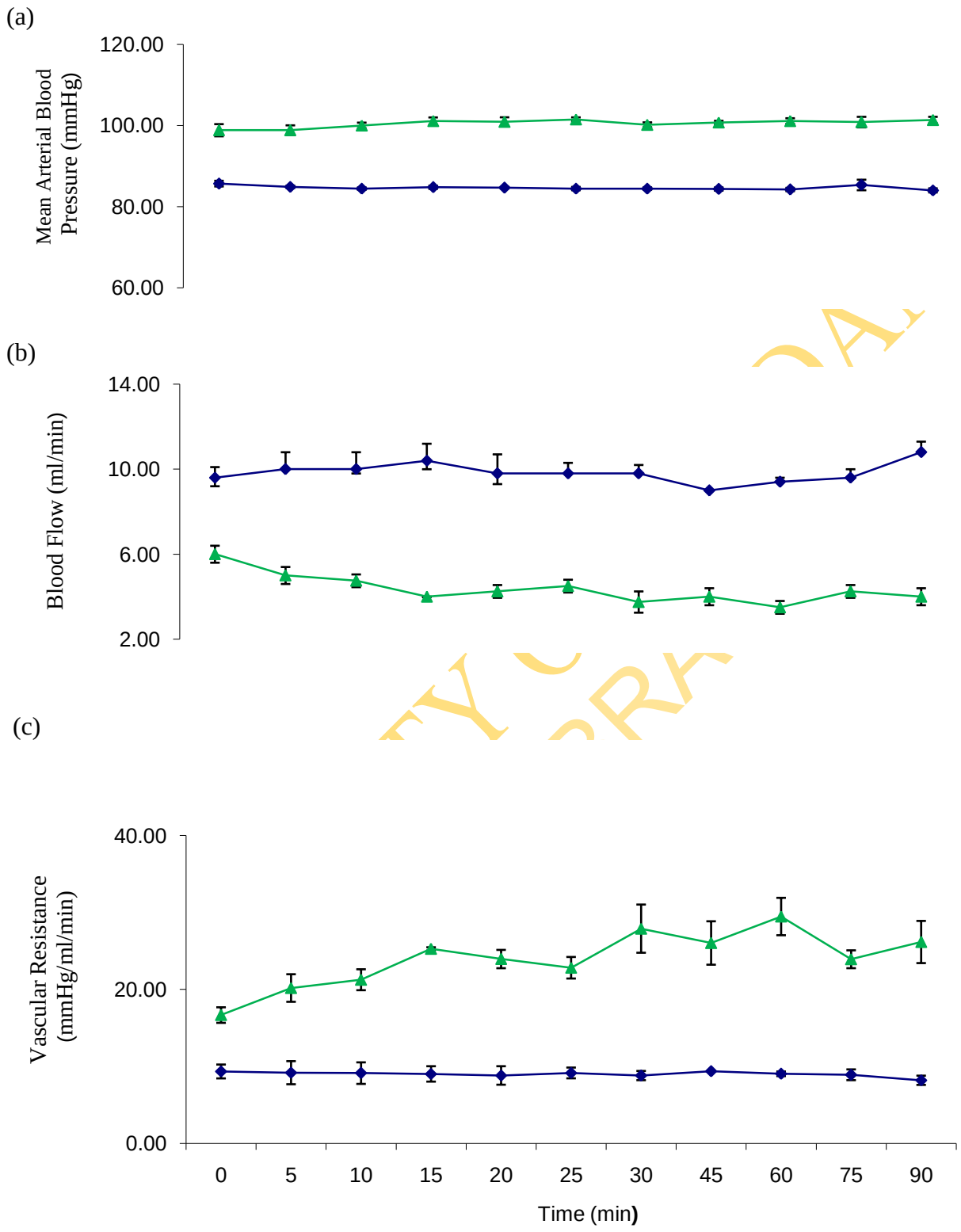


Figure 4-18: Effects of intravenous infusion of fructose on mean arterial blood pressure (a), blood flow (b) and vascular resistance (c) in untreated (◆- ◆) and dogs pre-treated with L-NAME **(▲-▲).**

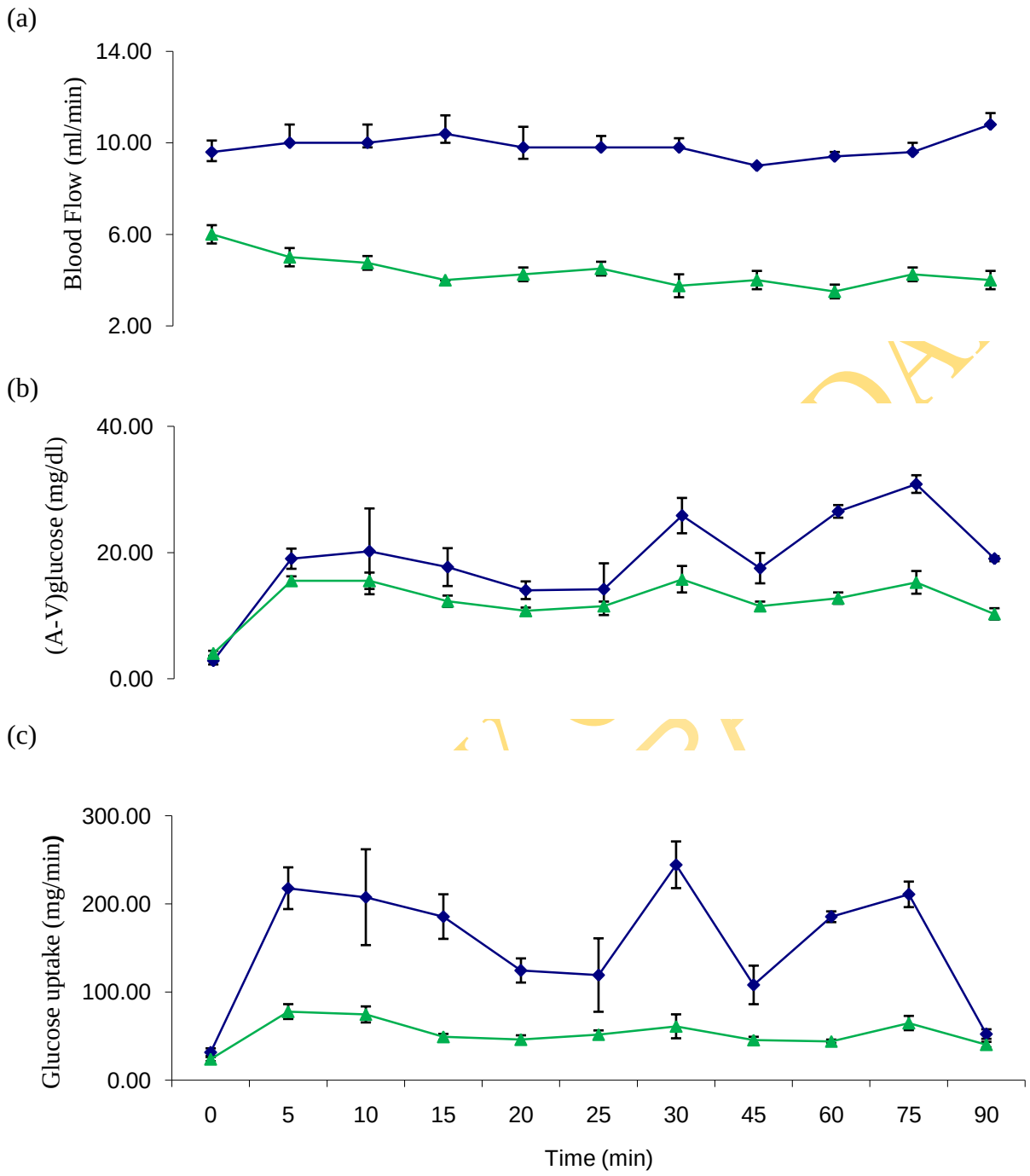


Figure 4-19: Effects of intravenous infusion of fructose on blood flow (a), (A-V) glucose and glucose uptake in untreated (◆-◆) and dogs pre-treated with L-NAME (▲-▲).

4.3 GALATOSE EXPERIMENTS

4.3.1.1 Effects of galactose on mean arterial blood pressure, intestinal blood flow and vascular resistance in dogs.

Galactose infusion had no effect on mean arterial blood pressure, intestinal blood flow and vascular resistance.

4.3.1.2 Effects of galactose on blood glucose levels, (A-V) glucose and intestinal glucose uptake.

The effects of intravenous infusion of galactose on arterial and venous blood glucose levels are shown in table 4-11. The three doses (0.15 mg/kg/min,0.55mg/kg/min and 1.1mg/kg/min.) of galactose administered significantly increased the arterial blood glucose levels. There was however no significant difference in the increases in the arterial blood glucose produced by the three doses. There were also moderate increases in venous blood glucose levels following galactose infusion. It is to be noted that generally that the venous blood glucose levels were lower than the arterial blood glucose levels with the three doses of galactose.

Table 4-12 shows the effects of the three doses of galactose on (A-V) glucose. There was no significant difference in the effects of galactose on (A-V) glucose in the two lower doses (0.15mg/kg/min and 0.55mg/kg/min). The effects of 1.1mg/kg/min of galactose on (A-V) glucose were however higher than the two lower doses.

Figure 4-20 shows the effect of infusion of galactose on intestinal glucose uptake. The three doses of galactose caused significant increases in intestinal glucose uptake. 1.1 mg/kg/min however caused the highest increase in intestinal glucose uptake followed by 0.15mg/kg.min while the 0.55 mg/kg/min of galactose produced the lowest increase. The maximum effect of galactose infusion on intestinal galactose uptake was about 350% which was caused by 1.1.mg/kg/min of galactose.

Table 4-11: Effects of intravenous infusion of galactose (0.15 mg/kg/min, 0.55 mg/kg/min and 1.1 mg/kg/min) on arterial and venous glucose concentrations in dogs. Values are expressed as mean ± SEM (N=5), (*P<0.05)

	Dose	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Arterial blood glucose	0.15 mg/kg/min	99.40 ±1.36	103.60 ±1.25	109.20 ±0.37*	108.80 ±0.58*	112.80 ±1.43*	110.80 ±2.96*	104.40 ±1.29	98.60 ±0.81	104.20 ±3.62	108.40 ±4.13	104.20 ±3.25
	0.55 mg/kg/min	93.20 ±1.16	103.20 ±2.97	99.80 ±1.32*	101.40 ±1.72*	100.60 ±2.56*	98.40 ±1.17*	102.00 ±1.58*	100.80 ±1.20	95.20 ±2.48	92.00 ±5.68	91.00 ±2.77
	1.1 mg/kg/min	96.20 ±1.16	102.60 ±1.50	106.40 ±1.29*	109.80 ±1.43*	105.20 ±1.39*	101.80 ±1.28*	99.00 ±1.05	91.20 ±1.07	89.60 ±1.03	86.00 ±0.55	85.80 ±1.16
Venous blood glucose	0.15 mg/kg/min	95.00 ±1.34	96.20 ±1.24	101.60 ±1.72*	105.20 ±0.73*	96.60 ±1.69	100.00 ±2.88	97.80 ±0.86	94.80 ±0.66	93.20 ±1.59	99.20 ±3.62	97.20 ±2.18
	0.55 mg/kg/min	89.00 ±1.52	94.40 ±1.72	92.80 ±1.69*	95.00 ±1.00	93.00 ±1.70*	91.80 ±1.24	92.00 ±0.89	91.60 ±2.25	87.60 ±3.60	80.80 ±4.27	82.00 ±2.77
	1.1 mg/kg/min	92.00 ±1.29	96.50 ±1.50	98.50 ±0.87*	97.75 ±2.39*	90.00 ±1.47	90.00 ±2.68	91.25 ±1.11	82.75 ±1.80	82.50 ±1.50	80.25 ±2.02	79.75 ±1.80

Table 4-12: Effects of intravenous infusion of galactose (0.15 mg/kg/min, 0.55 mg/kg/min, 1.1 mg/kg/min) on (A-V) glucose. (*****p<0.05, ******p<0.01).

	Dose	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
(A-V) glucose (mg/dl)	0.15 mg/kg/min	4.40 ±0.40	6.60 ±0.51*	5.60 ±0.24*	3.60 ±0.24	14.40 ±1.03**	10.80 ±1.02**	6.60 ±0.51*	3.80 ±0.20	11.00 ±2.28**	9.20 ±1.50**	7.00 ±1.52*
	0.55 mg/kg/min	4.20 ±0.37	8.80 ±1.50*	7.00 ±1.14*	6.40 ±0.87*	7.60 ±1.29*	6.60 ±1.03	10.00 ±1.14**	9.20 ±1.16*	7.60 ±1.36	11.20 ±2.92*	9.00 ±0.71*
	1.1 mg/kg/min	3.80 ±0.37	6.00 ±0.55*	7.80 ±0.66*	12.00 ±0.63**	15.20 ±0.86**	11.80 ±0.86**	7.60 ±0.81*	9.00 ±0.55*	7.80 ±0.80*	6.80 ±0.58*	6.20 ±0.49*

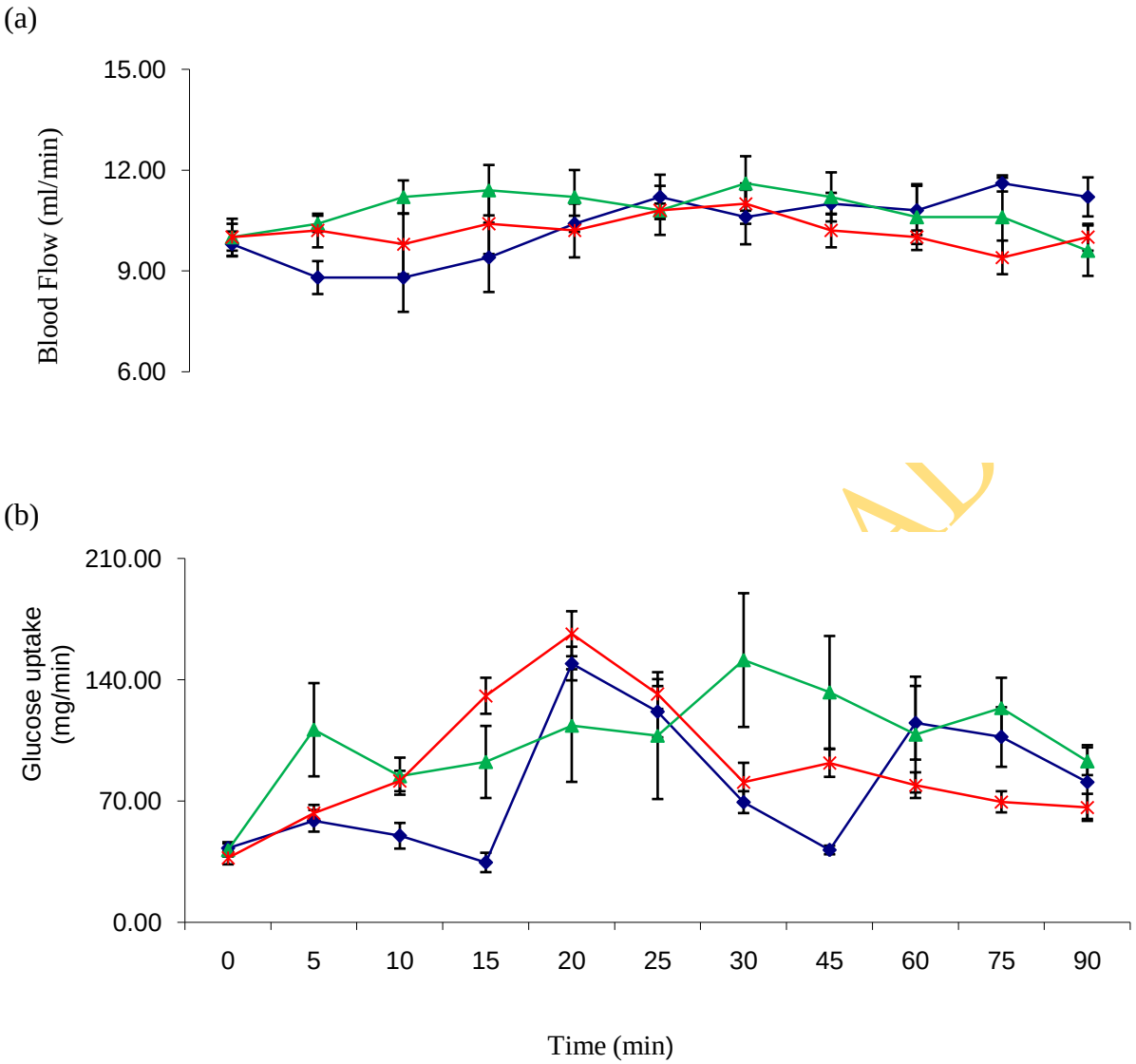


Figure 4-20: Effects of intravenous infusion of galactose (0.15- (♦-♦) ,0.55- (▲-▲),1.1 (×-×) mg/kg/min) on blood flow (a) and glucose uptake (b) in dogs.

4.3.2 Effects of adrenergic receptor blockers

4.3.2.1 Effects of galactose infusion on mean arterial blood pressure, blood flow and vascular resistance in dogs pretreated with adrenergic receptor blockers.

Propranolol caused a significant reduction in the basal mean arterial blood pressure and this low level of mean arterial blood pressure was sustained even when galactose was administered. Although, propranolol had no effect on the basal blood flow, it however caused significant decreases in blood flow following galactose administration. There was no change in vascular resistance.

Pretreatment with Prazosin also caused a significant decrease ($P<0.05$) in the basal mean arterial blood pressure. The mean arterial blood pressure dropped from 86.11±0.80 mmHg in the untreated dogs to 81.42 ± 0.67 mmHg in prazosin-treated dogs. This lower blood pressure was not affected following galactose infusion. Pretreatment of the dogs with prazosin also produced a small but significant decrease in intestinal blood flow during galactose infusion. That is, intestinal blood flow was higher in untreated dogs than in prazosin-treated dogs. However, there was no significant difference in vascular resistance between the prazosin-treated and untreated dogs infused with galactose.

The effects of pretreatment of the dogs with the combination of prazosin and propranolol followed by galactose infusion on mean arterial blood pressure, blood flow and vascular resistance are shown in figure 4-23. When the dogs were pretreated with prazosin and prapranolol, there was a significant decrease ($P<0.05$) in the basal mean arterial blood pressure. The basal mean arterial blood decreased from 86.10 ±0.80mmHg in the untreated dogs to 80.40±1.46 mmHg in the adrenergic blocker-treated dogs. This lower level of blood pressure was sustained even when galactose was infused.

Pretreatment of the dogs with two blockers also lowered the basal intestinal blood flow and the intestinal blood flow in response to galactose infusion. Although, pretreatment of the dogs with prazosin and propranolol had no effect on the basal vascular resistance, it however increased the vascular resistance during galactose infusion (Figure 4-23).

4.3.2.2. Effects of galactose infusion on blood glucose levels, (A-V) glucose and intestinal glucose uptake in dogs pretreated with adrenergic receptor blockers.

The effects of galactose on arterial and venous blood glucose levels in untreated and propranolol-treated dogs are shown in table 4-13. Although, galactose caused significant increases in blood glucose levels, these increases were not affected by pretreatment with propranolol. Figure 4-21b shows the effect of galactose on (A-V) glucose in untreated and propranolol-treated dogs. Pretreatment of the dogs with propranolol significantly decreased the galactose-induced increase in (A-V) glucose. This decrease in (A-V) glucose was about154%.

Figure 4-21c also shows the effect of galactose on intestinal glucose uptake in untreated and propranolol-treated dogs. Pretreatment of the dogs with propranolol reduced the galactose-induced increase in intestinal glucose uptake by as much as 208%.

Table 4-14 shows the effects of pretreatment of dogs with prazosin followed by infusion of galactose on blood glucose levels. There was no significant difference in both the arterial and venous blood glucose levels in untreated and prazosin-treated dogs given galactose. When the (A-V) glucose was calculated, prazosin abolished the significant increase in (A-V) glucose following galactose infusion (Figure 4- 22b). Prazosin also completely abolished the increase in intestinal glucose uptake induced by galactose infusion (figure 4-22c).

Table 4-15 shows the effect of galactose on arterial and venous blood glucose levels in untreated and dogs treated with a combination of propranolol and prazosin. The combination of

the two blockers did not have a significant effect on the galactose-induced increases in blood glucose levels. However the two adrenergic blockers completely abolished the galactose-induced increases in (A-V) glucose (figure 4-24b). The combination of propranolol and prazosin also abolished galactose-induced rise in intestinal glucose uptake.

Table 4-13: Effects of intravenous infusion of galactose (Gal) (1.1 mg/kg/min) on arterial and venous glucose concentrations (mg/dl) before and after pre-treatment with propranolol (Pro) (0.5 mg/kg).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Gal	Arterial blood glucose	96.00 ±1.47	102.75 ±1.93	106.25 ±1.65	109.75 ±1.84	105.25 ±1.80	102.00 ±1.63	99.00 ±1.35	91.75 ±1.18	90.25 ±1.03	86.00 ±0.71	86.00 ±1.47
Pro+Gal		96.25 ±1.75	107.75 ±1.11	112.25 ±1.03	114.75 ±2.14	101.50 ±2.53	98.50 ±0.65	92.75 ±3.92	94.25 ±3.79	96.25 ±0.48	96.75 ±0.48	94.00 ±1.68
Gal	Venous blood glucose	92.00 ±1.29	96.50 ±1.50	98.50 ±0.87	97.75 ±2.39	90.00 ±1.47	90.00 ±2.68	91.25 ±1.11	82.75 ±1.80	82.50 ±1.50	80.25 ±2.02	79.75 ±1.80
Pro+Gal		93.25 ±2.78	102.50 ±2.87	106.50 ±2.87*	107.25 ±4.61	97.25 ±3.09	93.50 ±2.06	88.75 ±5.06	90.50 ±4.70	90.00 ±2.80	90.25 ±2.53	88.50 ±3.43

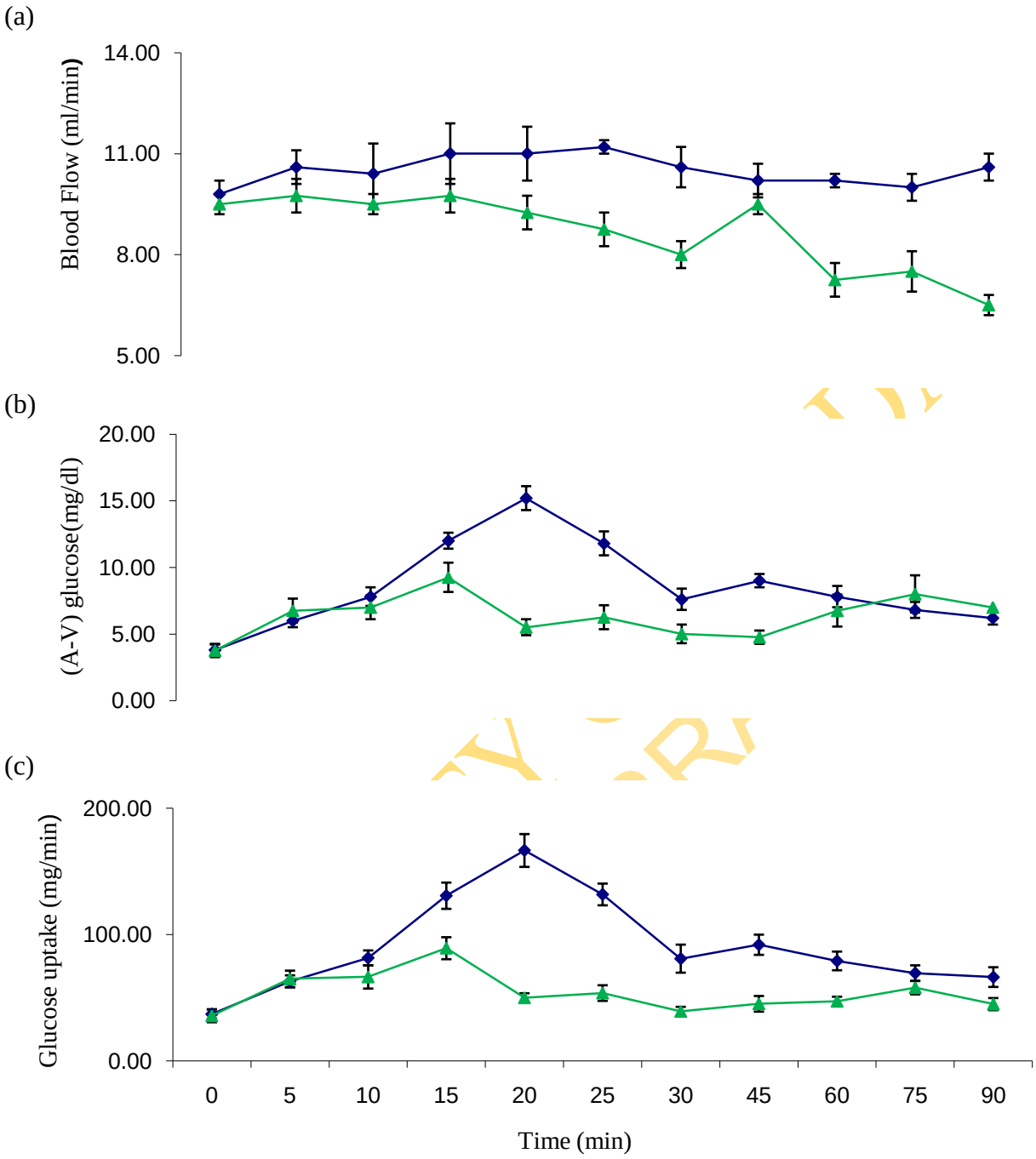


Figure 4-21: Effects of intravenous infusion of galactose on blood flow (a), (A-v) glucose (b) and glucose uptake (c) in untreated (◆- ◆) and dogs pre-treated with propranolol (▲-▲).

Table 4-14: Effects of intravenous infusion of galactose (Gal) (1.1 mg/kg/min) on arterial and venous glucose concentrations (mg/dl) before and after pre-treatment with prazosin (Pra) (0.2 mg/kg).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Gal	Arterial blood glucose	96.00 ±1.47	102.75 ±1.93	106.25 ±1.65	109.75 ±1.84	105.25 ±1.80	102.00 ±1.63	99.00 ±1.35	91.75 ±1.18	90.25 ±1.03	86.00 ±0.71	86.00 ±1.47
Pra+Gal		96.67 ±0.88	107.33 ±0.67	110.33 ±0.33	114.00 ±1.00	105.00 ±2.08	101.00 ±1.00	92.00 ±1.53	91.33 ±0.33	94.67 ±0.33	94.67 ±1.20	94.67 ±1.33
Gal	Venous blood glucose	92.00 ±1.29	96.50 ±1.50	98.50 ±0.87	97.75 ±2.39	90.00 ±1.47	90.00 ±2.68	91.25 ±1.11	82.75 ±1.80	82.50 ±1.50	80.25 ±2.02	79.75 ±1.80
Pra+Gal		93.00 ±0.58	99.67 ±0.88	103.33 ±0.88	105.33 ±1.20	99.00 ±2.31	94.00 ±0.58	86.33 ±1.33	86.33 ±0.33	89.67 ±0.33	89.33 ±0.88	89.67 ±1.33

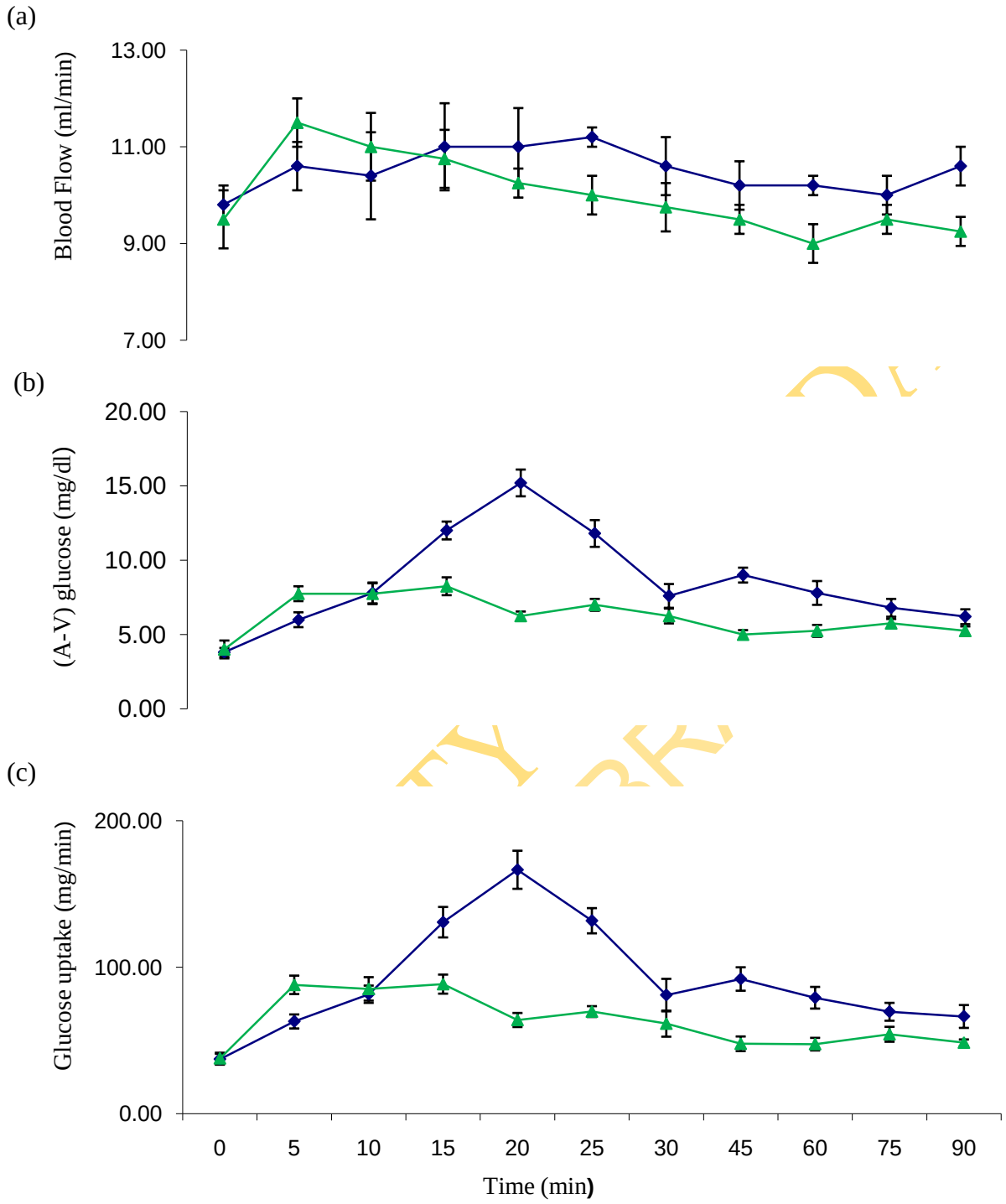


Figure 4-22: Effects of intravenous infusion of galactose on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated (◆-◆) and dogs pre-treated with prazosin (▲-▲).

Table 4-15: Effects of intravenous infusion of galactose (Gal) (1.1 mg/kg/min) on arterial and venous glucose concentration (mg/dl) before and after pre-treatment with propranolol (Pro) (0.5 mg/kg) and prazosin (Pra) (0.2 mg/kg).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Gal	Arterial blood glucose	96.00 ±1.47	102.75 ±1.93	106.25 ±1.65	109.75 ±1.84	105.25 ±1.80	102.00 ±1.63	99.00 ±1.35	91.75 ±1.18	90.25 ±1.03	86.00 ±0.71	86.00 ±1.47
Pro+Pra+Gal		99.50 ±2.90	100.75 ±2.87	108.25 ±2.29	112.50 ±2.63	107.75 ±3.52	99.75 ±0.85	99.00 ±1.78	98.00 ±2.48	96.00 ±0.82	98.75 ±4.21	96.50 ±0.65
Gal	Venous blood glucose	92.00 ±1.29	96.50 ±1.50	98.50 ±0.87	97.75 ±2.39	90.00 ±1.47	90.00 ±2.68	91.25 ±1.11	82.75 ±1.80	82.50 ±1.50	80.25 ±2.02	79.75 ±1.80
Pro+Pra+Gal		93.00 ±1.53	93.33 ±2.03	102.00 ±0.58	105.33 ±1.20	96.67 ±1.20	93.67 ±1.45	92.33 ±0.88	90.00 ±1.73	90.00 ±1.53	89.00 ±2.52	90.67 ±0.33

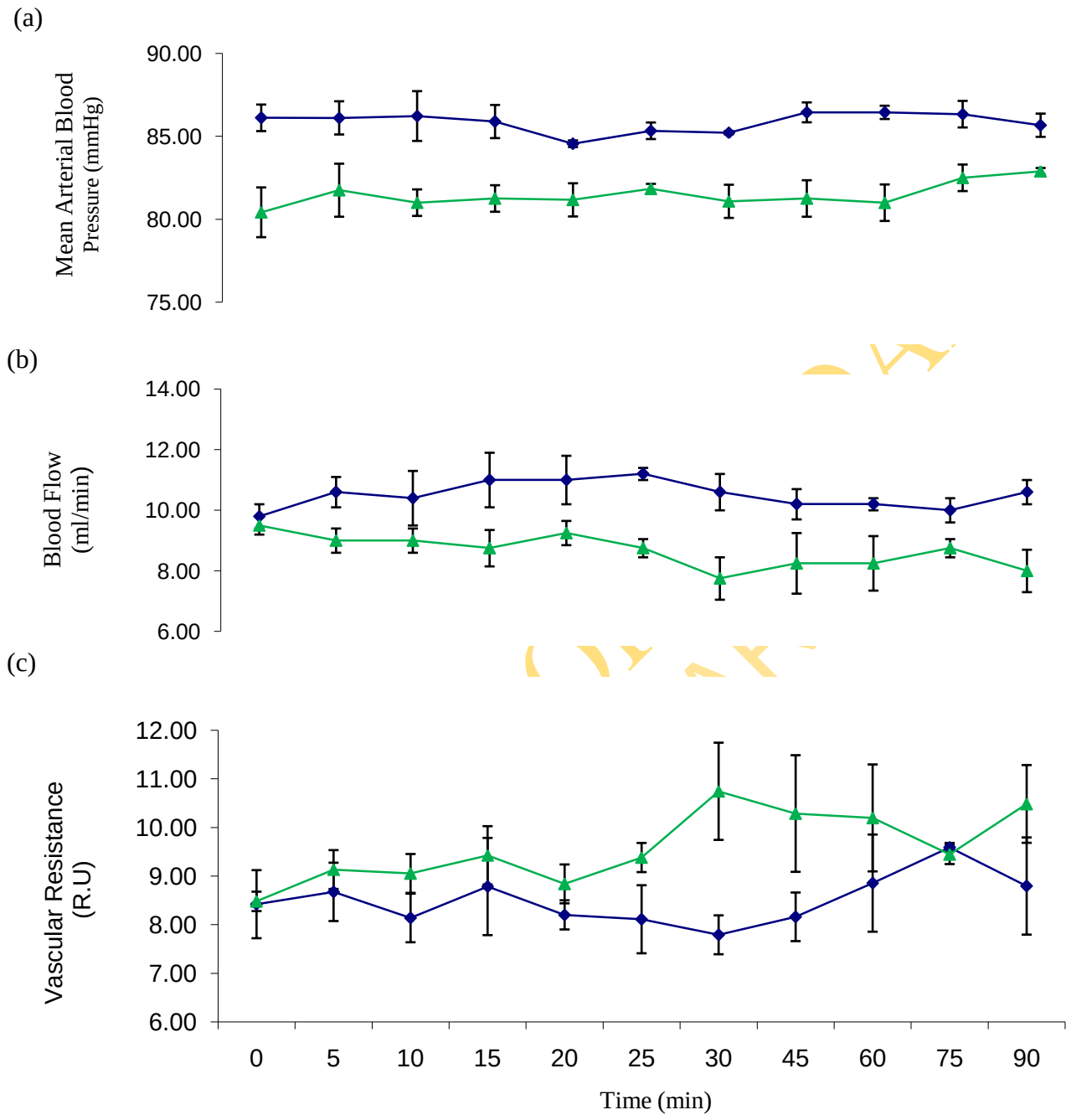


Figure 4-23: Effects of intravenous infusion of galactose on mean arterial blood pressure (a), blood flow (b) and vascular resistance (c) in untreated (◆-◆) and dogs pre-treated with propranolol and prazosin (▲-▲).

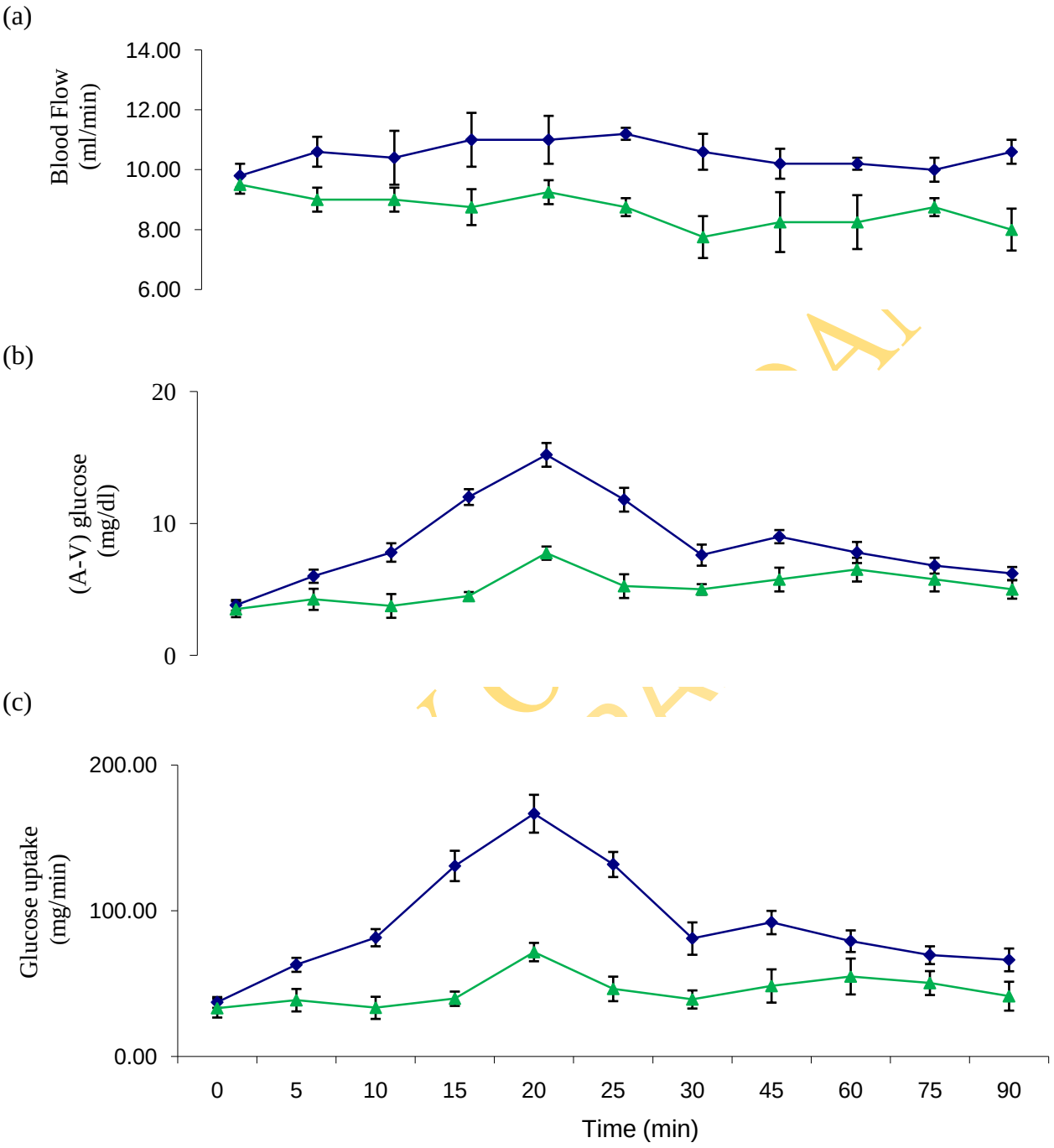


Figure 4-24:Effects of intravenous infusion of galactose on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated (◆- ◆) and dogs pre-treated with propranolol and prazosin (▲- ▲).

4.3.3. Effects of L-NAME

4.3.3.1 Effects of galactose on mean arterial blood pressure, blood flow and vascular resistance in dogs pretreated with L-NAME

Pretreatment with L-NAME reduced significantly the basal mean arterial blood pressure which was sustained even during and after infusion of galactose. Pretreatment with L-NAME also caused significant reduction in basal intestinal blood flow. For example, intestinal blood flow decreased from 9.75 ± 0.48ml/min in untreated dogs to 7.60 ± 0.44ml/min in L-NAME treated dogs. It is interesting to note that intestinal blood flow decreased further to 4.25 ± 0.25ml/min following galactose infusion.L-NAME pretreatment of the animal however produced a significant increase in basal vascular resistance. The basal vascular resistance of 8.42 ± 0.66 R.U in untreated dogs increased to 16.20 ± 0.77 R.U inL-NAME-treated dogs. Following infusion of galactose, the vascular resistance in L-NAME-treated dogs increased to a peak of 29.67 ± 3.83 R.U.

4.3.3.2 Effects of galactose on Blood glucose levels, (A-V) glucose and intestinal glucose uptake in dogs with L-NAME

The effects of galactose on arterials and venous blood glucose levels in dogs pretreated with L-NAME are shown in table 4-16. Pretreatment of the dogs with L-NAME did not produce any significant change in arterial and venous blood glucose levels following galactose infusion.

Pretreatment of the dogs with L-NAME also caused a significant ($P<0.05$) reduction in the peak of galactose-induced increase in (A-V) glucose (figure 4-25b). Pretreatment of the dogs with L-NAME completely abolished the huge increase in intestinal glucose uptake caused by galactose infusion (figure4-25c).

Table 4-16: Effects of intravenous infusion of galactose (Gal) (1.1 mg/kg/min) on arterial and venous glucose concentrations (mg/dl) before and after pre-treatment with L-Nitro-Arginine-Methyl-Esther (L-NAME) (35 mg/kg).(*p<0.05).

Treatment		0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Gal	Arterial blood glucose	96.00 ±1.47	102.75 ±1.93	106.25 ±1.65	109.75 ±1.84	105.25 ±1.80	102.00 ±1.63	99.00 ±1.35	91.75 ±1.18	90.25 ±1.03	86.00 ±0.71	86.00 ±1.47
L-NAME+Gal		99.50 ±2.90	100.75 ±2.87	108.25 ±2.29	112.50 ±2.63	107.75 ±3.52	99.75 ±0.85	99.00 ±1.78	98.00 ±2.48	96.00 ±0.82	98.75 ±4.21	96.50 ±0.65
Gal	Venous blood glucose	92.00 ±1.29	96.50 ±1.50	98.50 ±0.87	97.75 ±2.39	90.00 ±1.47	90.00 ±2.68	91.25 ±1.11	82.75 ±1.80	82.50 ±1.50	80.25 ±2.02	79.75 ±1.80
L-NAME+Gal		93.00 ±1.53	93.33 ±2.03	102.00 ±0.58	105.33 ±1.20	96.67 ±1.20	93.67 ±1.45	92.33 ±0.88	90.00 ±1.73	90.00 ±1.53	89.00 ±2.52	90.67 ±0.33

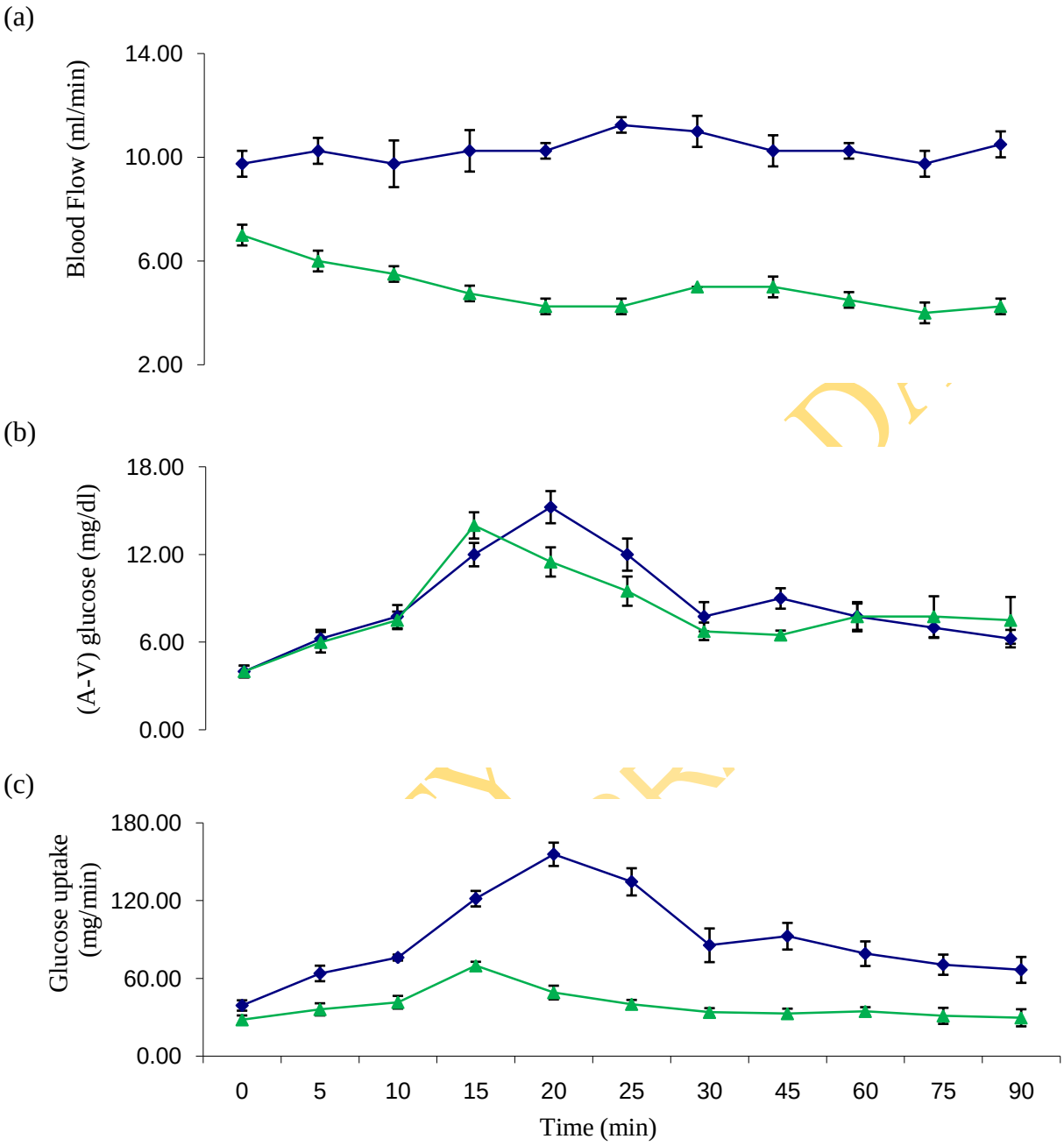


Figure 4-23: Effects of intravenous infusion of galactose on blood flow (a), (A-V) glucose (b) and glucose uptake (c) in untreated (◆-◆) and dogs pre-treated with L-NAME (▲-▲).

4.4. NORMAL SALINE EXPERIMENT

4.4.1 Effects of normal saline on mean arterial blood pressure, blood flow and vascular resistance in dogs.

Normal saline had no effect on the mean arterial blood pressure, blood flow and vascular resistance. Resting mean arterial blood pressure was 90.45 ± 1.78 mmHg, blood flow was 10.60 ± 0.75 ml/min while vascular resistance was 9.50 ± 0.80 R.U. (see appendix)

4.4.2 Effect of normal saline on blood glucose, (A-V) glucose intestinal glucose uptake in dogs.

Normal saline had no effect on arterial and venous blood glucose levels, (A-V) glucose and intestinal glucose uptake in dogs. The resting arterial and venous blood glucose level were 99.20 ± 0.37 mg/dl and 95.80±0.97mg/dl respectively. (A-V) glucose was 3.40 ± 0.68 mg/dl and intestinal glucose uptake in the dogs was 36.80 ± 8.26 mg/min (See appendix).

CHAPTER 5

DISCUSSION

The resting mean arterial blood pressure of 90.45 ± 1.78 mmHg observed in this study is consistent with similar blood pressure values reported in previous dog experiments under similar conditions (Grayson and Oyebola, 1981; Alada and Oyebola, 1996; 1997). In the present study, a physiographic two channel recorder (Gemini Model 7070, Ugo Basil, Italy) was used. Alada and Oyebola (1996) also used a dynograph recorder (Beckman R511A) to monitor blood pressure in dogs. All the results in the different studies are however consistent with each other.

The value of 10.60 ± 0.75 ml/min observed in this study for resting intestinal blood flow also agrees with that of Grayson and Oyebola (1981) and Alada and Oyebola (1996; 1997) in similar dog experiments. When the vascular resistance was calculated, a resting value of 9.50 ± 0.80 R.U was obtained and this value agrees with that of previous studies (Alada and Oyebola, 1996; 1997) in similar dog experiments.

The resting arterial blood glucose of 99.20 ± 0.37 mg/dl and venous blood glucose of 95.80 ± 0.97 mg/dl are similar to the arterial and venous blood glucose levels observed in dogs in previous studies. The resting (A-V) glucose and the intestinal glucose uptake observed in this study are also consistent with the (A-V) glucose and intestinal glucose uptake earlier reported in similar dog experiments (Alada and Oyebola, 1996; 1997; Alada *et al.*, 2005).

GLUCOSE EXPERIMENTS

The major haemodynamic effects of glucose infusion in this study is the significant increase in the jejunal blood flow. A similar observation was reported by

Alada and Oyebola (1996) in similar dog experiment. Grayson and Kinnear (1962) had earlier reported an increase in hepatic blood flow following glucose injection. The nature of this increase in blood flow is still not clear. There are reports of it being due to the vasodilatory action of insulin (Anderson *et al.*, 1991). In other words, glucose infusion causes hyperinsulinemia which could be responsible for the increase in blood flow. Insulin's vasodilatory action has been reported to be due to release of nitric oxide (Saskia and Peter, 1997). However, the transient nature of the increase in blood flow raises doubt on the possibility of the hyperemia being due to only insulin. It would seem unlikely that this response is related to the absolute level of glucose in the blood. It is more likely to be the result of the sudden change in blood glucose level. The decrease in the vascular resistance observed in this study may probably be a direct metabolic response of the blood vessels to the changes in blood glucose.

The failure of the alpha and beta adrenergic receptor blockers to alter the haemodynamic response to glucose infusion in the present study suggests that the effect is most probably not mediated through adrenergic receptors. The latter strengthens the possibility of a direct vascular effect. Indeed, the abolition of insulin-induced increase in blood flow by pretreatment of the dogs with L-NAME seems to confirm the role of nitric oxide in glucose-induced hyperemia in this study.

The increase in blood glucose levels following glucose infusion was comparable to the hyperglycemia induced by intravenous injection of other agents and glucose in previous studies (Grayson and Oyebola, 1983; Alada and Oyebola, 1996). In the present study, the venous blood glucose level was lower than the arterial blood glucose level in all the doses of glucose infused. The present result also showed that the (A-V) glucose

and intestinal glucose uptake increased during the period when the blood glucose was high. A low dose of 0.15 mg/kg/min increased blood glucose by 23% while the (A-V) glucose and intestinal glucose uptake increased by 53% and 74% respectively and at a higher dose (1.1 mg/kg/min), blood glucose increased by 42% while (A-V) glucose and intestinal glucose uptake increased by 476% and 670% respectively. These findings are similar to earlier findings with adrenaline (Grayson and Oyebola, 1983), glucagon (Alada and Oyebola, 1996), glucose (Alada and Oyebola, 1996) and nicotine (Grayson and Oyebola, 1985) where different doses of each of the drugs resulted in different levels of hyperglycemia and increase in glucose uptake by the small intestine was higher at higher levels of the increase in blood glucose. The results of this study therefore indicate beyond any doubt that the increase in glucose uptake by the intestine is related to the level of the increase in blood glucose and this is irrespective of the cause of hyperglycemia. This observation is totally in agreement with the earlier conclusion of Alada and Oyebola (1996) that the intestine is capable of taking up large quantities of glucose, especially when blood glucose is raised. Levine and Haft (1970) and Buttler and Rizza (1989) had reported that in both insulin-sensitive and insulin-insensitive tissues, the blood glucose concentration is a key factor determining the rate of glucose uptake. The transport mechanism of glucose across the cell membrane is saturable only at extremely high glucose concentration (Park et al 1968). Indeed, under conditions of complete absence of insulin (e.g diabetes) when blood glucose level rises to very high values, the absolute rate of glucose uptake has been reported to be as high as it is in a normal subject (Felig, 1980). In an earlier study by Alada *et al* (2005), intestinal glucose uptake was as high as about 1000% in diabetic dogs. The present result therefore leads to the conclusion that

the gastrointestinal tract glucose uptake is largely in response to the blood level of glucose. If the blood glucose is very high, glucose uptake is very high, whereas a moderate increase in blood glucose results in a moderate increase in glucose uptake.

The most interesting observation in this study on the role of adrenergic receptor in the increased intestinal glucose uptake is the effect of beta adrenergic blocker. It was noteworthy that prazosin had no effect on the glucose-induced increase in intestinal glucose uptake. However, propanolol reduced considerably (A-V) glucose and the peak of intestinal glucose uptake in this study. These findings are consistent with earlier observation on the effect of alpha and beta adrenoceptor blockers in similar dog experiments (Grayson and Oyebola, 1983; Alada and Oyebola, 1997; Salahdeen and Alada, 2009). The present study therefore suggests that the increase in intestinal glucose uptake in response to the high blood glucose caused by glucose infusion is most probably mediated by beta adrenergic receptor alone.

The significant reduction in intestinal glucose uptake observed in this study following pretreatment with L-NAME is most probably the result of the vasoconstriction effect and the decrease in blood flow caused by L-NAME. It is to be noted that intestinal glucose uptake is a product of both the (A-V) glucose and blood flow. Therefore,a significant reduction in blood flow most probably affect the intestinal glucose uptake. L-NAME is a well known vasoconstrictor. It acts as an inhibitor of nitric oxide synthase which is the enzyme responsible for the formation of nitric oxide (Bredt, 1999). The vasodilatory action of nitric oxide and its role in the regulation of blood flow to major organs in several animal species are well known (Iadecola *et al.*, 1994; Ito, 1995; McCall *et al.*, 1989; Umans and Levi, 1995). Other workers have also emphasized the role of

blood flow in tissue consumption of many substances including glucose (Adam and Erik, 2005).

FRUCTOSE EXPERIMENTS

The present findingswhich show that fructose infusion had no effect on blood pressure directly oppose a sizeable body of literature which reported that blood pressure was elevated following fructose infusion (Hwang *et al.*, 1987; Navarro-Cid *et al.*, 1995; Verma *et al.*, 1996; Juan *et al.*, 1998; Katovich *et al.*, 2001; Catena *et al.*, 2003; Hsieh, 2004). Different methods were used to measure blood pressure and these may result in contrasting results. The effects of measuring blood pressure using different methods were recently discussed in a report from a sub-committee of professional and public Association of the American Heart Association Council of high blood pressure research (Kurtz *et al.*, 2005). Most of the earlier studies have used tail cuff pletysmography which may involve some form of stress-mediated pressor response. In the present study, a direct blood pressure measurement was done by using a physiographic two channel recorder (Gemini Model 7070, Ugo Basil, Italy) and the animals were anaesthetized. This method therefore did not need any form of animal handling and tethering. In other words, aside from the normal animal husbandry, activity at the operating table was kept to a minimum so as to avoid any disturbance to the animal. A major draw back associated with the tail cuff measurement is the restraint and the thermal stress imposed on the animal leading to an increase in sympathetic output and consequently a rise in the mean arterial blood pressure. A major point of difference between this study and the previous ones is with the continuous monitoring instrumentation to monitor blood pressure for a long period.

Brands *et al.* (1994) also reported no effect of high fructose diet on mean arterial blood pressure.

The absence of any effect of fructose infusion on intestinal blood flow and vascular resistance agrees with the work of Brundin and Wahhren (1993) who also did not observe any effect of high fructose diet on blood flow. Unlike glucose, fructose is not known to stimulate insulin release which was earlier implicated in the effect of glucose on intestinal blood flow.

The hyperglycemia induced by fructose infusion is consistent with the well established effect of administration of fructose in many reported studies (Hue, 1987; Anundi *et al*., 1987). Fructose has been reported to be converted to glucose at a high rate (Anundi *et al*., 2007; Hue, 1987) through the process of gluconeogenesis. Although, factors regulating fructose metabolishm are still not understood, unlike many other gluconoegenic precursors which are metabolized in the mitochondria, fructose bypasses many enzymes involved in gluconeogenesis (e.g Phosphoenolpyruvate carboxykinase). Fructose is converted to triose phosphates by fructokinase, fructose biphosphate aldolase and triokinase resulting in accumulation of fructose-1-phosphate in the liver (Van den Berghe and Kjerulf-jensen, 1942; Youn *et al.*, 1986) which can easily be converted into glucose. The significant increases in (A-V) glucose and intestinal glucose uptake following fructose infusion in this study seem to be a metabolic response to the rise in the blood glucose. Although, the level of increase in blood glucose during and after fructose infusion was not as high as that produced in glucose-induced hyperglycemia, other agents have also been reported to exhibit the same phenomenon. For instance, following adrenaline injection, there was about 100% increase in the blood glucose level resulting

in 900% increase in intestinal glucose uptake (Alada and Oyebola, 1996). Also, glucagon injection produced 46% increase in blood glucose and a corresponding 700% increase in intestinal glucose uptake (Alada and Oyebola, 1996). In the present study, the maximum dose of fructose (1.1.mg/kg/min) caused 17% increase in blood glucose resulting in 670% increase in intestinal glucose uptake. At a lower dose of fructose(0.55 mg/kg/min) of fructose when there was 15% increase in blood glucose level, the (A-V) glucose and intestinal glucose uptake increased by about 295% and 324% respectively. Since there was no linear correlation between the doses of fructose infused and the level of hyperglycemia induced viz-a-viz the intestinal glucose uptake, it may therefore be reasonable to conclude that fructose had a direct effect on intestinal glucose uptake. A similar correlation between the level of blood glucose and the percentage increase in intestinal glucose uptake had earlier been explored (Alada *et al.,* 2005) whereby no direct correlation was established between the percentage change in the blood glucose level and the increase in the intestinal glucose uptake. The latter therefore, concluded that the factor responsible for the increase in intestinal glucose uptake may be more than blood glucose alone. The results of the present study on the effect of fructose on intestinal glucose uptake is constistentwith this observation. In other words, it is the administration of fructose to the dogs that is responsible for the rise in intestinal glucose uptake. Another clear observation that is noted in the present study is that at a lower dose of fructose, the intestinal glucose uptake is less than that at a higher fructose dose even though the percentage changes in blood glucose produced by the two doses of fructose are not significantly different. From the result of this study, it is clear that the gastrointestinal tract is responding to fructose infusion in a similar manner to that of glucose infusion.

Fructose and glucose are isomers with the same number of carbon atoms (Meyer, 1999). The main difference between the two hexoses is that they have different structures.

The significant reduction in fructose-induced increase in (A-V) glucose and intestinal glucose uptake by propranolol seems to suggest that the fructose effect on (A-V) glucose and IGU was mediated through beta adrenergic receptors. It is to be noted also that propranolol also reduced significantly both the arterial and venous blood glucose levels in this study. Therefore, the decrease in intestinal glucose uptake could as well be a consequence of the significant reduction in fructose-induced hyperglycemia.

The absence of any effects of prazosin pretreatment on fructose induced-increase in (A-V) glucose and intestinal glucose uptake suggests that the fructose effects are most probably not mediated through alpha adrenergic receptors.

The observed effect of pretreatment of the dogs with L-NAME on fructose-induced increase in intestinal glucose uptake is quite interesting. L-NAME completely abolished fructose-induced increase in intestinal glucose uptake. The decrease in intestinal glucose uptake caused by L-NAME pretreatment is most probably due to its vasoconstrictor effect and the significant reduction in intestinal blood flow as observed in this study. Although, there was a slight increase in blood glucose level, the hyperglycemia was not enough to counteract the significant reduction in blood flow.

It is therefore reasonable to say that the blood flow plays a major role in the fructose-induced increase in intestinal glucose uptake as observed in this study. Another possibility is that the fructose-induced increase in intestinal glucose uptake could be mediated through nitric oxide. Nitric oxide has been implicated in glucose uptake in some tissues such as the heart (Ji *et al.*, 2004) and skeletal muscle (Higaki *et al.*, 2001).

GALACTOSE EXPERIMENTS

The effect of galactose infusion on the haemodynamics is essentially similar to that of fructose in which galatose had no effect on mean arterial blood pressure, blood flow and vascular resistance. Some other workers (DiPette *et al*., 1988) had reported an initial decrease in mean arterial blood pressure followed by a slight increase. However, the doses of galactose administered by DiPette *et al.* (1988) were far more than what was administered in this study. Similarly, DiPette *et al*. (1988) reported a significant decrease in the absolute blood flow in almost every organ examined with the exception of the kidney and the forearm muscle in response to high dose of galactose administered. Interestingly, blood flow to the gastrointestinal tract was not one of the parameters determined by DiPette *et al.* (1988). Consistent with the present study is a report by Ward-Hartly and Jaine (1987) which showed no significant change in blood vessel diameter following galactose injection in rabbits. Therefore, it will not be unreasonable to conclude that galactose exhibit little or no vascular effect.

In this study galactose infusion produced a slight but significant increase in blood glucose. The observed hyperglycemia following galactose infusion agrees with earlier report in dogs (Meyer, 1923) and Pigs (Bird and Hartman, 1994). In several species of neonatal animals, galactose is incorporated into hepatic glycogen more rapidly than glucose and the activity of galatokinase in the liver is greater than that of either hexokinase or glucokinase (Kliegman and Sparks, 1985). Galactose is finally converted to an intermediate uridine diphosphoglucose in glucose metabolism in the liver (Bosch, 2006).

The significant increase in (A-V) glucose and intestinal glucose uptake following galactose infusion as observed in this study is most probably a metabolic response to the increase in the blood glucose since galactose had no effect on blood flow. This observation is similar to what was observed in glucose-induced hyperglycemia, fructose-induced hyperglycemia and hyperglycemia induce by other agents (Alada and Oyebola, 1996; Patrick *et al*., 1994; Grayson and Oyebola, 1985). From these findings, it may be reasonably concluded that the gastrointestinal tract increases its glucose uptake whenever there is an increase in blood glucose level irrespective of the cause of hyperglycemia. Although, there is no linear correlation between the percentage change in blood glucose level and the degree of increase in intestinal glucose uptake, it is very clear from these findings that even a slight increase in blood glucose level resulted in a huge rise in intestinal glucose uptake. It is therefore, noteworthy that though the three sugars, that is glucose, fructose and galactose caused different levels of hyperglycemia, they also produced different levels of increases in (A-V) glucose and intestinal glucose uptake. A cursory look at the levels of hyperglycemia in response to the three hexoses showed that there were 42%, 17% and 16% increases in blood glucose levels for glucose, fructose and galactose respectively. On the other hand, the corresponding increases in intestinal glucose uptake were 670%, 670% and 350% for glucose, fructose and galactose respectively. These huge increases in intestinal glucose uptake are definitely far more than what the hyperglycemia could account for. The possibility of the three hexoses using a common transport mechanism to produce the observed effects cannot be ruled out. Indeed, apical glucose transporter GLUT 2 has been shown to transport each of the three hexoses (George and Edith, 2005) during absorption. Although, the present study has not

determined the level of involvement of the three sugars in the activity of glucose transporters, there is strong evidence from this study to show that the presence of any of the hexoses in blood circulation may be responsible for the huge amount of glucose that was taken up by the gastrointestinal tract.

It is also important to note that while the changes in blood flow contribute significantly to the increase in intestinal glucose uptake produced by glucose, intestinal blood flow had no effect on the increase in intestinal glucose uptake caused by fructose and galactose. The differences observed in their effects on intestinal glucose uptake may therefore be due to allosteric differences in their structures.

The observed reduction in galactose induced increases in (A-V) glucose and intestinal glucose uptake following pretreatment of the dogs with either an alpha blocker or a beta blocker seem to suggest the involvement of alpha and beta adrenergic receptors in the mechanism of galactose-induced increases in (A-V) glucose and intestinal glucose uptake. The complete abolition of galactose-induce increase in (A-V) glucose and intestinal glucose uptake following pretreatment with alpha and beta blockers seem to suggest the involvement of alpha and beta adrenoceptors in galactose-induce increase in intestinal glucose uptake.

The observed significant reduction of galactose-induced increase in intestinal glucose uptake following pretreatment of the dogs with L-NAME seem to suggest the involvement of nitric oxide in the mechanism for the galactose-induced rise in intestinal glucose uptake. The involvement of nitric oxide in glucose uptake of some other tissues had earlier been reported (Higaki *et al*., 2001; Ji *et al.*, 2004). The significant decrease in the blood flow following pretreatment of the dogs with L-NAME seem to confirm the

contributorty role of intestinal blood flow in the galactose–induced increase in intestinal glucose uptake. The absence of L-NAME effect on the increase in (A-V) glucose caused by galactose shows that nitric oxide is mainly involved in the intestinal blood flow.

In conclusion, the present study has shown that infusion of glucose, galactose or fructose caused significant increases in intestinal glucose uptake most probably independent of increases in blood glucose levels. There is no correlation between the hyperglycemia produced by the three hexoses and the intestinal glucose uptake. The study also showed that the contribution of the haemodynamics in the effect of glucose was very significant while the haemodynamic parameters played little or no role in the effects of fructose and galactose. The present finding showed that beta adrenergic receptors are involved in increasing intestinal glucose uptake produced by glucose, fructose and galactose and nitric oxide plays an important role in the vasodilatory effect of glucose.

CONTRIBUTIONS OF THIS STUDY TO KNOWLEDGE

1. The study showed that infusion of fructose and galactose into dogs produced significant increases in intestinal glucose uptake in a similar manner as glucose infusion.
2. The study also showed that the peaks of the intestinal glucose uptake following infusion of the three hexoses occurred at different times.
3. The study also showed that the increases in intestinal glucose uptake induced by the three sugars were mediated through beta adrenoceptors and nitric oxide.
4. The study also showed that only glucose has a vasodilatory effect which is nitric oxide-dependent.

FURTHER STUDIES

There is need to investigate further on the following questions:

(a) What is the fate of the huge glucose that is taken up by the intestine following infusion of glucose, fructose and galactose?

(b) Is this phenomenon limited to the upper jejunum of the intestine?

(c) What actually triggers on the intestinal glucose uptake?

(d) What is the role and which glucose transport protein is involved in the intestinal glucose uptake in postabsorptive state?

(e) What will happen if fructose and/or galactose is infused in diabetic animal. Will an increase in intestinal glucose uptake ameliorate the hyperglycemia?

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APPENDIX

Appendix i : Effects of intravenous infusion of glucose (0.15 mg/kg/min, 0.55 mg/kg/min, 1.1 mg/kg/min) on mean arterial blood pressure in dogs

	Dose	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Blood pressure (mmHg)	0.15 mg/kg/min	87.67 ±0.84	87.45 ±0.40	88.89 ±0.62	87.67 ±1.26	88.44 ±0.80	88.00 ±1.45	86.67 ±0.38	89.11 ±1.56	88.11 ±0.95	88.55 ±0.97	87.89 ±0.29
	0.55 mg/kg/min	91.33 ±2.65	91.22 ±1.74	92.00 ±3.10	91.78 ±3.42	93.44 ±3.31	91.33 ±1.65	90.44 ±1.56	90.89 ±2.75	90.89 ±2.32	92.45 ±3.27	91.67 ±3.17
	1.1 mg/kg/min	88.44 ±0.29	88.44 ±1.44	89.67 ±0.38	88.44 ±1.42	89.67 ±1.02	86.78 ±1.06	87.11 ±1.56	87.22 ±1.93	85.78 ±1.57	87.11 ±1.18	88.00 ±0.67

Appendix ii: Effects of intravenous infusion of glucose on mean arterial blood pressure in untreated and dogs pretreated with propranolol (pro) and prazosin (pra) or L-Nitro-Arginine-Methyl-Ester (L-NAME).

	Treatment	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Blood pressure (mmHg)	Glu	88.44 ±0.29	88.44 ±1.44	89.67 ±0.38	88.44 ±1.42	89.67 ±1.02	86.78 ±1.06	87.11 ±1.56	87.22 ±1.93	85.78 ±1.57	87.11 ±1.18	88.00 ±0.67
	Pro + Glu	83.42 ±1.62*	82.00 ±0.36*	81.42 ±1.16*	81.42 ±1.02*	81.75 ±1.02*	82.17 ±1.09*	82.92 ±1.07*	83.50 ±1.13	83.17 ±0.78	83.33 ±1.29	83.83 ±1.65*
	Pro+Pra+Glu	80.17 ±1.29*	80.33 ±1.40*	80.42 ±1.18*	80.33 ±1.37*	81.25 ±1.11*	81.08 ±0.97*	81.50 ±1.22*	82.42 ±1.05	82.17 ±0.95	82.42 ±1.24*	82.59 ±1.20*
	L-NAME + Glu	95.67 ±1.14*	95.58 ±1.79*	94.67 ±1.34*	95.00 ±0.65*	95.42 ±1.42*	95.84 ±1.46*	94.67 ±1.61*	95.75 ±1.84*	95.00 ±0.76*	94.00 ±1.11*	95.58 ±1.58*

Appendix iii : Effects of intravenous infusion of fructose on mean arterial blood pressure and vascular Resistance.

	Dose	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Blood pressure (mmHg)	0.15 mg/kg/min	87.45 ±0.62	87.22 ±0.73	87.00 ±0.51	86.56 ±0.11	86.22 ±0.29	86.56 ±0.29	85.33 ±0.67	86.11 ±0.22	85.45 ±0.91	85.22 ±1.28	85.67 ±1.00
	0.55 mg/kg/min	86.44 ±0.73	86.33 ±1.17	86.44 ±0.29	85.89 ±0.29	86.56 ±0.59	86.11 ±0.99	86.67 ±0.51	85.56 ±0.80	86.00 ±0.88	87.00 ±0.84	86.11 ±0.87
	1.1 mg/kg/min	85.67 ±0.67	84.89 ±0.29	84.44 ±0.29	84.78 ±0.45	84.67 ±0.19	84.45 ±0.40	84.44 ±0.29	84.33 ±0.38	84.22 ±0.40	85.33 ±1.35	84.00 ±0.51
Vascular resistance (R.U)	0.15 mg/kg/min	8.48 ±0.21	8.23 ±0.42	7.96 ±0.47	7.16 ±0.75	7.64 ±1.01	8.02 ±0.83	9.32 ±1.43	8.67 ±1.20	7.60 ±0.51	7.78 ±0.32	9.00 ±0.76
	0.55 mg/kg/min	7.02 ±0.24	7.47 ±0.48	7.97 ±0.80	8.57 ±1.10	7.43 ±0.27	7.88 ±0.44	8.18 ±0.44	8.30 ±0.33	7.42 ±1.67	7.95 ±0.35	9.79 ±0.99
	1.1 mg/kg/min	9.35 ±0.91	9.19 ±1.48	9.13 ±1.43	9.03 ±1.04	8.83 ±1.20	9.16 ±0.74	8.82 ±0.59	9.37 ±0.04	9.05 ±0.33	8.92 ±0.67	8.20 ±0.55

Appendix iv : Effects of intravenous infusion of fructose on mean arterial blood pressure in untreated and dogs pretreated with propranolol (pro), prazosin (pra) or combination of propranolol and prazosin. (*p<0.05).

	Treatment	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Blood pressure (mmHg)	Fru	85.67 ±0.67	84.89 ±0.29	84.44 ±0.29	84.78 ±0.45	84.67 ±0.19	84.45 ±0.40	84.44 ±0.29	84.33 ±0.38	84.22 ±0.40	85.33 ±1.35	84.00 ±0.51
	Pro + Fru	81.11 ±0.22*	81.55 ±0.22*	80.67 ±0.69*	80.22 ±0.97*	80.89 ±1.28*	81.00 ±1.53*	81.33 ±1.86	82.22 ±1.42	84.00 ±3.17	84.22 ±3.07	84.55 ±2.89
	Pra + Fru	79.33 ±2.33*	78.92 ±2.32*	79.00 ±2.42*	79.08 ±2.42*	79.09 ±2.51*	79.17 ±2.44	79.59 ±2.47	79.25 ±2.51	79.25 ±2.51	79.42 ±2.33	78.58 ±2.55
	Pro + Pra + Fru	79.02 ±1.08*	79.22 ±0.49*	79.56 ±0.59*	80.11 ±0.40*	80.22 ±0.45*	78.22 ±1.82*	77.56 ±1.83*	77.89 ±1.64*	79.11 ±1.56*	80.11 ±0.91*	80.00 ±0.39*

Appendix v: Effects of intravenous infusion of fructose on vascular resistance in untreated and dogs pretreated with propranolol (pro), prazosin (pra) or combination of propranolol and prazosin.

	Treatment	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Vascular resistance (R.U)	Fru	9.35 ±0.91	9.19 ±1.48	9.13 ±1.43	9.03 ±1.04	8.83 ±1.20	9.16 ±0.74	8.82 ±0.59	9.37 ±0.04	9.05 ±0.33	8.92 ±0.67	8.20 ±0.55
	Pro + Fru	8.17 ±0.48	7.66 ±0.27	8.13 ±0.54	8.61 ±0.21	7.59 ±0.17	8.70 ±0.39	8.43 ±0.23	8.83 ±0.38	8.70 ±0.30	9.36 ±0.34	9.09 ±0.55
	Pra + Fru	10.04 ±0.82	9.98 ±0.78	10.56 ±0.36	10.24 ±0.44	10.95 ±0.52	10.25 ±0.47	10.35 ±0.52	9.35 ±0.38	9.72 ±0.71	9.63 ±0.16	7.71 ±0.40
	Pro + Pra + Fru	10.23 ±0.60	10.84 ±0.42	10.88 ±0.40	10.12 ±0.73	9.65 ±0.35	8.39 ±0.22	8.71 ±0.77	8.65 ±0.18	9.22 ±0.60	9.10 ±0.89	8.92 ±0.50

Appendix vi : Effects of intravenous infusion of galactose on mean arterial blood pressure and vascular Resistance.

	Dose	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Blood pressure (mmHg)	0.15 mg/kg/min	86.89 ±0.40	87.33 ±0.58	87.78 ±0.40	87.66 ±0.67	87.34 ±0.67	87.32 ±0.59	87.89 ±1.28	87.78 ±0.78	88.00 ±0.51	87.89 ±0.67	87.56 ±0.29
	0.55 mg/kg/min	88.00 ±1.39	87.78 ±1.61	88.44 ±1.55	89.33 ±0.84	91.11 ±3.29	89.78 ±1.61	89.67 ±1.76	88.67 ±1.95	88.78 ±2.33	88.22 ±1.61	89.22 ±2.51
	1.1 mg/kg/min	86.11 ±0.80	86.11 ±0.97	86.22 ±1.50	85.89 ±0.97	84.55 ±0.22	85.33 ±0.51	85.22 ±0.11	86.44 ±0.59	86.44 ±0.44	86.33 ±0.77	85.67 ±0.67
Vascular resistance (r.u)	0.15 mg/kg/min	8.75 ±0.51	10.20 ±1.08	10.81 ±0.16	11.08 ±0.87	8.74 ±0.07	7.31 ±0.31	8.46 ±1.00	7.76 ±0.17	9.12 ±0.29	7.55 ±0.23	7.59 ±0.58
	0.55 mg/kg/min	9.13 ±0.47	8.50 ±0.12	7.88 ±0.51	7.80 ±0.69	8.28 ±1.03	9.24 ±0.33	7.34 ±0.49	7.75 ±0.73	7.79 ±0.89	8.47 ±1.26	9.13 ±0.90
	1.1 mg/kg/min	8.42 ±0.66	8.67 ±0.56	8.14 ±0.47	8.78 ±0.97	8.20 ±0.27	8.11 ±0.66	7.79 ±0.40	8.16 ±0.47	8.85 ±1.03	9.59 ±0.09	8.79 ±0.99

Appendix vii: Effects of intravenous infusion of galactose on mean arterial blood pressure and vascular resistance in untreated and dogs pretreated with propranolol (pro) or L-Nitro-Arginine-Methyl-Esther (L-NAME). (*p<0.05).

	Treatment	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Blood pressure (mmHg)	Gal	86.11 ±0.80	86.11 ±0.97	86.22 ±1.50	85.89 ±0.97	84.55 ±0.22	85.33 ±0.51	85.22 ±0.11	86.44 ±0.59	86.44 ±0.44	86.33 ±0.77	85.67 ±0.67
	Pro + Gal	76.33 ±1.74*	75.42 ±1.93*	76.50 ±1.16*	73.67 ±1.86*	75.08 ±1.95*	77.17 ±1.19*	76.83 ±0.78*	76.67 ±0.95*	75.51 ±1.09*	79.17 ±1.10*	78.84 ±1.17*
	L-NAME + Gal	111.17 ±1.57*	112.34 ±1.78*	112.33 ±2.22*	111.33 ±2.55*	111.33 ±3.01*	110.75 ±3.38*	110.50 ±3.38*	109.67 ±3.90*	109.75 ±3.97*	110.17 ±3.84*	110.83 ±3.57*
Vascular resistance (R.U)	Gal	8.42 ±0.66	8.42 ±0.56	8.14 ±0.47	8.78 ±0.97	8.20 ±0.27	8.11 ±0.66	7.79 ±0.40	8.16 ±0.47	8.85 ±1.03	9.59 ±0.09	8.79 ±0.99
	Pro + Gal	8.06 ±0.32	7.80 ±0.48	8.07 ±0.24	7.59 ±0.30	8.17 ±0.38	8.88 ±0.36	9.67 ±0.43*	8.09 ±0.21	10.53 ±0.58*	10.77 ±0.84*	12.68 ±0.56*
	L-NAME + Gal	16.02 ±0.78*	19.39 ±1.51*	21.07 ±1.10*	24.24 ±1.66*	27.18 ±1.44*	27.13 ±1.30*	22.78 ±0.62*	23.38 ±2.48*	25.77 ±2.29*	29.67 ±3.83*	27.29 ±1.67*

Appendix viii : Effect of normal saline on mean arterial blood pressure, blood flow and vascular resistance

	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Mean Arterial blood pressure (mmHg)	90.45 ±1.78	90.11 ±0.87	90.89 ±1.18	89.66 ±0.88	90.00 ±1.33	90.00 ±2.37	89.11 ±1.85	89.44 ±1.16	89.67 ±1.07	89.77 ±0.00	88.89 ±1.11
Blood flow (ml/min)	10.60 ±0.75	11.00 ±0.63	10.40 ±0.75	10.40 ±0.75	10.80 ±0.37	10.60 ±0.51	11.40 ±0.24	11.40 ±0.51	10.80 ±0.73	10.20 ±0.80	10.40 ±0.93
Vascular resistance (r.u)	9.50 ±0.80	8.79 ±0.53	8.72 ±0.88	9.31 ±1.24	8.24 ±0.55	8.80 ±0.56	8.10 ±0.17	8.18 ±0.49	8.79 ±0.69	8.75 ±1.33	8.47 ±1.41

Appendix ix: Effect of intravenous infusion of normal saline on arterial and venous blood glucose levels, (A-V) glucose and intestinal glucose uptake.

	0min	5min	10min	15min	20min	25min	30min	45min	60min	75min	90min
Arterial blood glucose (mg/dl)	99.20 ±0.37	98.60 ±0.51	98.20 ±0.66	97.40 ±1.25	97.80 ±0.86	96.40 ±1.08	97.20 ±1.66	96.60 ±1.40	97.00 ±1.05	96.60 ±1.86	97.00 ±1.05
Venous blood glucose (mg/dl)	95.80 ±0.97	95.20 ±0.58	94.60 ±0.24	94.80 ±0.86	94.20 ±1.88	92.40 ±1.75	93.40 ±1.66	92.60 ±1.50	93.40 ±0.81	93.40 ±1.57	93.40 ±0.51
(A-V) glucose (mg/dl)	3.40 ±0.68	3.40 ±0.93	3.60 ±0.68	3.60 ±0.68	3.60 ±1.12	4.00 ±1.05	3.80 ±0.73	4.00 ±0.55	3.80 ±0.66	3.20 ±0.73	3.60 ±0.68
Glucose uptake (mg/min)	36.80 ±8.26	39.00 ±13.16	38.40 ±7.34	38.00 ±7.54	37.80 ±10.73	43.20 ±12.11	43.40 ±8.79	46.40 ±7.50	41.60 ±7.68	32.60 ±7.56	37.00 ±6.77